Glyphosate

DOCUMENT M-CA, Section 5

TOXICOLOGICAL AND METABOLISM STUDIES ON THE ACTIVE SUBSTANCE

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Version history[1]

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[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 how to revise an Assessment Report

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CA 5 TOXICOLOGICAL AND METABOLISM STUDIES ON THE ACTIVE SUBSTANCE

Commission Directive 2001/99/EC included glyphosate as an active substance in Annex I to Council Directive 91/414/EEC. Following a peer review organised by the European Commission, glyphosate was included in Annex I of Council Directive 91/414/EEC with Commission Directive 2001/99/EC, entering into force on 01st July 2002. According to Regulation (EU) No 540/2011, glyphosate was deemed for approval under Regulation (EC) No 1107/2009 as well.

In agreement with Article 4 of Regulation (EC) No 1141/2010 Monsanto Europe S.A./N.V. (now Bayer Agriculture BV) on behalf of the then European Glyphosate Task Force submitted an application to Germany as RMS and Slovakia as Co-RMS notifying the intention to renew the existing approval of glyphosate on 24th March 2011 during the AIR 2 process. A collective supplementary dossier from the Glyphosate Task Force comprising 24 applicants was submitted on 25th May 2012.

On 12th November 2015, the European Food Safety Authority (EFSA) published its conclusions on the peer review of the pesticide risk assessment of the active substance glyphosate in the framework of the renewal of the approval under Commission Regulation (EU) No 1141/2010 (EFSA Journal 2015;13(11):4302)[1].

EFSA was requested by the European Commission (EC) to consider available information on the potential endocrine activity of the pesticide active substance glyphosate in accordance with Article 31 of Regulation (EC) No 178/2002. The assessment concluded that the weight of evidence indicates glyphosate does not possess endocrine disrupting properties via oestrogen, androgen, thyroid or steroidogenesis modes of action based on a comprehensive database available in the toxicology area.

On 17th March 2016, the rapporteur Member State, Germany, submitted a dossier to the European Chemical Agency for harmonised classification and labelling of the substance glyphosate. The proposal document was prepared in accordance with Article 37 of Regulation (EC) No 1272/2008 of the European Parliament and of the Council.

The Committee for Risk Assessment (RAC) assessed the hazards presented by glyphosate against the criteria in the Classification, Labelling and Packaging Regulation[2]. The RAC concluded that the available cientific evidence did not meet the criteria in the CLP Regulation and that glyphosate would not be classified as possessing STOT (specific target organ toxicity), carcinogenicity, mutagenicity or reproductive toxicity.

The AIR 2 process at EU level, concluded that it has been established with respect to one or more representative uses of at least one plant protection product containing the active substance glyphosate that the approval criteria provided for in Article 4 of Regulation (EC) No 1107/2009 are satisfied. Thus, the approval criteria of demonstrating a safe use were deemed to be satisfied. It was therefore appropriate to renew the active substance glyphosate[3]. Glyphosate was renewed (date of approval) on 16th December 2017 with the expiration of approval set up for 15th December 2022.

Bayer Agriculture BV[4] submits the dossier on behalf of the Glyphosate Renewal Group (GRG) for the AIR 5 process.
In the frame of the pre-submission meeting held between the GRG and the Assessment Group on

[1] Conclusion on the peer review of the pesticide risk assessment of the active substance glyphosate in the framework of the renewal of the approval under Commission Regulation (EU) No 1141/2010; EFSA Journal 2015;13(11):4302, 107 pp;doi:10.2903/j.efsa.2015.4302.
[2] RAC Opinion proposing harmonised classification and labelling at EU level of glyphosate (ISO); N (phosphono-methyl)glycine. CLH-O-0000001412-86-149/F. Adopted 15 Mar 2017.
[3] COMMISSION IMPLEMENTING REGULATION (EU) 2017/2324.
[4] Due to the Bayer-Monsanto acquisition in 2018, the legal entity name Monsanto Europe S.A. / N.V. has been changed to Bayer Agriculture BV.

Glyphosate (AGG) on 27th September 2019, the AGG provided a reference document to GRG on the process to be considered when summarizing studies from past submissions in the June 2020 renewal dossier[5].

In 1995, glyphosate active substance dossiers were submitted by both task force and individual companies comprising a total of 19 applicants. The majority of applicants of the 1995 submissions did not join the 2012 Glyphosate Task Force (GTF) nor the GRG submitting the AIR 5 dossier in 2020. The GRG was not able to get access to a total of 46 study reports from three companies that were part of the submissions in 1995 (for details please refer to the Document B, Doc ID: 110054-B-GRG_Jun_2020). Because some of the companies involved in the submissions in 1995 have subsequently been acquired by/merged with other companies or have since exited the market. Therefore, the GRG contacted Germany as the former RMS for glyphosate to discuss options available in order for AGG to get access to all said 46 study reports. A list of all these studies was sent to BVL (letter from 03rd March 2020). BVL replied to this request on 24th March 2020, advising the AGG to send a "request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009)" to the BVL. Then, BVL will forward the respective studies directly to the AGG. In the present AIR 5 Dossier, information on those inaccessible studies has been summarised based on the 2000 monograph documents[6] and are identified (as Category 4a and 4b) in the present AIR 5 dossier[7]. In these cases, GRG was unable to provide updated Appendix E summaries due to lack of access to these studies.

A number of new regulatory studies, generated after the previous EU renewal process and/or not previously submitted at EU level, are presented as part of the data package of this AIR 5 dossier. To date, those new studies have not been peer-reviewed at EU level (please refer to the Application document Rev 2 Dated May 2020 – Document F, Doc ID: 110054-F-GRG_Jun_2020).

A literature search for the active substance glyphosate and metabolites was performed in accordance with the provisions of the EFSA Guidance "Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) 1107/2009" and according to the updated Appendix to this Guidance document[8]. The scientific literature review was performed for the period of 01st January 2010 until 31st December 2019, and total of 60 relevant and reliable articles were identified for the toxicology and metabolism section. The identified relevant and reliable articles are presented as appendix E summaries in this M-CA section. For further detailed information on the Literature Review Report (LRR) and the corresponding evaluation, please refer to M-CA Section 9 "Literature". In the frame of the pre-submission meeting held on 27th September 2019, the AGG provided a reference document to GRG on the process to be considered when presenting literature in the June 2020 submission dossier[9].

During the former EU processes, public literature data was evaluated, listed and reported by the RMS. An annex, containing information about all previously submitted and/or included public literature articles from the former EU process is presented, for sake of completeness, as Annex to this M-CA section.

Analytical methods used in the toxicology studies within MCA 5 are described in detail in Section 4 Analytical Methods (MCA 4).

[5] AGG_Advice to GTF2_Literature search_Final Oct 2019 "HOW TO SUMMARISE STUDIES IN DOSSIERS FROM 1998 AND 2012 IN THE DOSSIER TO BE SUBMITTED JUNE 2020"
[6] Monograph and Addendum to the monograph EU 2001: Glyphosate monograph
[7] In the AIR 5 dossier, in each M document, a category has been assigned to each regulatory study included in the AIR 5 dossier (for details please refer to the Doc ID: 110054-B-GRG_Jun_2020).
[8] Administrative guidance on submission of dossiers and assessment reports for the peer-review of pesticide active substances approved 27 March 2019 (doi: 10.2903/sp.efsa.2019.EN-1612)
[9] AGG_Advice to GTF2_Literature search_Final Oct 2019 "ADVICE TO GTF2: HOW TO PRESENT THE LITERATURE SEARCH IN THE DOSSIER TO BE SUBMITTED JUNE 2020"

CA 5.1 Studies on Absorption, Distribution, Metabolism and Excretion in Mammals

The mammalian metabolism of glyphosate has been well assessed in numerous studies investigating the absorption, distribution, metabolism and excretion of glyphosate in rats. Across a large number of studies a consistent ADME profile is observed for glyphosate. Following oral administration, approximately 10 - 35 % of the glyphosate dose is absorbed and excreted predominantly unchanged in the urine irrespective of dose, sex or exposure duration. The unabsorbed glyphosate is rapidly excreted unchanged in faeces. No significant quantities of glyphosate are eliminated in bile or via the pulmonary route of elimination. Overall, glyphosate metabolism in mammals has been shown to be very limited, with only a small proportion (approx. 0.5 %) of the metabolite AMPA observed in urine.

For the AIR 5 process, the assessment of the mammalian metabolism of glyphosate includes detailed reports of two studies which were not available for the AIR 2 renewal process in 2012 (CA 5.1.1/001; CA 5.1.2/001). The studies are summarised together with the remaining toxicokinetic studies already evaluated in 2001 and 2012 in the table below.

Absorption, distribution and elimination

Following intraveneous dosing excretion was predominantly via urine (>75 %) (CA 5.1.1/008, CA 5.1.1/012, CA 5.1.1/014) and the extent of biliary excretion in rats was low (<0.1 %) irrespective of dose or sex (CA 5.1.1/002, CA 5.1.1/007), therefore following oral administration the amount excreted via the urinary route was considered absorbed. Glyphosate is absorbed from the gastrointestinal tract with peak plasma levels (T_{max}) ranging from 2 to 8 h (CA 5.1.1/002, CA 5.1.1/010, CA 5.1.1/011) after exposure. Absorption after oral application is limited and independent of dose, exposure duration and sex. Approximately 10 - 35 % of the glyphosate dose is absorbed and excreted predominantly unchanged in the urine. Correcting the urinary excretion after oral application for the urinary excretion after i.v. application it is determined that approximately 20 % of orally applied glyphosate is absorbed.

Elimination of ingested glyphosate via faeces and urine is rapid and is nearly complete within 48 h as shown in the majority of studies. The major route of excretion for the unabsorbed fraction is via the faeces and via the urine for the absorbed fraction. The pulmonary route of elimination is negligible (<0.2 %, CA 5.1.1/006 and CA 5.1.1/010). The systemic available amount (given as AUC) correlates with the dose level upon single gavage application. Repeated dietary application resulted in comparably lower maximum plasma concentrations. Maximum blood plasma concentration in rats after repeated dietary application of 72 and 385 mg glyphosate/kg bw/day were 0.84 and 5.31 µg/mL for male and 0.64 and 4.69 µg/mL for female rats, respectively (CA 5.1.1/001). After a single gavage application of 1 and 100 mg glyphosate/kg bw maximum plasma concentrations of 0.02 and 8.91 µg/mL for male rats and 0.036 and 7.63 µg/mL for female rats (CA 5.1.1/002). After a single gavage application of 10 and 600 mg glyphosate/kg bw maximum plasma concentrations of 0.22 and 26 µg/mL for male rats and 0.28 and 29 µg/mL for female rats (CA 5.1.1/0010). In another single gavage application study with 30 mg glyphosate/kg bw the a mean maximum plasma concentration (mean of 3 males) was 1.2 µg/mL (CA 5.1.1/011). Several studies investigated distribution of glyphosate at different dose levels into the organs and tissues after oral dose (CA 5.1.1/006, CA 5.1.1/010 - CA 5.1.1/012). The absorbed glyphosate distributes rapidly however, only low residues were found in organs and tissues at termination. After a period of 3 - 7 days following oral administration, total body burden accounted for less than 1 % of the applied radioactivity. The highest residues were measured in bone, followed by kidney and liver. There is no evidence of a potential for accumulation in animals based on residue analysis in organs and tissues after 3 - 7 days. Elimination from bone is slower than from other tissues. However, the amount of radiolabel in bone after 7 days after a single oral dose was relatively low at 0.02 - 0.03 % of the applied dose. The pattern of absorption, distribution and elimination was not significantly changed either by single high doses administered or by repeated administration of low doses. Similarly, the pattern of absorption, distribution and elimination was irrespective of the sex. The pattern of distribution of radioactivity in whole-body autoradiograms showed the greatest intensity of radioactivity to be in bone and gastrointestinal tract up to 24 h after dosing which

was reduced to negligible amounts within 48 h (CA 5.1.1/006). Elimination from blood and plasma was rapid with no evidence of accumulation in blood cells. A biphasic pattern of elimination of radiolabel in plasma has been suggested from the plasma radiolabel in a range of studies and terminal half-lives were 6 – 12 h and independent of dose level (CA 5.1.1/003, CA 5.1.1/011). The terminal half-lives were comparable (11 and 13 h at low and high dose, respectively) when glyphosate was applied via diet at 14 consecutive days (CA 5.1.1/001).

Radiolabel in plasma was negligible after 24 h and not detected at 168 h upon single application. Upon repeated application for 14 consecutive days via diet blood plasma concentrations were higher but rapidly declined within 48 h.

Metabolism

In the rat metabolism studies the metabolism of glyphosate is very limited. Most of the parent is eliminated unchanged and a small amount, just under 0.5 % of the applied dose, is eliminated as aminomethylphosphonic acid (AMPA). Low AMPA concentrations were detected in faeces and urine upon intravenous application of glyphosate (CA 5.1.1/015). Following 14 days of dietary administration of 72 and 385 mg/kg bw/day glyphosate to rats no AMPA was detected in plasma of the rats at the low dose. AMPA was only detected in plasma at 385 mg/kg bw/day and only accounted for 0.6 % of the systemic exposure (AUC0 - 48h) for glyphosate (CA 5.1.1/001). Maximum blood plasma concentration of AMPA in rats after repeated dietary application for 14 consecutive days of 385 mg glyphosate/kg bw/day were about 0.04 µg/mL, respectively. The half-life of AMPA was approximately 7 h.

Metabolism of glyphosate by other mammals was also confirmed to be limited. At least 97 % of the applied radioactivity was identified as glyphosate when cryo-preserved hepatocytes from human, rat, dog, mouse and rabbit were incubated with ^{14}C-glyphosate. No AMPA was detected in hepatocytes of any species at any time point and likewise no human unique metabolites were detected (CA 5.1.2/001). Studies in rabbits (CA 5.1.1/017), goats and laying hen also demonstrated a similar pattern of toxicokinetics parameters and limited metabolism.

Overall, glyphosate metabolism in mammals has been shown to be very limited.

Percutaneous absorption of glyphosate is very limited and is discussed in Section CP 7.3.

CA 5.1.1 Absorption, distribution, metabolism and excretion by oral exposure

Table 5.1-1: Overview of ADME studies with glyphosate

Annex Point	Study	Scope of study	Substance(s)	Reference list- related category[1]	Result
CA 5.1.1/001	[redacted] 2020	Determination of toxicokinetic characteristics of glyphosate when given via diet at 72 and 385 mg/kg bw/day for 14 consecutive days to Sprague Dawley rats.	Glyphosate (Batch: 11493988, Purity: 97.7 %)	Valid, Category 1	Systemic exposure to glyphosate and AMPA appeared to be independent of sex. Systemic exposure to AMPA was ca. 0.6 % of the systemic exposure for glyphosate following administration of 385 mg glyphosate /kg bw/day. AMPA was not detected in plasma samples from rats treated with 72 mg glyphosate/kg bw/day. $T_{1/2}$ for glyphosate 11 and 13 h, for AMPA 7.3 h
CA 5.1.1/002	[redacted] 1996	Determination of elimination of radiolabel in urine, faeces, and residual carcass. Plasma concentrations and determination of pharmacokinetic parameters; biliary excretion measurement; quantitative tissue distribution; Identification of major urinary and faecal metabolites. Single oral gavage to fasted rats of 1 or 100 mg glyphosate /kg bw.	Glyphosate (Batch: 08808TG; H95D161A, Purity: 96 %; 95.3 %)	Valid, Category 2a	Absorption, distribution, metabolism and excretion were independent of dose level and sex. Absorption was limited and distribution was rapid and extensive. Metabolism was negligible. Elimination was essentially complete within 48 h, with the majority of radioactivity recovered in faeces, likely being the unabsorbed dose. The remaining radioactivity was excreted with the urine. T_{max} 4 h for males and 8 h for females at low dose. 4 h for both sexes at high dose.
CA 5.1.1/003	[redacted] 1996	Examination of urinary and faecal excretion, plasma concentrations as well as tissue retention in selected tissues and residual carcasses; these studies corroborate findings from other studies. Single oral gavage of 10 mg glyphosate/kg bw to rats.	Glyphosate acid (Batch: Y04707/045, Purity: 99.2 %) [^{14}C]-Glyphosate acid (Batch: Y04707/047, Purity: >98 %)	Valid, Category 2a	Glyphosate was excreted rapidly and extensively, predominantly in faeces.
CA 5.1.1/004	[redacted], 1996	Examination of urinary and faecal excretion, plasma concentrations as well as tissue retention in selected tissues and residual carcasses; these studies	Glyphosate acid (Batch: Y04707/048, Purity: 99.5 %) [^{14}C]-Glyphosate acid	Valid, Category 2a	No apparent sex difference in distribution and excretion of glyphosate following oral administration. Excretion was rapidly and extensively, predominantly in the faeces.

Table 5.1-1: Overview of ADME studies with glyphosate

Annex Point	Study	Scope of study	Substance(s)	Reference list- related category[1]	Result
		corroborate findings from other studies. Single oral gavage of 1000 mg glyphosate /kg bw to rats.	(Batch: Y04707/047, Purity: >98 %)		
CA 5.1.1/005	[redacted] 1996	Examination of urinary and faecal excretion as well as residual radioactivity in blood, selected tissues and residual carcasses; this study corroborates findings from other studies obtained at the same dose level but with pre-administration of unlabelled test substance to rat.	Glyphosate acid (Batch: Y04707/045, Purity: 99.2 %) [^{14}C]-Glyphosate acid (Batch: Y04707/047, Purity: >98 %)	Valid, Category 2a	Excretion was rapid for rats of both sexes and most of the administered dose was eliminated, principally in faeces, within 24 h.
CA 5.1.1/006	[redacted], 1996	Performance of whole body autoradiogram; examination of urinary, faecal and pulmonary excretion. Single oral gavage dose of 10 mg/kg bw [^{14}C]-glyphosate to rats.	Glyphosate acid (Batch: Y04707/045, Purity: 99.2 %) [^{14}C]-Glyphosate acid (Batch: Y04707/047, Purity: >96 %)	Supportive, Category 2a	Glyphosate was excreted rapidly and predominantly in the faeces. 24 and 48 h after dosing the greatest intensity of radiolabelling were found in the bone and intestinal tract and contents.
CA 5.1.1/007	[redacted] 1996	Examination of biliary elimination after a single high oral dose and extent of systemic absorption; Identification of urinary and faecal metabolites. Single oral gavage to rats of 1000 mg glyphosate /kg bw.	Glyphosate acid (Batch: Y04707/048, Purity: 99.5 %) [^{14}C]-Glyphosate acid (Batch: Y04707/047, Purity: 97.8 %)	Valid, Category 2a	Ca. 10 - 20 % of the dose was absorbed. The unabsorbed glyphosate was excreted unchanged in faeces. The absorbed dose was excreted in urine as glyphosate and trace amounts of AMPA. No significant quantities of glyphosate were eliminated in bile.
CA 5.1.1/008	[redacted] 1995	Provides information on the distribution of radiolabel in excreta and in tissues and organs over 168 h via the oral and via a non-parenteral route. I.v. study was also used to facilitate the estimation of oral absorption from the oral route study. Single oral gavage dose of 0.2 or 200 mg/kg bw glyphosate and single	Glyphosate (Batch: UN-NO: 1759, Purity: 98 %) [^{14}C]-Glyphosate (data not given in the report)	Supportive[2], Category 2a	Absorption of glyphosate after oral application is low, rapid via faeces and independent of dose level. The majority of glyphosate is excreted unchanged within the first 24 h after application. After i.v. application the majority is excreted via urine.

Table 5.1-1: Overview of ADME studies with glyphosate

Annex Point	Study	Scope of study	Substance(s)	Reference list- related category[1]	Result
		intravenous 0.2 mg/kg bw glyphosate to rats.			
CA 5.1.1/009	[redacted] 1995	Provides information of metabolite profiles in urine and faeces and forms part of the preceding studies by Leuschner.	Glyphosate (Batch: 32140, Purity: 98 %) [^{14}C]-Glyphosate (data not given in the report)	Supportive, Category 2a	Please refer to CA 5.1.1/008
CA 5.1.1/010	[redacted] 1995	Elimination of radiolabel in urine, faeces, pulmonary route and residual carcass, determined. Plasma concentrations and determination of pharmacokinetic parameters; Quantitative tissue distribution; Identification of major urinary and faecal metabolites. Single oral gavage of 10 or 600 mg glyphosate /kg bw to rats.	HR-001 (Batch: 061221, Purity: 98.9 %) [^{14}C]-Glyphosate (Purity: >98 %; Batch data not given in the report)	Valid, Category 2a	Absorption of glyphosate after oral application is at least about 20 % based on renal excretion. Radioactivity is distributed to various body compartments with certain affinity to the bones. Excretion is virtually complete and metabolism occurs only very minor. T_{max} was observed 2 – 6 h after dosing. $T_{½}$ was 8 and 6 h, respectively.
CA 5.1.1/011	[redacted], 1992	Provides information on the distribution of radiolabel in plasma with time and by whole body autoradiography. Single oral gavage dose of 30 mg glyphosate /kg bw to rats.	Glyphosate (Batch: 206-JaK-25-1, Purity: 98.6 %) [^{14}C]-Glyphosate (Batch: CFA 745 C4, Purity: 94.3 %)	Supportive, Category 2a	Distribution of radioactivity into tissues is limited. Except for radioactivity in bone and bone marrow, there were negligible tissue concentrations 24 h post-dose. T_{max} was 4 h. $T_{1/2}$ was 6 and 12 h.
CA 5.1.1/012	[redacted] 1992	Provides information on the distribution of radiolabel in excreta and in tissues and organs over 168 h via the oral and via a non-parenteral route. Metabolites in urine and faeces from all dose groups investigated by HPLC and TLC and quantitative investigation of parent glyphosate and potential metabolite AMPA. 30 mg/kg bw single and multiple oral gavage dose, 1000 mg/kg bw single gavage dose and 20 mg/kg bw i.v.	Glyphosate (Batch: 206-JaK-25-1, Purity: 98.6 %) [^{14}C]-Glyphosate (Batch: CFQ.6228, Purity: 94.3 %)	Valid, Category 2a	After an oral single low dose, ca. 34 - 36 % were absorbed. Absorption profile did not change on repeated daily low administration or at a single high dose. Most of the dose was excreted in faeces. After i.v. administration, renal excretion was rapid and predominated. Tissue residues were low, higher concentrations in bone were noted. Concentrations in bones increased with dose but not with frequency of dosing. The pattern of absorption, distribution and excretion appeared to be independent of sex, and no

Table 5.1-1: Overview of ADME studies with glyphosate

Annex Point	Study	Scope of study	Substance(s)	Reference list- related category[1]	Result
		application of glyphosate to rats.			radiolabelled metabolites were characterised in urine or faecal samples. Only low levels (<2 %) of radioactivity co-chromatographed with the AMPA reference standard.
CA 5.1.1/013	[redacted] 1990	Previous Monograph considered the study supplementary. Provides some information on the distribution of radiolabel in excreta and tissues and organs and the distribution of parent and metabolites.	Glyphosate no information available	Invalid, Category 4b	Glyphosate is poorly absorbed and levels of glyphosate in different tissues are negligible after 15 days of oral feeding.
CA 5.1.1/014	[redacted], 1988	Orally dosed rats provide information on balance of distribution of radiolabel in excreta and in tissues and organs over 168 h after repeat dosing. Intravenously dosed rats provide information on the distribution of radiolabel in excreta and tissues and organs after administration via a non-parenteral route and was also used to facilitate the estimation of oral absorption from the oral route study. 10 mg/kg bw single and multiple oral gavage dose, 1000 mg/kg bw single gavage dose and 10 mg/kg bw i.v. application of glyphosate to rats.	Glyphosate (Batch: not given, Purity: 99.8 %) [^{14}C]-Glyphosate (Batch: not given, Purity: >99 %)	Valid, Category 2a	Glyphosate is poorly absorbed and rapidly eliminated following a single oral dose at 10 or 1000 mg/kg bw and accounted for approx. 30 %. Upon i.v. application the majority of radioactivity was excreted via urine. The plasma half-life ranged between 5.3 - 7.5 h after oral administration irrespective of dose level and number of dosing. The plasma half-life after i.v. application ranged between 2 - 5 h.
CA 5.1.1/015	[redacted], 1988	Metabolism study. Analysis of excreta, blood, tissues and organs for the for the presence and distribution of glyphosate and metabolites using HPLC and mass spectroscopy. Oral and intravenous administration of glyphosate to rats (refer to Section CA	Glyphosate (Batch: not given, Purity: 99.9 %) [^{14}C]-Glyphosate (Batch: not given, Purity: 99.85 %)	Valid, Category 2a	Predominant radioactive component with at least 97.5 % of the urine or faecal extract-contained radioactivity was [^{14}C]-glyphosate. Only small amounts (<1 %) of AMPA were found. Small amounts of AMPA were also detected upon i.v. application ind urine and faeces.

Table 5.1-1: Overview of ADME studies with glyphosate

Annex Point	Study	Scope of study	Substance(s)	Reference list- related category[1]	Result
		5.1.1/014).			
CA 5.1.1/016	[redacted], 1973	Noted to be the only study of this kind to investigate metabolism after admixture in diet and considered to provide supplementary information. Previous Monograph considered dose levels to be too low.	Glyphosate (additional data not given)	Supportive ~~Valid~~, Category 2a	By Day 4 of dosing, combined excretion in urine and faeces exceeded 90 % of the cumulative intake. After six days the body load had plateaued at a level directly proportional to the administered dose. Upon withdrawal from dosed feed, the excretion of radioactivity dropped significantly but plateaued temporarily after four days, possibly due to mobilization of previously established body loads. Most tissues reached maximum residue levels in 10 days or less and were clearly not bound. Highest tissue concentrations were found in kidneys and spleen (bone was not investigated).
CA 5.1.1/017	[redacted], 1973	Orally dosed male rabbits provide information on toxicokinetics of glyphosate in non-rodents after low dose administration (5.7 - 8.8 mg/kg bw).	Glyphosate (additional data not given)	Supportive ~~Valid~~, Category 2a	The results indicate similarities and differences between species such as a lower urinary clearance rate and higher tissue retention in rabbits as compared to the rat.
CA 5.1.1/018	[redacted], 1998	Examination of absorption and distribution in some tissues. Only imprecise results given.	Glyphosate (additional data not given)	Invalid, Category 3b	Glyphosate is poorly absorbed and levels of glyphosate in different tissues are negligible after 15 days of oral feeding.

[1] The category describes the acceptability/reliability of the study within the AIR 5 submission. This category does not describe hazard classification. (for details please refer to the Doc ID: 110054-B-GRG_Jun_2020)
[2] This study was performed a[redacted]

1. Information on the study

Data point	CA 5.1.1/001
Report author	[redacted]
Report year	2020
Report title	A GLP 14-Day Oral (Dietary) Toxicokinetic Study of Glyphosate in Sprague Dawley Rats
Report No	00050502
Document No	Not reported
Guidelines followed in study	None reported
Deviations from current test guideline	Only evaluation of plasmakinetics; clearance not calculated
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive summary

Glyphosate (97.7 % purity) was administered as dietary preparation for 14 consecutive days to four male and four female Sprague-Dawley rats to determine plasma toxicokinetic parameters of glyphosate and aminomethylphosphonic acid (AMPA). The target dose levels were 75 and 400 mg/kg bw/day in the low and high dose group, respectively. Thereafter, animals received basal diet for up to 3 days during which toxicokinetic samples were taken. Blood was sampled at 0.5, 2, 5, 8, 14, 24, 36 and 48 h after end of dietary administration.

All animals survived to the scheduled euthanasia. No abnormal clinical observations were noted and there were no differences in body weight or food consumption between test substance-treated groups.
The mean achieved dose levels were 72 and 385 mg/kg bw/day in the low and high dose group, respectively.

Systemic exposure to glyphosate and aminomethylphosphonic acid (AMPA) appeared to be independent of sex. Following 14 days of dietary administration of glyphosate, C_{max} and $AUC_{0\text{-}48h}$ values for glyphosate increased with increasing dose in an approximately dose-proportional manner. Systemic exposure ($AUC_{0\text{-}48h}$) for AMPA was only noted at 385 mg/kg bw/day and was approximately 0.6 % of the systemic exposure ($AUC_{0\text{-}48h}$) for glyphosate following 14 days of dietary administration.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	
Identification:	Glyphosate
Description:	White powder
Lot/Batch #:	11493988
Purity:	97.7 %
Stability of test compound:	Expiry date 2020-04-01
2. Reference standards:	
Identification:	Glyphosate
Lot/Batch #:	107671 (GLP-1507-24072-A)
Expiration date:	2021-08-31
Storage Conditions:	Kept in a controlled temperature area set to maintain 21°C and desiccated.
Identification:	Aminomethylphosphonic acid (AMPA)
Lot/Batch #:	107785 (GLP-1510-24158-A)
Expiration date:	2021-09-30
Storage Conditions:	Kept in a controlled temperature area set to maintain 21°C and desiccated.
3. Internal standards:	
Identification:	Glyphosate-$^{13}C_3$,^{15}N
Lot/Batch #:	107369 (GLP-0904-19814-A)
Expiration date:	2021-04-30
Storage Conditions:	Kept in a refrigerator set to maintain 4°C.
Identification:	AMPA-^{13}C,^{15}N,D_2
Lot/Batch #:	107360 (GLP-1002-20244-A)
Expiration date:	2020-01-31
Storage Conditions:	Kept in a refrigerator set to maintain 4°C.
4.Vehicle and/ or positive control:	PMI Nutrition International, LLC Lab Diet Certified Rodent Diet 5002
5. Test animals:	
Species:	Rat
Strain:	Sprague-Dawley; Crl:CD(SD)
Source:	[redacted], [redacted]
Age:	Approx. 8 weeks
Sex:	Males and females
Weight at dosing:	272 – 331 g (males), 190 – 238 g (females)
Acclimation period:	At least 14 days
Diet/Food:	PMI Nutrition International, LLC Lab Diet Certified Rodent Diet 5002, *ad libitum*
Water:	Tap water, *ad libitum*

Housing:	Individual housing in polycarbonate, solid-bottom cages containing appropriate bedding material.
Environmental conditions:	Temperature: 20 - 26°C Humidity: 30 - 70 % Air changes: not reported 12-hour light/dark cycle (except during designated procedures)

B. STUDY DESIGN

In life dates: 2019-07-08 to 2019-08-22

Animal assignment and treatment

Eight rats (4 male, 4 female) received repeated oral target doses of either 75 (Group 1) or 400 mg/kg bw/day (Group 2) by dietary administration for 14 consecutive days. Thereafter, animals received basal diet for up to 3 days during which toxicokinetic samples were taken.
Observations for mortality were made at least twice daily (once daily on days of receipt and necropsy), beginning upon arrival through termination/release. Cageside observations were made at least once daily beginning on Day 0 except for the days of detailed clinical observations. Detailed clinical observations were made within 4 days of receipt, on day of randomization, Day 0, weekly (± 2 days) thereafter and on the day of scheduled euthanasia.
Individual body weights were recorded within 4 days of receipt, on day of randomization, Days 0, 3, 7, 14 and on the day of scheduled euthanasia. Food consumption was examined on Days 0, 7, and 14.
Blood samples (0.5 mL/time point) were taken from the tail vein into heparinised tubes from each group at 0.5, 2, 5, 8, 14, 24, 36 and 48 h on Study Day 14. Each group was sacrificed upon completion of the specified sampling schedule.

Food evaluation

Homogeneity and concentration of the test material in the dietary preparations were assessed via HPLC. The initial concentrations of test substance in the diet were based on historical data for this age, strain, and sex of animal and were corrected for purity. Concentrations were adjusted weekly based on the expected average body weight and food consumption in order to meet target dose levels for each treatment group. The mean amounts of glyphosate consumed (mg/kg bw/day) by each sex per dose group were calculated from the mean food consumed (g/kg bw/day) and the appropriate target concentration of test substance in the food (mg/kg bw/day). Food efficiency (body weight gained as a percentage of food consumed) was calculated and reported.

Sample Processing

Blood samples were mixed gently centrifuged. The resultant plasma was separated, transferred to duplicate uniquely labelled polypropylene tubes, kept on ice packs until verified, and then transferred on dry ice to storage. Samples were stored in a freezer set to maintain a target of -70°C.

Measurement of glyphosate and aminomethylphosphonic acid (AMPA)

Bioanalytical samples were analysed for concentration of glyphosate and aminomethylphosphonic acid (AMPA) using a validated LC-MS/MS method (Nethero, Method No. 50.501A.RP).
Study design parameters were as following:

Parameters	
Analytes	Glyphosate Aminomethylphosphonic acid (AMPA)
Internal Standards (ISs)	Glyphosate-$^{13}C_3$,^{15}N AMPA-^{13}C,^{15}N,D_2
Species / Matrix	Rat plasma
Anticoagulant	K_2EDTA
Assay Volume	0.05 mL
Extraction Procedure	Protein filtration and solid phase extraction (SPE)
Instrumentation	UHPLC-MS/MS (Sciex API 5500)
Detection	Electrospray ionization (negative-ion mode)
Regression, Weighting	Quadratic, $1/x^2$
Calibration Curve Concentrations	10.0 to 5000 ng/mL

In addition to the experimental samples, each analytical run consisted of a minimum of duplicate calibration standards at nine concentrations, at least one blank matrix sample, at least one blank matrix with internal standard (IS) sample, and at least duplicate quality control (QC) samples at a minimum of three concentrations. The acceptance criteria described in SOP BAC-008 were required to be met for an analytical run to be accepted. The back-calculated concentrations of the calibration samples should be within ± 15 % (20 % at the lower limit of quantitation (LLOQ)) of the nominal value and at least 75 % of the calibration samples, with a minimum of 6 calibration sample levels to fulfil these criteria.

The calculated concentration of at least two-thirds of the QC samples must be within ± 15 % of their nominal concentrations. At least half of the replicates at each QC concentration must be within ± 15 % of the nominal concentration.

Concentrations less than the LLOQ (<10 ng/mL for glyphosate and AMPA) were set to 0 for toxicokinetic analysis.

Calculations

For each dose group, maximum observed plasma concentration (C_{max}), time of maximum observed plasma concentration (T_{max}) and area under the plasma concentration-time curve (AUC) were calculated.

The AUC from time 0 – 48 h ($AUC_{0\text{-}48h}$), the AUC from time 0 - 24 h ($AUC_{0\text{-}24h}$), and the AUC from time 0 to the time of the final quantifiable sample (AUC_{Tlast}) were calculated by the linear trapezoidal method for all dose groups with at least 3 consecutive quantifiable concentrations. For Day 14, C_{min} was used as an estimate of the 0-hour concentration.

Half-life values ($T_{1/2}$) were reported for composite plasma concentration-time profiles with sufficient plasma concentrations in the terminal elimination phase (at least 3 samples not including T_{max}) and an adjusted R^2 of ≥0.9. The female to male exposure ratio (F:M) and the AMPA:Glyphosate ratio were calculated for each dose group using the following formulas:

$$F{:}M = \frac{AUC_{0-48h\ Female}}{AUC_{0-48h\ Male}}$$

$$AMPA{:}Glyphosate = \frac{AUC_{0-48h\ AMPA}}{AUC_{0-48h\ Glyphosate}}$$

When T_{last} did not equal the last collection interval, the percent of AUC extrapolated (% AUC_{Extrap}) for $AUC_{0\text{-}24h}$ or $AUC_{0\text{-}48h}$ was calculated as:

$$\% AUC_{Extrap} = \frac{AUC_{0-24h} - AUC_{Tlast}}{AUC_{0-24h}} \times 100$$

$$\% AUC_{Extrap} = \frac{AUC_{0-48h} - AUC_{Tlast}}{AUC_{0-48h}} \times 100$$

All $AUC_{0\text{-}24h}$ and $AUC_{0\text{-}48h}$ values were calculated with less than 25 % extrapolation. The % AUC_{Extrap} data are not reported but are maintained in the study file.

Data exclusion and additional information

All AMPA plasma concentrations were below the LOQ (<10 ng/mL) for Group 1 (72 mg/kg bw/day) and therefore were excluded from the toxicokinetic data analysis. No other data exclusions were performed for toxicokinetic data analysis.

There was insufficient volume for analysis of glyphosate and AMPA from the sample obtained from one male dosed at 385 mg/kg bw/day glyphosate (Animal no. 6140) on Day 14 at the 5 h collection time point. $AUC_{0\text{-}24h}$ values were reported for informational purposes only to reflect the daily glyphosate dosing interval; however, were not discussed.

II. RESULTS AND DISCUSSION

A. MORTALITY

All animals survived until scheduled euthanasia.

B. CLINICAL OBSERVATIONS

There were no abnormal clinical observations noted during the study.

C. BODY WEIGHT

There were no differences in body weights or body weight gains between treated groups.

D. FOOD AND TEST SUBSTANCE CONSUMPTION DATA

From Day 0 to Day 14, food consumption and food efficiency were similar between the 75 and 400 mg/kg bw/day groups.

The average achieved glyphosate dose was determined using the food consumption data from Days 0 to 14 and resulted in 74 and 389 mg/kg bw/day for 75 and 400 mg/kg bw/day group males, and 71 and 382 mg/kg bw/day for 75 and 400 mg/kg bw/day group females. The average test substance consumption for combined sexes amounted to 72 and 385 mg/kg bw/day for the 75 and 400 mg/kg bw/day group, respectively (please refer to the table below). These average achieved glyphosate doses were used for the toxicokinetic evaluations.

Table 5.1-2: A GLP 14-Day Oral (Dietary) Toxicokinetic Study of Glyphosate in Sprague Dawley Rats ([redacted] 2020): Mean (± SD) food consumption data (mg/animal/day)

aiDose level	75 mg/kg bw/day		400 mg/kg bw/day	
Day	Males	Females	Males	Females
0 - 7	22 ± 2.0	16 ± 2.0	22 ± 2.3	16 ± 1.2
7 - 14	23 ± 1.5	16 ± 1.8	22 ± 1.9	16 ± 1.7
0 - 14	23 ± 1.7	16 ± 1.9	22 ± 2.1	16 ± 1.4

Table 5.1-3: A GLP 14-Day Oral (Dietary) Toxicokinetic Study of Glyphosate in Sprague Dawley Rats ([redacted] 2020): Average test substance consumption data (mg/kg bw/day)

Target dose levels [mg/kg bw/day]	Average achieved dose levels [mg/kg bw/day]		
	Males	Females	Combined
75	74	71	72
400	389	382	385

E. TOXICOKINETIC EVALUATIONS

Systemic exposure to glyphosate and AMPA appeared to be independent of sex. There were no consistent differences in individual plasma concentrations, C_{max}, and $AUC_{0\text{-}48h}$ values between males and females (female to male $AUC_{0\text{-}48h}$ ratios ranged from 0.784 to 0.867). Please refer to tables below. Therefore, the following discussion is based on the data for males and females combined.

Glyphosate

The variability in mean glyphosate plasma concentrations, as measured by coefficient of variation (CV) values, ranged from 10.3 - 54.5 % following 14 days of dietary administration of 72 and 385 mg/kg bw/day glyphosate.

Glyphosate was quantifiable up to 48 h and peak glyphosate plasma concentrations were observed at 0.5 h following 14 days of dietary administration of 72 and 385 mg/kg bw/day glyphosate. The mean plasma concentration declined exponentially in male and female rats irrespective of the dose level (based on a coefficient of determination (R^2) between 0.974 and 0.997 calculated for males and females at high and low dose for the logarithmic regression).

Following 14 days of dietary administration of glyphosate, C_{max} and $AUC_{0\text{-}48h}$ values for glyphosate were dose dependendly increased at the high dose. A 5.3-fold increase in glyphosate dose resulted in an approximate 6.8-fold increase in glyphosate C_{max} values and an approximate 5.4-fold increase in glyphosate $AUC_{0\text{-}48h}$ values.

The half-lives ($T_{1/2}$) for glyphosate were 11.0 and 13.0 h at 72 and 385 mg/kg bw/day, respectively.

AMPA

AMPA concentrations were all below the limit of quantitation (BQL) for the 72 mg/kg bw/day glyphosate dose level. The variability in mean AMPA plasma concentrations, as measured by CV values, ranged from 17.4 - 84.3 % following 14 days of dietary administration of 385 mg/kg bw/day glyphosate. The higher variability observed (84.3 %) was the result of BQL values converted to 0 for parameter estimates and averaged with quantifiable results. The variability in mean AMPA plasma concentrations without this value ranged from 17.4 - 27.5 %.

AMPA concentrations were below the limit of quantitation within 24 h but was detected up to 14 h after termination o ftreatment Peak AMPA plasma concentrations were observed at 0.5 h following 14 days of dietary administration of 385 mg/kg bw/day glyphosate.

C_{max} and $AUC_{0\text{-}48h}$ values for AMPA were 39.2 ng/mL and 302 h × ng/mL, respectively.

Systemic exposure ($AUC_{0\text{-}48h}$) for AMPA was less than the systemic exposure of glyphosate following 14 days of dietary administration of 385 mg/kg bw/day glyphosate. The AMPA:Glyphosate ratio, based on $AUC_{0\text{-}48h}$, was 0.00596 (i.e. 0.6 %). The $T_{1/2}$ value for AMPA was 7.32 h at 385 mg/kg bw/day.

Table 5.1-4: A GLP 14-Day Oral (Dietary) Toxicokinetic Study of Glyphosate in Sprague Dawley Rats ([redacted] 2020): Mean (± SD) glyphosate and AMPA plasma concentrations (ng/mL) in male and female rats after dietary administration of glyphosate at 72 and 385 mg/kg bw/day for 14 days

Time Point [h]	72 mg/kg bw/day				385 mg/kg bw/day			
	Male		Female		Male		Female	
	Glyphosate	AMPA	Glyphosate	AMPA	Glyphosate	AMPA	Glyphosate	AMPA
	Plasma concentrations in ng/mL							
0.5	841 ± 145	BQL[3]	636 ± 74.0	BQL	5310 ± 521	40.6 ± 13.1	4690 ± 294	37.8 ± 8.48
2	674 ± 232	BQL	523 ± 134	BQL	4280 ± 320	28.8 ± 3.68	3510 ± 472	24.3 ± 4.73
5	599 ± 79.9	BQL	520 ± 73.5	BQL	3500 ± 230	23.0 ± 3.73	2560 ± 500	18.2 ± 6.31
8	438 ± 155	BQL	307 ± 125	BQL	2240 ± 411	16.2 ± 5.81	2040 ± 509	16.9 ± 3.81
14	282 ± 36.3	BQL	272 ± 37.5	BQL	1520 ± 135	9.65 ± 6.66	1030 ± 300	7.28 ± 8.41
24	115 ± 53.2	BQL	66.1 ± 35.7	BQL	491 ± 100	0.00[2] ± N/A[4]	385 ± 87.9	0.00 ± N/A
36	51.9 ± 18.2	BQL	41.4 ± 12.1	BQL	255 ± 94.2	0.00 ± N/A	176 ± 32.5	0.00 ± N/A
48	22.5 ± 10.3	BQL	17.2 ± 6.86	BQL	134 ± 31.3	0.00 ± N/A	110 ± 12.2	0.00 ± N/A

[1]All AMPA plasma concentrations were below the limit of quantitation for Group 1 (72 mg/kg bw/day) and therefore excluded from the toxicokinetic data analysis.
[2]All concentrations less than the lower limit of quantitation (LLOQ <10.0 ng/mL for glyphosate and AMPA) were set to 0 for toxicokinetic analysis.
[3] BQL below the limit of quantitation
[4] N/A not applicable

Table 5.1-5: A GLP 14-Day Oral (Dietary) Toxicokinetic Study of Glyphosate in Sprague Dawley Rats ([redacted], 2020): Toxicokinetic parameters in plasma after dietary administration of glyphosate (<u>72 mg/kg bw/day</u>) for 14 days

Parameter		Male		Female		Combined	
		Glyphosate	AMPA	Glyphosate	AMPA	Glyphosate	AMPA
C_{max}	[ng/mL]	841	N/A[1]	636	N/A	738	N/A
C_{max}/dose	[kg × ng/mL/mg]	11.7	N/A	8.83	N/A	10.3	N/A
T_{max}	[h]	0.5	N/A	0.5	N/A	0.5	N/A
T_{last}	[h]	48	N/A	48	N/A	48	N/A
AUC_{Tlast}	[h × ng/mL]	10400	N/A	8260	N/A	9330	N/A
$AUC_{0\text{-}24h}$	[h × ng/mL]	8950	N/A	7270	N/A	8110	N/A
$AUC_{0\text{-}24h}$/dose	[h × kg × ng/mL/mg]	124	N/A	101	N/A	113	N/A
$AUC_{0\text{-}48h}$	[h × ng/mL]	10400	N/A	8260	N/A	9330	N/A
$AUC_{0\text{-}48h}$/dose	[h × kg × ng/mL/mg]	144	N/A	115	N/A	130	N/A
F:M[2]		N/A	N/A	0.795	N/A	N/A	N/A
AMPA:Glyphosate[3]	-	N/A	N/A	N/A	N/A	N/A	N/A
$T_{1/2}$	[h]	10.2	N/A	10.2	N/A	11.0	N/A

[1] N/A not applicable
[2] $F{:}M = \frac{AUC_{0-48h\ Female}}{AUC_{0-48h\ Male}}$
[3] $AMPA{:}Glyphosate = \frac{AUC_{0-48h\ AMPA}}{AUC_{0-48h\ Glyphosate}}$

Table 5.1-6: A GLP 14-Day Oral (Dietary) Toxicokinetic Study of Glyphosate in Sprague Dawley Rats ([redacted] 2020): Toxicokinetic parameters in plasma after dietary administration of glyphosate (385 mg/kg bw/day) for 14 days

Parameter		Male		Female		Combined	
		Glyphosate	AMPA	Glyphosate	AMPA	Glyphosate	AMPA
C_{max}	[ng/mL]	5310	40.6	4690	37.8	5000	39.2
C_{max}/dose	[kg × ng/mL/mg]	13.8	N/A[1]	12.2	N/A	13.0	N/A
T_{max}	[h]	0.5	0.5	0.5	0.5	0.5	0.5
T_{last}	[h]	48	14	48	14	48	14
AUC_{Tlast}	[h × ng/mL]	57000	276	44700	245	50700	260
$AUC_{0\text{-}24h}$	[h × ng/mL]	50200	325	39600	281	44700	302
$AUC_{0\text{-}24h}$/dose	[h × kg × ng/mL/mg]	130	N/A	103	N/A	116	N/A
$AUC_{0\text{-}48h}$	[h × ng/mL]	57000	325	44700	281	50700	302
$AUC_{0\text{-}48h}$/dose	[h × kg × ng/mL/mg]	148	N/A	116	N/A	132	N/A
F:M[2]	-	N/A	N/A	0.784	0.867	N/A	N/A
AMPA:Glyphosate [3]	-	N/A	0.00569	N/A	0.00629	N/A	0.00596
$T_{1/2}$	[h]	12.8	7.48	8.86	7.04	13.0	7.32

[1] N/A not applicable

[2] $F:M = \frac{AUC_{0-48h\ Female}}{AUC_{0-48h\ Male}}$

[3] $AMPA:Glyphosate = \frac{AUC_{0-48h\ AMPA}}{AUC_{0-48h\ Glyphosate}}$

III. CONCLUSIONS

Systemic exposure to glyphosate and AMPA after dietary application appeared to be independent of sex. Following 14 days of dietary administration of glyphosate, C_{max} and $AUC_{0\text{-}48h}$ values for glyphosate increased with increasing dose in an approximately dose proportional manner.

Systemic exposure ($AUC_{0\text{-}48h}$) for AMPA was approximately 0.6 % of the systemic exposure ($AUC_{0\text{-}48h}$) for glyphosate following 14 days of dietary administration of 385 mg/kg bw/day glyphosate. AMPA was not detected in plasma samples from rats treated with 72 mg/kg bw/day.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The aim of the study was to evaluate plasma kinetic parameters for two days following repeated dietary administration of glyphosate at target concentrations of 75 and 400 mg/kg bw/day for 14 consecutive days.

The average test substance consumption for combined sexes amounted to 72 and 385 mg/kg bw/day. Systemic exposure to glyphosate and AMPA appeared to be independent of sex indicated by the calculated male/female $AUC_{0\text{-}48h}$ ratios of 0.8 for glyphosate at both dose levels and the male/female $AUC_{0\text{-}48h}$ ratios of 0.9 for AMPA at the high dose. No AMPA was detected in plasma at the low dose. Following 14 days of dietary administration of glyphosate, C_{max} and $AUC_{0\text{-}48h}$ values for glyphosate were dose-dependendly increased at the high dose. The plasma concentration of AMPA was low, i.e. approximately 0.6 % of the systemic exposure ($AUC_{0\text{-}48h}$) for glyphosate following 14 days of dietary administration of 385 mg/kg bw/day glyphosate. Plasma elimination of glyphosate was fast with half-life values of 11.0 and 13.0 h at 72 and 385 mg/kg bw/day, respectively. Plasma elimination of AMPA was fast with a half-life of 7.3 h.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.1.1/002
Report author	[redacted]
Report year	1996
Report title	[^{14}C]-Glyphosate: Absorption, distribution, metabolism and excretion following oral administration to the rat
Report No	1413/2-1011
Document No	Not reported
Guidelines followed in study	Japanese MAFF, 59 NohSan, Notification No. 4200 (1995)
Deviations from current test guideline	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive summary

[^{14}C]-labelled glyphosate was administered by gavage, as a single dose of 1 or 100 mg/kg bw to fasted male and female Sprague-Dawley rats to determine tissue distribution, excretion of [^{14}C] and plasma [^{14}C]-concentrations. The biliary excretion was measured directly *via* bile duct cannulation. The proportion and ratio of metabolites were also investigated.

Absorption from the gut was slow and limited. Mean maximum plasma concentrations of 0.016 µg equiv./mL (male) and 0.0362 µg equiv./mL (female) were observed between 1.5 and 6 h (males, mean 3.9 h) and 4 and 12 h (females, mean 8 h) after dosing at a nominal dose level of 1 mg/kg bw. Following oral administration at a nominal dose level of 100 mg/kg bw maximum plasma levels were 8.909 µg equiv./mL (male) and 7.634 µg equiv./mL (female) at about 4 h following administration. A comparison of the AUC_{0-24} values shows an approximate 100-fold increase with a corresponding increase in dose, indicating that absorption is independent of the dose level. The distribution of [^{14}C]-glyphosate was rapid and widespread. Maximum tissue concentrations were observed between 4 and 12 h post-dose (1 mg/kg bw) and about 6 h post-dose (100 mg/kg bw). Examination of tissue levels indicated that [^{14}C]-glyphosate was not retained in the tissues with the exception of less than 1 % in bone at 72 h post-dose.

Metabolite profiles of pooled urine and faecal samples were investigated by HPLC analysis. Only one major peak was detected in urine and faeces (>90 % of total activity) which was subsequently identified

as glyphosate by LC-MS/MS in representative samples. A minor component was observed in the radiochromatogram, which had a similar retention time to AMPA, however, due to the very low levels this could not be definitively identified. Within 168 h the mean total recovery was 98.31 % (male) and 98.81 % (female) at a nominal dose level of 1 mg/kg bw, with 72.62 % (male) and 62.39 % (female) being recovered in faeces and 18.44 % (male) and 27.15 % (female) being detected in urine. At the high dose level (100 mg/kg bw) elimination *via* urine (39.42 % male; 43.08 % female) was quantitatively more significant than for the low dose group. Faecal elimination accounted for 41.23 % (male) and 42.38 % (female). Negligible amounts of radioactivity were detected in the bile (<0.08 µg equiv./g) at the dose level of 1 mg/kg bw, thus indicating that radioactivity recovered in faeces is most likely unabsorbed material. The increase in renal elimination following administration at 100 mg/kg bw suggest an increase in absorption. However, a high inter-animal variation is also observed which prevents a firm conclusion being drawn. Furthermore, the pharmacokinetic data indicates that absorption is linear which contradicts the suggestion of increased absorption. The highly ionisable nature of the test substance could be a contributory factor to the variability in the degree of absorption.

I. MATERIALS AND METHODS

A. MATERIALS

1. Non-labelled test material:

Identification:	Glyphosate
Description:	Not reported
Lot/Batch #:	08808TG and H95D161A
Purity:	96 % and 95.3 %, respectively
Stability of test compound:	Not reported

2. Radiolabelled test material:

Identification:	[^{14}C]-Glyphosate
Position of radiolabel:	N-(phosphono[^{14}C]methyl)glycine
Lot/Batch #:	24, Lot 3 and 25, Lot 4-7
Radiochemical purity:	>99 % (HPLC and TLC)
Specific activity:	310 µCi/mg, 53 mCi/mmol
Stability of test compound:	Stable over 24 h under the conditions of the study

3. Reference substance:

Identification:	Aminomethylphosphonic acid (AMPA)
Description:	Not reported
Lot/Batch #:	50526010
Purity:	Not reported
Stability of test compound:	Not reported

4. Vehicle and/ or positive control: Deionised water

5. Test animals:

Species:	Rat
Strain:	Sprague-Dawley (Crl:CD BR)
Source:	[redacted], [redacted]
Age:	6 - 10 weeks

Sex:	Males and females
Weight at dosing:	179 – 280 g (males) and 167 – 205 g (females)
Acclimation period:	Approximately 1 week
Diet/Food:	SQC Rat and Mouse Maintenance Diet No. 1, Expanded (Special Diet Services, Stepfield, Witham, Essex, UK), *ad libitum* Diet was removed the evening before and returned 4 h after administration
Water:	Tap water, *ad libitum*
Housing:	During acclimatisation: Groups of 5 per cage, in wire floor polypropylene cages suspended over polypropylene dirt trays containing wood saw dust After dosing: Excretion-balance experiments - individually in glass metabolism cages Blood/plasma kinetics - in wire floor cages Tissue distribution - in wire floor cages
Environmental conditions:	Temperature: 21 ± 2°C (24 and 26°C on two consecutive days) This deviation did not affect the study outcome Humidity: 40 - 70 % Air changes: not reported 12-hour light/dark cycle

B. STUDY DESIGN

In life dates: not reported

Animal assignment and treatment: Preliminary excretion study

Four fasted rats (2 males, 2 females) received single oral doses of 100 mg/kg bw by gavage and were placed in glass metabolism cages immediately thereafter. Urine was collected at 0 - 12, 12 - 24, and every 24 h for 7 days in receivers cooled with solid CO_2. Faeces were collected every 24 h for 7 days. Expired air was passed through duplicate traps containing an ethanolamine/2-ethoxyethanol mixture (1:3, v/v). These traps were changed 12, 24, 48 and 72 h after dosing. The interiors of the cages were rinsed with water after each collection time. At the end of the collection period cages were rinsed with water and methanol. Samples were analysed accordingly.

Animal assignment and treatment: Excretion studies

In two independent experiments 10 fasted rats (5 males, 5 females) received single oral doses of either 1 or 100 mg/kg bw by gavage and were placed in glass metabolism cages immediately thereafter. Urine and faeces were collected as described in the preliminary study.

Animal assignment and treatment: Plasma concentrations

In two independent experiments 10 fasted rats (5 males, 5 females) received single oral doses of either 1 or 100 mg/kg bw by gavage. Blood samples (0.1 mL) were taken from the tail vein into heparinised tubes at the following times from each animal:

Prior to administration and 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 12, 24, 36, 48 and 72 h after administration.
Blood was centrifuged to separate plasma and radioactivity was determined in plasma.

Animal assignment and treatment: Quantitative tissue distribution

In two independent experiments 24 fasted rats (12 male, 12 female) received single oral doses of either 1 or 100 mg/kg bw by gavage. The animals were divided into four groups of six (3 per sex) and sacrificed 4, 12, 24 and 72 (low dose) or 4, 6, 24 and 72 h (high dose) after dosing. Animals were exsanguinated under halothane anaesthesia. Following tissues/organs were taken or sampled for radioactivity measurements:

Adrenals, bone, brain, fat (abdominal), gastrointestinal tract, heart, kidneys, liver, lungs, muscle (quadriceps), gonads, plasma, skin, spleen, and residual carcass.

The contents of the gastrointestinal tract were analysed separately.

Animal assignment and treatment: Biliary excretion study

16 rats were cannulated (8 males, 8 females), of which 14 received single oral doses of 1 mg/kg bw/day by gavage. Following incision animals were placed in metabolism cages and allowed to recover for 24 h. Fully recovered animals were dosed after they were fasted overnight, and bile, urine and faeces were taken from the animals at the following times:

Bile: Prior to administration, 0 - 1, 1 - 4, 4 - 6, 6 - 12, 12 - 24 and 24 – 48 h p.a.
Urine, faeces: 0 - 24 and 24 – 48 h p.a. in vessels cooled with solid CO_2.

The interiors of the cages were rinsed with water after each collection time. At the end of the collection period cages were rinsed with water and methanol. Samples were analysed accordingly.

Measurement of radioactivity

Pooled faecal samples were extracted with water prior to solid phase extraction. Urine samples were diluted with water prior to solid phase extraction. Solid phase extraction was performed using columns, conditioned with methanol and de-ionised water. The samples were loaded onto the cartridge washed with de-ionised water, methanol and again water. Radioactivity was eluted using formic acid (5 % v/v). The eluate was freeze dried and reconstituted in water prior to HPLC analysis and where appropriate LC-MS (samples of 100 mg/kg bw dose group).

A suitable volume of solubilising agent was added to tissue samples. After an appropriate incubation time, liquid scintillant was added and samples were subjected to liquid scintillation counting (LSC). Samples of faecal residues, cage debris, homogenates, blood and bone were combusted, absorbed, mixed with scintillation cocktail and analysed by LSC thereafter. Combustion and trapping efficiencies were found to be in excess of 96 % and all reported data are, therefore, uncorrected. Radioassays were performed in duplicates.

Isolation of the major urinary and faecal metabolites

Samples of urine and faecal extracts from male and female rats of the excretion studies were pooled and analysed directly by HPLC. Representative samples were then submitted for analysis by mass spectrometry. The samples were analysed for the presence of glyphosate and the potential metabolite aminomethyl phosphonic acid (AMPA). Following samples were pooled and analysed for each dose group and sex:

Excretion study: Urine 12 - 24 h, Faeces 24 – 48 h
Biliary excretion study: Urine 24 – 48 h, Faeces 24 – 48 h

High performance liquid chromatography (HPLC)

The gradient elution method was used for sample analysis (column: Sperisorp SAX 250 x 4.6 mm id, eluent

A: water, eluent B: 0.75 M KH_2PO_4, pH 3.35). The system was linked to a radio-detector. Following HPLC analysis, representative samples were submitted for analysis by mass spectroscopy (samples of 100 mg/kg bw dose group).

Liquid chromatography - Mass Spectrometry (LC - MS)

A VG Quattro triple quadrupole mass spectrometer with electrospray LCMS interface connected to a Jasco ternary gradient HPLC system and a Lablogic β-Ram radio detector were used.

Mode: positive ion electrospray
Scan range: m/z 50 – 250
Mobile phases: water or 1 M formic acid

Glyphosate was detected using Multiple Reaction Monitoring (MRM) of m/z 170 → 88. AMPA was detected using Selected Ion Recording (SIR) of m/z 112.

II. RESULTS AND DISCUSSION

A. EXCRETION AND RETENTION OF RADIOACTIVITY

In a preliminary study with a single dose of 100 mg/kg bw (two rats/sex) the mean total recovery of radioactivity within 7 days was 100.3 % (male) and 95.15 % (female). No relevant radioactivity could be detected in expired air or carcass.

The initial observation was confirmed in the main study with 10 rats per dose. Please refer to table given below.

Mean total recovery of radioactivity in rats receiving a single dose of 1 mg/kg bw was 98.31 % in males and 98.81 % in females. Elimination of radioactivity was almost complete within the first 48 h after dosing. The major route of elimination after oral dosing was faeces with 72.62 % and 62.39 % recovered in males and females, respectively, with most of the radioactivity being excreted within the first 24 h after dosing, suggesting this proportion of the dose was not systemically absorbed. During the 7 days observation period 24.92 % (male) and 34.86 % (female) of radioactivity were recovered in the urine, representing the systemically absorbed dose.

After administration of 100 mg/kg bw to rats mean total recovery of radioactivity was 96.31 % in males and 98.50 % in females. Elimination of radioactivity in the urine (including cage wash 53.27 % in males and 55.04 % in females) was quantitatively more significant compared to the low dose group. Faecal elimination accounted for 41.23 % in males and 42.37 % in females. Again most of the radioactivity was recovered within the first 48 h after dosing.

Table 5.1-7: [^{14}C]-Glyphosate: Absorption, distribution, metabolism and excretion following oral administration to the rat [redacted] 1996): Excretion balance (in mean % of applied dose) at 168 h post dosing

Balance/Excretion	1 mg/kg bw		100 mg/kg bw	
	Males	Females	Males	Females
Urine 0 - 12	9.52	15.47	31.30	34.93
Urine 12 - 24	6.14	7.59	4.68	4.46
Urine 24 - 48	2.10	3.03	2.40	2.32
Urine 48 - 72	0.35	0.56	0.46	0.71
Urine 72 - 96	0.15	0.20	0.27	0.33

Table 5.1-7: [^{14}C]-Glyphosate: Absorption, distribution, metabolism and excretion following oral administration to the rat ([redacted] [redacted] 1996): Excretion balance (in mean % of applied dose) at 168 h post dosing

Balance/Excretion	1 mg/kg bw		100 mg/kg bw	
	Males	Females	Males	Females
Urine 96 - 120	0.09	0.14	0.14	0.15
Urine 120 - 144	0.06	0.10	0.10	0.10
Urine 144 - 168	0.04	0.06	0.08	0.07
Cage wash	6.48	7.71	13.85	11.96
Subtotal urine + cage wash	**24.92**	**34.86**	**53.27**	**55.04**
Faeces 0 - 24	63.93	49.69	30.46	32.28
Faeces 24 - 48	7.21	10.93	9.96	4.46
Faeces 48 - 72	0.65	1.46	0.55	1.10
Faeces 72 - 96	0.09	0.16	0.12	4.42
Faeces 96 - 120	0.03	0.06	0.06	0.06
Faeces 120 - 144	ND	0.07	0.06	0.04
Faeces 144 - 168	0.71	0.02	0.03	0.01
Subtotal faeces	**72.62**	**62.39**	**41.23**	**42.37**
Cage debris	0.03	0.58	0.98	0.10
Carcass	0.75	0.98	0.84	0.98
Total	**98.31**	**98.81**	**96.31**	**98.50**

B. BILIARY EXCRETION OF RADIOACTIVITY

Biliary excretion was determined in biliary cannulated rats receiving 1 mg/kg bw. Within 48 h 94.63 % and 95.99 % of radioactivity were recovered in males and females, respectively. Major route of elimination was faeces. Negligible amounts of radioactivity were detected in the bile, providing strong evidence that low doses of systemic glyphosate are eliminated almost exclusively in the urine. Please refer to table given below.

Table 5.1-8: [^{14}C]-Glyphosate: Absorption, distribution, metabolism and excretion following oral administration to the rat ([redacted] [redacted] 1996): Excretion balance (in mean % of applied dose) at 48 h post dosing in biliary excretion study

Balance/Excretion	1 mg/kg bw	
	Males	Females
Urine	27.45	24.21
Faeces	55.33	60.97
Bile	**0.031**	**0.076**
Cage wash	6.571	6.769
Cage debris	0.262	0.146
Carcass	4.989	3.817
Total	**94.63**	**95.99**

C. CONCENTRATION OF RADIOACTIVITY IN THE PLASMA

Following a single oral dose of 1 mg/kg bw of the test substance low levels of radioactivity were detected in plasma. Concentrations of radioactivity declined rapidly such that the levels of radioactivity were below the detection limit in most animals by 24 h. The mean terminal elimination half-lives were 10.86 h and 8.07 h with corresponding AUC of 0.319 and 0.340 µg equiv./mL × h in males and females, respectively. As the elimination half-lives could not be calculated for several animals of the high dose group, mean AUC_{0-24} (0.257 and 0.338 µg equiv./mL × h in males and females, respectively) were calculated to compare the results of both groups.

Following a single oral dose of 100 mg/kg bw of the test substance mean maximal plasma concentration of 8.91 (male) and 7.63 µg equiv./mL (female) were observed 2 - 4 h post-dose in males and 4 h post dose in females. Mean AUC_{0-24} were 58.2 and 50.7 µg equiv./mL × h in males and females, respectively. Levels of radioactivity were below the detection limit in males by 48 h and in females by 72 h. Please refer to table given below.

Table 5.1-9: [^{14}C]-Glyphosate: Absorption, distribution, metabolism and excretion following oral administration to the rat ([redacted] 1996): Kinetic parameters in plasma after single oral dose of 1 or 100 mg/kg bw (n=5)

	1 mg/kg bw		100 mg/kg bw	
	Males	**Females**	**Males**	**Females**
C_{max} (µg equiv./mL)	0.016	0.037	8.909	7.634
T_{max} (h)	3.900	8.000	3.600	4.000
AUC_{0-24} (µg equiv./mL × h)	0.257	0.338	58.200	50.700
AUC (µg equiv./mL × h)	0.319	0.340	[1]	[1]
Terminal half life (h)	10.860	8.065	[1]	[1]

[1] Could not be calculated

D. DISTRIBUTION OF RADIOACTIVITY IN TISSUE

After administration of 1 mg/kg bw [^{14}C]-labelled glyphosate radioactivity was detected in all tissues, except brain within 4 hours (see Table 5.1-10). Apart from the gastrointestinal tract (and content) and carcass, the kidney and bone were the only tissue with a notable content of radioactivity throughout the observation period ranging from 0.012 - 0.463 µg equiv/g and 0.062 - 0.201 µg equiv/g, respectively. By 72 h post-dose concentrations had decreased or plateaued to less than 2 % of the administered dose in all tissues of either sex, with carcass containing most of the remaining radioactivity (see Table 5.1-12). After administration of 100 mg/kg bw was detected in all tissues within 4 h (see Table 5.1-11). Again, apart from the gastrointestinal tract and carcass the kidney and bone were the only tissue with a notable content of radioactivity throughout the observation period ranging from 1.19 - 132.2 µg equiv/g and 10.42 - 35.45 µg equiv/g, respectively. By 72 h post-dose concentrations had decreased or plateaued to less than 2 % of the administered dose in all tissues of either sex, with carcass containing most of the remaining radioactivity(see

Table **5.1-13**).

Table 5.1-10: [^{14}C]-Glyphosate: Absorption, distribution, metabolism and excretion following oral administration to the rat ([redacted] [redacted] 1996): Radioactivity in tissues after single oral dose of 1 mg/kg bw (in mean µg equiv./g)

Tissue	Males				Females			
	4 h	12 h	24 h	72 h	4 h	12 h	24 h	72 h
Adrenals	0.014	0.024	0.020	0.009	0.023	0.031	0.022	0.009
Blood	0.010	0.015	0.001	0.002	0.020	0.009	0.002	<0.001
Bone	0.062	0.105	0.201	0.123	0.091	0.140	0.134	0.112
Brain	<0.001	0.003	0.003	0.002	0.002	0.001	0.002	0.002
Carcass	0.021	0.028	0.049	0.016	0.035	0.076	0.045	0.024
Fat	0.022	0.005	0.003	0.002	0.013	0.010	0.006	0.002
GIT + contents	13.040	1.333	1.272	0.026	11.630	3.531	1.314	0.075
Heart	0.006	0.004	0.003	0.002	0.010	0.006	0.004	0.001
Kidney	0.463	0.380	0.307	0.020	0.424	0.387	0.129	0.012
Liver	0.012	0.013	0.022	0.012	0.016	0.018	0.015	0.012
Lung	0.009	0.009	0.013	0.006	0.019	0.013	0.009	0.006
Muscle	0.003	0.001	0.002	<0.001	0.006	0.003	0.002	0.001
Ovaries	-	-	-	-	0.031	0.018	0.021	0.007
Plasma	0.017	0.011	0.006	<0.001	0.027	0.015	0.004	<0.001
Skin	0.010	0.026	0.016	0.006	0.029	0.016	0.106	0.014
Spleen	0.004	0.009	0.010	0.005	0.010	0.009	0.010	0.005
Testes	0.004	0.002	0.001	0.001	-	-	-	-

Table 5.1-11: [^{14}C]-Glyphosate: Absorption, distribution, metabolism and excretion following oral administration to the rat ([redacted] [redacted] 1996): Radioactivity in tissues after single oral dose of 100 mg/kg bw (in mean µg equiv./g)

Tissue	Males				Females			
	4 h	6 h	24 h	72 h	4 h	6 h	24 h	72 h
Adrenals	2.936	5.610	1.856	0.338	8.161	7.244	1.522	0.504
Blood	4.545	4.900	0.016	ND	5.719	1.923	0.218	ND
Bone	24.660	31.360	18.600	11.140	35.450	24.420	17.010	10.420
Brain	0.344	0.699	0.269	0.221	0.619	0.630	0.293	0.215
Carcass	6.097	26.530	4.978	1.843	10.910	38.410	7.206	3.057
Fat	1.366	1.547	0.290	0.120	3.826	2.042	0.393	0.115
GIT+contents	1155.000	544.600	47.750	1.279	1057.000	401.800	59.580	4.320
Heart	2.063	3.424	0.363	0.140	3.704	2.282	0.314	0.092
Kidneys	105.500	127.700	17.440	1.433	132.200	55.770	10.800	1.191
Liver	2.942	4.970	1.831	1.165	5.105	5.564	1.552	0.981
Lung	3.495	4.206	1.069	0.423	6.476	4.623	0.999	0.443
Muscle	0.827	0.887	0.168	0.026	1.698	1.141	0.213	0.051
Ovaries	-	-	-	-	7.532	5.407	1.260	0.438
Plasma	6.479	5.406	0.359	ND	10.830	3.033	0.403	ND

Table 5.1-11: [[14]C]-Glyphosate: Absorption, distribution, metabolism and excretion following oral administration to the rat ([redacted] [redacted] 1996): Radioactivity in tissues after single oral dose of 100 mg/kg bw (in mean µg equiv./g)

Tissue	Males				Females			
	4 h	6 h	24 h	72 h	4 h	6 h	24 h	72 h
Skin	2.884	3.520	1.293	0.313	6.106	22.480	1.543	0.435
Spleen	1.277	2.678	0.974	0.479	2.337	1.237	0.937	0.395
Testes	0.949	0.942	0.203	0.104	-	-	-	-

ND not detected

Table 5.1-12: [[14]C]-Glyphosate: Absorption, distribution, metabolism and excretion following oral administration to the rat ([redacted] [redacted] 1996): Radioactivity in tissues after single oral dose of 1 mg/kg bw (in mean % of applied dose)

Tissue	Males				Females			
	4 h	12 h	24 h	72 h	4 h	12 h	24 h	72 h
Adrenals	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Brain	<0.001	0.003	0.002	0.002	0.002	0.001	0.002	0.002
Carcass	1.236	1.668	3.048	1.045	1.887	4.115	2.542	1.405
GIT + contents	94.310	17.670	12.990	0.342	89.940	41.740	13.760	0.910
Heart	0.002	0.001	0.001	0.001	0.005	0.003	0.002	0.001
Kidney	0.392	0.304	0.255	0.016	0.348	0.341	0.110	0.011
Liver	0.040	0.050	0.113	0.059	0.055	0.070	0.073	0.057
Lung	0.005	0.005	0.007	0.003	0.012	0.008	0.005	0.004
Ovaries	-	-	-	-	0.002	0.001	0.001	<0.001
Spleen	0.001	0.002	0.002	0.001	0.002	0.002	0.002	0.001
Testes	0.004	0.002	0.002	0.001	-	-	-	-

Table 5.1-13: [[14]C]-Glyphosate: Absorption, distribution, metabolism and excretion following oral administration to the rat [redacted]., [redacted] 1996): Radioactivity in tissues after single oral dose of 100 mg/kg bw (in mean % of applied dose)

Tissue	Males				Females			
	4 h	6 h	24 h	72 h	4 h	6 h	24 h	72 h
Adrenals	0.001	0.001	<0.001	<0.001	0.002	0.001	<0.001	<0.001
Brain	0.003	0.005	0.002	0.002	0.006	0.006	0.003	0.002
Carcass	4.620	8.549	2.402	1.014	8.288	5.879	2.752	1.254
GIT + contents	85.430	64.870	5.456	0.199	75.050	48.910	7.509	0.676
Heart	0.009	0.015	0.001	0.001	0.016	0.009	0.001	<0.001
Kidney	0.870	1.109	0.151	0.012	1.165	0.535	0.096	0.011
Liver	0.104	0.180	0.110	0.060	0.183	0.214	0.088	0.050
Lung	0.027	0.024	0.006	0.003	0.040	0.027	0.007	0.003
Ovaries	-	-	-	-	0.004	0.002	0.001	<0.001
Spleen	0.003	0.007	0.002	0.001	0.006	0.003	0.003	0.001
Testes	0.011	0.010	0.003	0.001	-	-	-	-

E. METABOLITE PROFILING

After analysis of the pooled samples by HPLC, comparison of chromatograms indicated that the metabolism of the compound was not influenced by the sex or dose level. The peak with the majority of radioactivity could be allocated to [^{14}C]-glyphosate standard. A peak with <1 % of the total radioactivity was thought to correspond to AMPA. The presence of glyphosate could be confirmed by mass spectroscopy, whereas the presence of AMPA could not be verified due to technical problems.

F. RECOVERY OF RADIOACTIVITY

The mean total recovery of radioactivity within 168 h was about 94 - 99 % at the low dose and the high dose.

III. CONCLUSIONS

In conclusion, there was no apparent sex difference in the absorption, metabolism, distribution and excretion of [^{14}C]-glyphosate following oral administration at both dose levels. A time-dependent decrease of radioactivity was observed for all investigated tissues, which indicates that accumulation rather does not occur.

After oral administration of glyphosate absorption, distribution, metabolism and excretion were independent of dose level and sex. Absorption was limited and distribution was rapid and extensive. Metabolism was negligible. Elimination was essentially complete within 48 h, with the majority of radioactivity recovered in faeces, likely being the unabsorbed dose. The remaining radioactivity was excreted with the urine.

3. Assessment and conclusion

Assessment and conclusion by applicant:

After single oral administration of 10 or 100 mg/kg bw glyphosate to both male and female rats at least about 25 % are absorbed (based on the amount excreted *via* urine including cage wash). About 62 - 73 % (low dose) and 41 - 42 % (high dose) of the dose are excreted *via* faeces and 25 - 35 % (low dose) and 53 - 55 % (high dose) urine including cage wash, respectively, mainly as the unchanged parent compound within 7 days after administration. The presence of aminomethyl phosphonic acid (AMPA) could not be verified due to technical issues.
Biliary excretion experiments confirmed that bioavailability is rather low and can be calculated from the amount recovered in urine and cage wash. Furthermore, bioavailable glyphosate is excreted completely within 48 h, whereby almost no biotransformation occurs.

Investigation of pharmacokinetics in plasma revealed that plasma peak levels were reached 4 h (males) and 8 h (females) after administration of the low dose and 4 h (males and females) after administration of the high dose. No further significant gender-dependent differences were noted.

Determination of concentrations in tissue revealed overall low levels at all investigated sampling time points and dose levels, except the GI tract and contents. For all other investigated tissues and organs the levels were each below or equal to 0.39 % and 1.17 % (both determined for kidney) of the administered low and high dose, respectively. Radioactivity levels decreased time-dependently or reached the plateau levels to <2 %. Albeit repeated dosing was not investigated within the present study, the findings do not indicate an accumulation, which is also supported by observations made within other studies.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.1.1/003
Report author	[redacted]
Report year	1996
Report title	Glyphosate acid: Excretion and tissue retention of a single oral dose (10 mg/kg) in the rat
Report No	[redacted]/P/4940
Document No	Not reported
Guidelines followed in study	MAFF (Japan) Metabolism Study (1985), OECD 417 (1984), US-EPA FIFRA 85-1, EEC B.36 (1987)
Deviations from current test guideline	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive summary

A single oral dose of 10 mg/kg bw of [14C]-labelled glyphosate acid (glyphosate, >98 % radiochemical purity) was administered as aqueous solution by gavage to Alpk:APfSD rats (5 per sex) to determine tissue distribution, excretion of [14C] and plasma [14C] concentrations 72 h post administration.

Excretion of radioactivity was rapid for rats of both sexes and most of the administered dose was eliminated, principally in the faeces, within 24 h. Males excreted means of 13.3 % and 88.5 % of the dose in urine and faeces, respectively, over 72 h. Females excreted means of 11.1 % and 88.7 % of the dose in urine and faeces, respectively, over the same period. At the end of the observation period (72 h) a low percentage of radioactivity was present in all tissues examined, with highest concentrations found in the bone and intestinal tract plus contents.

In conclusion, after oral application glyphosate acid was excreted rapidly and extensively, predominantly in faeces.

I. MATERIALS AND METHODS

A. MATERIALS

1. Non-labelled test material:

Identification: Glyphosate acid (N-phosphonomethyl glycine)
Description: White solid
Lot/Batch #: Y04707/045
Purity: 99.2 %
Stability of test compound: Stable throughout the experiment

2. Radiolabelled test material:

Identification:	[^{14}C]-Glyphosate acid
Position of radiolabel:	N-(phosphono[^{14}C]methyl)glycine
Lot/Batch #:	Y04707/047
Radiochemical purity:	>98 %
Specific activity:	1.580 GBq/mMol
Stability of test compound:	Stable throughout the experiment

3. Vehicle and/or positive control: Deionised water

4. Test animals:

Species:	Rat
Strain:	Alpk:AP$_f$SD
Source:	[redacted]
Age:	Not reported, but at least 6 weeks according to body weight
Sex:	Male/female
Weight at dosing:	195 - 235 g
Acclimation period:	At least 5 days
Diet/Food:	PCD rat diet (SDS Ltd. Stepfield, Witham, Essex, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	During acclimatisation: Groups of the same sex, in stock rat cages, 24 h prior to dosing transfer individually into metabolism cages After dosing: Excretion-balance experiments - individually in metabolism cages
Environmental conditions:	Temperature: 21 ± 2°C Humidity: 40 - 70 % Air changes: 12/hour 12-hour light/dark cycle

B. STUDY DESIGN

In life dates: not reported

Animal assignment and treatment: Excretion study and quantitative tissue distribution

Ten rats (5 male, 5 female) received a single oral dose of 10 mg [^{14}C]-glyphosate acid/kg bw (10 mL/kg, 0.6 MBq/kg of dosing solution) by gavage and were placed back in glass metabolism cages immediately thereafter. Urine was collected at 0 - 6, 6 - 12, 12 - 24, 24 - 36, 36 - 48 and 48 - 72 h hafter dosing in receivers cooled with solid CO_2. Faeces were collected at 0 - 12, 12 - 24, 24 - 36, 36 - 48 h and 48 - 72 h intervals. The interior of the cages were washed with water after each collection time. At the end of the study, cages were washed with ethanol/water 1:1 (v/v). Samples were stored at -20°C until analysis.
Animals were exsanguinated by cardiac puncture under halothane anaesthesia. One blood sample was retained and divided into two portions samples were collected in heparinised vials. One was centrifuged to separate plasma. Following tissues/organs were taken or sampled for radioactivity measurements: Bone (femur), brain, fat (abdominal), gastrointestinal tract and its contents, gonads, heart, kidneys, liver, lungs, muscle (femoral), spleen, salivary glands and residual carcass.

Dosing Formulation Analysis

The radiochemical concentration of the dosing preparation was determined by liquid scintillation counting. The (radiochemical) purity of the [^{14}C]-labelled test substance prior to and following formulation in the vehicle was determined by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC).

Measurement of radioactivity

Samples of urine, cage wash and plasma were taken, without further processing, for liquid scintillation counting (LSC). Faecal samples were ground with an approximately equal weight of anhydrous magnesium sulphate until homogeneous. Samples were analysed by sample oxidation followed by LSC. Bone (cut into pieces) and whole blood were analysed by sample oxidation followed by LSC. Liver, fat, residual carcass and gastrointestinal tract (GIT) plus contents were homogenised. Liver, fat and residual carcasses were than solubilised in Soluene-350® whereas GIT plus contents were oxidised. All other tissues were solubilised without prior homogenisation.

Sample oxidation

Samples were oxidised in a Packard Tricarb sample oxidiser. The [^{14}C]-carbon dioxide generated was absorbed into Carbo-sorb E® and mixed with Permafluor E+® scintillant prior to analysis by LSC.

Liquid scintillation counting (LSC)

Samples and dilutions of the dosing preparation were mixed with Optiphase Hi-Safe 3® and counted for [^{14}C]-radioactivity to a 1 % standard deviation of the count or for a maximum of 10 min, whichever occurred first. The results obtained were corrected for background activity and counting efficiency using [^{135}Ba] as the external source.

Data evaluation

Data were processed using the Debra (Version 4.1) computerised acquisition and processing system. The limit of detection (LOD) of radioactivity measurement during this study was taken as 50 dpm per sample which was twice the liquid scintillation counter's background rate. For the purpose of calculating group mean results, individual values below the LOD are accepted as being equal to the limit of detection. Means which include one or more values which are below the LOD are reported as "<" the mean result and without a standard deviation. The limit of detection obtained for all tissues in this study was 0.004/µg equivalents glyphosate acid/g of tissue (µg equiv/g). This value is based upon a sample size of 200 mg of all determinations. Organs of less than this weight were analysed as a single sample and hence this figure represent a limiting value.

II. RESULTS AND DISCUSSION

A. EXCRETION AND RETENTION OF RADIOACTIVITY

After a single oral dose to rats, excretion was rapid for both sexes with most of the radioactivity being eliminated in the faeces during the first 24 h after dosing (means of 77.8 % in males, and 80.7 % in females). In the urine, means of 11.5 % and 9.4 % of the radioactivity were eliminated in the first 24 h in male and female rats respectively. Within the observation period of 72 h means of 101.8 % (male) and 99.6 % (female) of the administered radioactivity were excreted. There were no differences in the cumulative excretion patterns between the sexes. Please refer to table given below.

Table 5.1-14: Glyphosate acid: Excretion and tissue retention of a single oral dose (10 mg/kg) in the rat ([redacted] 1996): Excretion balance (in mean % of applied dose) at 72 h post dosing

Balance/Excretion	10 mg/kg bw (oral gavage)	
	Males	Females
Urine 0 - 6	3.7	3.5
Urine 6 - 12	4.5	3.3
Urine 12 - 24	3.3	2.6
Urine 24 - 36	0.8	0.7
Urine 36 - 48	0.4	0.4
Urine 48 - 72	0.3	0.2
Cage wash	0.3	0.4
Subtotal urine + cage wash*	**13.3**	**11.1**
Faeces 0 - 12	42.3	48.1
Faeces 12 - 24	35.5	32.6
Faeces 24 - 36	6.6	3.9
Faeces 36 - 48	2.8	2.9
Faeces 48 - 72	1.3	1.2
Subtotal faeces*	**88.5**	**88.7**
Total*	**101.8**	**99.6**

* Minor numerical deviations may occur due to rounding

B. DISTRIBUTION OF RADIOACTIVITY IN TISSUE

The highest tissue concentration of radioactivity was found in the bone with a mean concentration of 0.51 µg equiv./g (male) and 0.40 µg equiv./g (female), followed by the gastrointestinal tract (GIT) plus contents with 0.15 µg equiv./g (males and females). Lower mean concentrations between 0.01 and 0.07 µg equiv./g were found in kidneys, liver, lungs, spleen, salivary glands and ovaries. Mean concentrations of approximately 0.06 µg equiv./g were found in the residual carcass (which also included the skeletal bone) of either sex. All other concentrations were either similar to or lower than the corresponding blood concentrations. The mean total percentage of administered radioactivity present in all tissues examined and the residual carcass was 0.6 % for males and 0.5 % for females. The amounts in the intestinal tract plus contents were about 0.2 % for both sexes. Please refer to table given below.

Table 5.1-15: Glyphosate acid: Excretion and tissue retention of a single oral dose (10 mg/kg) in the rat ([redacted] 1996): Radioactivity in tissues after a single oral dose of 10 mg/kg bw at 72 h

Tissue	Males		Females	
	% of dose	µg equiv./g	% of dose	µg equiv./g
Blood	N/A	0.011	N/A	0.009
Bone (femur)	N/A	0.511	N/A	0.395
Brain	0.001	0.011	0.001	0.009
Fat (abdominal)	N/A	0.007	N/A	<0.004
Heart	<0.001	0.012	<0.001	0.011
Kidneys	0.007	0.068	0.004	0.049
Liver	0.036	0.059	0.022	0.044
Lungs	0.002	0.031	0.001	0.026
Muscle (femoral)	N/A	0.007	N/A	0.006
Ovary	-	-	<0.001	0.024
Plasma	N/A	N/A	N/A	<0.004

Table 5.1-15: Glyphosate acid: Excretion and tissue retention of a single oral dose (10 mg/kg) in the rat [redacted] 1996): Radioactivity in tissues after a single oral dose of 10 mg/kg bw at 72 h

Residual Carcass	0.542	0.062	0.458	0.056
Salivary glands	<0.001	0.017	<0.001	0.018
Spleen	0.001	0.026	0.001	0.024
Testes	0.001	0.007	-	
Total	**0.590**	**N/A**	**0.488**	**N/A**
GIT plus contents	0.186	0.152	0.172	0.152

N/A not applicable

C. RECOVERY OF RADIOACTIVITY

The total mean recovery, including excreta, tissues and residual carcass, was 102.6 % for male and 100.3 % for female rats.

III. CONCLUSIONS

After a single oral dose, glyphosate acid was excreted rapidly and predominantly in faeces. The remaining radioactivity was excreted with the urine. Elimination was essentially complete within 72 h. Negligible traces of radioactivity (<0.6 %) were still present in the tissues and residual carcass at 72 h, with bone having the highest tissue radioactivity.

3. Assessment and conclusion

Assessment and conclusion by applicant:

After single oral administration of 10 mg/kg bw glyphosate to male and female rats, at least 11 % was absorbed (based on the amount excreted *via* urine including cage wash). Urinary excretion is mainly completed within 24 h. About 89 % of the dose are excreted *via* faeces, whereby again main portions are excreted within 24 h. No gender difference was noted.

Determination of concentrations in tissue 72 h after application revealed overall low levels each below or equal to 0.6 % of the dose (residual carcass) or 0.51 µg equiv./g (bone) for both genders. Albeit repeated dosing was not investigated within the present study, the findings do not indicate an accumulation, which is also supported by observations made within other studies.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.1.1/004
Report author	[redacted]
Report year	1996
Report title	Glyphosate acid: Excretion and tissue retention of a single oral dose (1000 mg/kg) in the rat
Report No	[redacted]/P/4942
Document No	Not reported

Guidelines followed in study	Japanese MAFF, 59 NohSan, Notification No. 4200 (1985), OECD 417 (1984), US-EPA FIFRA 85-1, EEC B.36 (1987)
Deviations from current test guideline	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive summary

[^{14}C]-labelled glyphosate acid (glyphosate, >98 % radiochemical purity) was administered by gavage, as a single dose of 1000 mg/kg bw to non-fasted Alpk:AP$_f$SD rats (5 per sex) to determine tissue distribution, excretion of [^{14}C] and plasma [^{14}C] concentrations 72 h post administration.

Excretion of radioactivity was rapid for rats of both sexes and most administered dose was eliminated, principally in faeces, within 24 h. Males excreted means of 16.7 % and 89.6 % of the dose in urine and faeces respectively over 72 h. Females excreted means of 17.5 % and 84.5 % of the dose in urine and faeces respectively over the same period. The rates of excretion were thus similar for both sexes. Upon termination of the experiment (72 h) the mean total percentage of administered radioactivity present in all tissues examined and the residual carcass was 0.5 % for males and 0.6 % for females.

In conclusion, there was no apparent sex difference in distribution and excretion of [^{14}C]-glyphosate acid following oral administration at 1000 mg/kg bw. Glyphosate acid was excreted rapidly and extensively, predominantly in the faeces.

I. MATERIALS AND METHODS

A. MATERIALS

1. Non-labelled test material:

Identification:	Glyphosate acid (N-phosphonomethyl glycine)
Description:	White solid
Lot/Batch #:	Y04707/048
Purity:	99.5 %
Stability of test compound:	Stable throughout the experiment

2. Radiolabelled test material:

Identification:	[^{14}C]-Glyphosate acid
Position of radiolabel:	N-(phosphono[^{14}C]methyl)glycine
Lot/Batch #:	Y04707/047
Radiochemical purity:	>98 %
Specific activity:	1.580 GBq/mMol
Stability of test compound:	Stable throughout the experiment

3. Vehicle and/or positive control:	Deionised water
4. Test animals:	
Species:	Rat
Strain:	Alpk:AP$_f$SD
Source:	[redacted]
Age:	Not reported, but at least 6 weeks according to body weight
Sex:	Male/female
Weight at dosing:	182 - 235 g
Acclimation period:	At least 4 days
Diet/Food:	PCD rat diet (SDS Ltd. Stepfield, Witham, Essex, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	During acclimatisation: Groups of 6 per cage and sex, in stock rat cages After dosing: Excretion-balance experiments - individually in metabolism cages
Environmental conditions:	Temperature: 21 ± 2°C Humidity: 40 - 70 % Air changes: 12/hour 12-hour light/dark cycle

B. STUDY DESIGN

In life dates: not reported

Animal assignment and treatment: Excretion study and quantitative tissue distribution

Ten non-fasted rats (5 male, 5 female) received single oral doses of 1000 mg [^{14}C]-glyphosate acid/kg bw (10 mL/kg, 6 MBq/kg) by gavage and were placed in stainless steel metabolism cages immediately thereafter. Urine was collected at 0 - 6, 6 - 12, 12 - 24, 24 - 36, 36 - 48 and 48 - 72 h after dosing in receivers cooled with solid CO_2. Faeces were collected at 0 - 12, 12 - 24, 24 - 36, 36 - 48 h and 48 - 72 h intervals. The interior of the cages were washed with water after each collection time. At the end of the study, cages were washed with ethanol/water 1:1 (v/v). Samples were stored at -20°C until analysis.
Animals were exsanguinated by cardiac puncture under halothane anaesthesia. One blood sample separated into two portions was collected in heparinised vials. One was centrifuged to separate plasma. Following tissues/organs were taken or sampled for radioactivity measurements: Bone (femur), brain, fat (abdominal), gastrointestinal tract and its contents, gonads, heart, kidneys, liver, lungs, muscle (femoral), spleen, salivary glands and residual carcass.

Dosing Formulation Analysis

The radiochemical concentration of the dosing preparation was determined by liquid scintillation counting. The (radiochemical) purity of the [^{14}C]-labelled test substance prior to and following formulation in the vehicle was determined by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC).

Measurement of radioactivity

Samples of urine, cage wash and plasma were taken, without further processing, for liquid scintillation

counting (LSC). Faecal samples were ground with an approximately equal weight of anhydrous magnesium sulphate until homogeneous. Samples were analysed by sample oxidation followed by LSC. Bone (cut into pieces) and whole blood were analysed by sample oxidation followed by LSC. Liver, fat, residual carcasses were and gastrointestinal tract (GIT) plus contents were homogenised. Liver and fat were than solubilised in Soluene-350®, whereas GIT and residual carcass were oxidised. All other tissues were solubilised without prior homogenisation.

Sample oxidation

Samples were oxidised in a Packard Tricarb sample oxidiser. The [^{14}C]-carbon dioxide generated was absorbed into Carbo-sorb E® and mixed with Permafluor E+® scintillant prior to analysis by LSC.

Liquid scintillation counting (LSC)

Samples and dilutions of the dosing preparation were mixed with Optiphase Hi-Safe 3® and counted for [^{14}C]-radioactivity to a 1 % standard deviation of the count or a maximum of 10 min in Packard Tricarb instruments, whichever occurred first. The results obtained were corrected for background activity and counting efficiency using [^{135}Ba] as the external source.

Data evaluation

Data were processed using the Debra (Version 4.1) computerised acquisition and processing system. The limit of detection (LOD) of radioactivity measurement during this study was taken as 50 dpm per sample, which was twice the liquid scintillation counter's background rate. For the purpose of calculating group mean results, individual values below the LOD are accepted as being equal to the limit of detection. Means which include one or more values which are below the LOD are reported as "<" the mean result and without a standard deviation. The limit of detection obtained for all tissues in this study was 0.004/µg equivalents glyphosate acid/g of tissue (µg equiv/g). This value is based upon a sample size of 200 mg of all determinations. Organs of less than this weight were analysed as a single sample and hence this figure represent a limiting value.

II. RESULTS AND DISCUSSION

A. EXCRETION AND RETENTION OF RADIOACTIVITY

After a single oral dose to rats, excretion was rapid for both sexes with most of the radioactivity being eliminated in the faeces during the first 24 h after dosing (78.7 % in males, and 71.3 % in females). In the urine, means of 15.3 % and 16.0 % of the radioactivity were eliminated in the first 24 h in males and females, respectively. Within the observation period of 72 h, means of 106.4 % (male) and 102.3 % (female) of the administered radioactivity were excreted. There were no differences in the cumulative excretion patterns between the sexes. Please refer to table given below.

Table 5.1-16: Glyphosate acid: Excretion and tissue retention of a single oral dose (1000 mg/kg) in the rat ([redacted] 1996): Excretion balance (in mean % of applied dose) at 72 h post dosing

Balance/Excretion	1000 mg/kg bw	
	Males	Females
Urine 0 - 6	7.9	9.7
Urine 6 - 12	5.0	3.9
Urine 12 - 24	2.5	2.4
Urine 24 - 36	0.7	0.8
Urine 36 - 48	0.4	0.5

Table 5.1-16: Glyphosate acid: Excretion and tissue retention of a single oral dose (1000 mg/kg) in the rat [redacted]., 1996): Excretion balance (in mean % of applied dose) at 72 h post dosing

Balance/Excretion	1000 mg/kg bw	
	Males	Females
Urine 48 - 72	0.3	0.3
Cage wash	0.1	0.2
Subtotal urine + cage wash*	**16.9**	**17.8**
Faeces 0 - 12	36.4	19.7
Faeces 12 - 24	42.2	51.6
Faeces 24 - 36	6.6	8.5
Faeces 36 - 48	2.9	3.5
Faeces 48 - 72	1.4	1.3
Subtotal faeces*	**89.6**	**84.5**
Total*	**106.4**	**102.4**

*Minor numerical deviations may occur due to rounding

B. DISTRIBUTION OF RADIOACTIVITY IN TISSUE

The highest tissue concentration of radioactivity was found in the bone with a mean concentration of 49.8 µg equiv./g (male) and 44.9 µg equiv./g (female), followed by the gastrointestinal tract (GIT) pluscontents with 13.3 µg equiv./g (male and 16.3 µg equiv./g (female). Lower mean concentrations between 1.1 and 6.6 µg equiv./g were found in kidneys, liver, heart, lungs, spleen, brain, gonads and salivary glands of both sexes. Mean concentrations of 4.8 and 5.9 µg equiv./g were found in the residual carcass (which also included the skeletal bone) of males and females respectively. All other concentrations were either similar to or lower than the corresponding blood concentrations. The mean total percentage of administered radioactivity present in all tissues examined and the residual carcass was < 0.6 % for both sexes. The amounts in the intestinal tract plus contents were about 0.2 % for both sexes. Please refer to table given below.

Table 5.1-17: Glyphosate acid: Excretion and tissue retention of a single oral dose (1000 mg/kg) in the rat ([redacted] 1996): Radioactivity in tissues after single oral dose of 1000 mg/kg bw at 72 h

	Males		Females	
Tissue	**% of dose**	**µg equiv./g**	**% of dose**	**µg equiv./g**
Blood	N/A	0.894	N/A	0.803
Bone	N/A	49.792	N/A	44.925
Brain	0.001	1.233	0.001	1.164
Fat	N/A	0.536	N/A	0.496
GIT plus contents	0.2	13.276	0.219	16.329
Heart	0.001	1.111	0.001	1.254
Kidneys	0.007	6.511	0.005	6.046
Liver	0.039	5.480	0.029	5.226
Lungs	0.002	2.870	0.002	3.535
Muscle	N/A	0.816	N/A	0.825
Ovary	-	-	<0.001	2.940

Plasma	N/A	<0.392	N/A	<0.396
Residual carcass	0.466	4.772	0.537	5.858
Salivary glands	<0.001	1.811	<0.001	2.089
Spleen	0.001	2.441	0.001	3.106
Testes	0.001	0.905	-	
Total*	0.518	N/A	0.576	N/A

N/A not applicable
*Minor numerical deviations may occur due to rounding

C. RECOVERY OF RADIOACTIVITY

The total mean recovery, including excreta, tissues and residual carcass, was 107.1 % for male and 103.1 % for female rats.

III. CONCLUSIONS

Oral doses of glyphosate acid were excreted rapidly and predominantly in the faeces. The remaining radioactivity was excreted with the urine. Elimination was essentially complete within 72 h. Negligible traces of radioactivity (<0.6 %) were still present in the tissues and residual carcass at 72 h, with bone having the highest tissue radioactivity.

3. Assessment and conclusion

Assessment and conclusion by applicant:

After single oral administration of 1000 mg/kg bw glyphosate to male and female rats, at least 17 % was absorbed (based on the amount excreted *via* urine including cage wash). Urinary excretion is mainly completed within 24 h. About 85 % of the dose are excreted *via* faeces, whereby again main portions are excreted within 24 h. No gender difference was noted.

Determination of concentrations in tissue 72 h after application revealed overall low levels each below 0.6 % of the dose (residual carcass) or 49.79 µg equiv./g (bone) for both genders. Albeit repeated dosing was not investigated within the present study, the findings do not indicate an accumulation, which is also supported by observations made within other studies.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.1.1/005
Report author	[redacted]
Report year	1996
Report title	Glyphosate acid: Excretion and Tissue Retention of a Single Oral Dose (10 mg/kg) in the Rat Following Repeat Dosing
Report No	[redacted]/P/4944
Document No	Not reported
Guidelines followed in study	Japanese MAFF, 59 NohSan, Notification No. 4200 (1985), OECD 417 (1984), US-EPA FIFRA 85-1, EEC B.36 (1987)

Deviations from current test guideline	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive summary

Eight male and eight female non-fasted rats received a single oral dose of 10 mg unlabelled glyphosate acid/kg bw daily for 14 days by gavage. 24 h after the 14th dose of unlabelled glyphosate acid, five rats/sex received a single oral dose of 10 mg/kg bw of [^{14}C]-phosphonomethyl-labelled glyphosate acid. After monitoring the excretion of radioactivity in urine and faeces for 72 h after dosing, the rats were sacrificed and the residual radioactivity was measured in blood, selected tissues and in the residual carcasses.
The results showed that excretion of radioactivity was rapid for rats of both sexes and most of the administered dose was eliminated, principally in faeces, within 24 h. Over 72 h, males and females excreted means of 86.6 % and 90.7 % of the dose in faeces, respectively.

I. MATERIALS AND METHODS

A. MATERIALS

1. Non-labelled test material:

Identification:	Glyphosate acid (N-phosphonomethyl glycine)
Description:	White solid
Lot/Batch #:	Y04707/045
Purity:	99.2 % w/w
Stability of test compound:	Not reported

2. Radiolabelled test material:

Identification:	[^{14}C]-Glyphosate acid
Position of radiolabel:	N-(phosphono[^{14}C]methyl)glycine
Lot/Batch #:	Y04707/047
Radiochemical purity:	>98 %
Specific activity :	1.580 GBq/mMol
Stability of test compound:	The test substance was shown to be stable in the vehicle for longer than a period of use during the study

3. Vehicle and/or positive control: Deionised water

4. Test animals:

Species:	Rat
Strain:	Alpk:AP$_f$SD
Source:	[redacted]
Age:	Not reported, but at at least 6 weeks according to body weight

Sex:	Males and females
Weight at dosing (radiolabelled dose) :	225 - 328 g
Acclimation period:	At least 4 days prior to the study start and 24 h prior to dosing with the radiolabelled preparation
Diet/Food:	Pelleted PCD rat diet (Special Diets Services Ltd., Stepfield, Wiltham, Essex, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	During acclimatisation and treatment with unlabelled dose: Groups of the same sex, in stock rat cages, 24 h prior to dosing [^{14}C]-phosphonomethyl-labelled glyphosate acid transfer individually into metabolism cages After administration of [^{14}C]-phosphonomethyl-labelled glyphosate acid: Individually in stainless steel metabolism cages
Environmental conditions:	Temperature: 21 ± 2°C Humidity: 40 - 70 % Air changes: At least 12/hour 12-hour light/dark cycle

B. STUDY DESIGN

In life dates: 1995-10-16 to 1996-03-26

Animal assignment and treatment

Eight male and eight female non-fasted rats received a single oral dose of 10 mg unlabelled glyphosate acid/kg bw daily for 14 days by gavage. 24 h after the 14th dose of unlabelled glyphosate acid, five rats/sex received a single oral dose of 10 mg/kg bw of [^{14}C]-phosphonomethyl-labelled glyphosate acid. 72 h after dosing, the rats were sacrificed and the residual radioactivity was measured in blood, selected tissues and in the residual carcasses.

Dosing Formulation Analysis

The radiochemical concentration of the dosing preparation was determined by liquid scintillation counting. The (radiochemical) purity of the [^{14}C]-labelled test substance prior to and following formulation in the vehicle was determined by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC).

Collection of excreta

Urine was collected at 0 - 6, 6 - 12, 12 - 24, 24 - 36, 36 - 48 and 48 - 72 h h after dosing in receivers cooled with solid CO_2. Faeces were collected at 0 - 12, 12 - 24, 24 - 36, 36 - 48 h and 48 - 72 h intervals.
Urine collections comprised rinsing of each cage at each time point with water together with a thorough washing at the end of the study using ethanol/water 1:1 (v/v).

Collection of blood and tissues

72 h after dosing, animals were exsanguinated by cardiac puncture under halothane anaesthesia.A blood sample was retained and divided into two portions. A portion of each blood sample was centrifuged to obtain plasma, which was analysed by liquid scintillation counting. Whole blood was analysed by sample oxidation.

The following tissues/organs were taken or sampled for radioactivity measurements: Bone (femur), brain, fat (abdominal), gastrointestinal tract and its contents, gonads, heart, kidneys, liver, lungs, muscle (femoral), spleen, salivary glands and residual carcass.

Dosing Formulation Analysis

The radiochemical concentration of the dosing preparation was determined by liquid scintillation counting. The (radiochemical) purity of the [^{14}C]-labelled test substance prior to and following formulation in the vehicle was determined by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC).

Measurement of radioactivity

Samples of urine, cage wash and plasma were taken, without further processing, for liquid scintillation counting (LSC). Faecal samples were ground with an approximately equal weight of anhydrous magnesium sulphate until homogeneous. Samples were analysed by sample oxidation followed by LSC. Bone (cut into pieces) and whole blood were analysed by sample oxidation followed by LSC. Liver, fat, residual carcass and gastrointestinal tract (GIT) plus contents were homogenised. Liver, fat and residual carcasses were than solubilised in Soluene-350® whereas GIT plus contents were oxidised. All other tissues were solubilised without prior homogenisation.

Liquid scintillation counting

Radioactivity was measured by liquid scintillation counting by means of Packard Tricarb instruments. The results obtained were corrected for background activity and counting efficacy using [^{133}Ba] as the external source. Disintegrations per minute (dpm) values were calculated using the appropriate quench curve data entered into instrument's computer.

Where sample oxidation had to be performed, samples were oxidised in a Packard Tricarb sample oxidiser.

Thin layer chromatography (TLC)

TLC was conducted using a normal phase silica-gel (60F_{254}) with the following solvent system: methanol: water: 28 % ammonium hydroxide: 10 % trichloroacetic acid (60: 30: 15:5 v/v/v/v).

Radioactivity on the TLC plate was measured using a Berthold Tracemaster linear analyser. Unlabelled glyphosate acid was visualised by spraying the TLC plate with a 0.2 % ethanoic ninhydrin solution.

High performance liquid chromatography (HPLC)

To facilitate analysis a mixture of the unlabelled and radiolabelled test substance was derivatised. Sample analysis was performed by a Hichrom S5NH column (250 x 4.6 mm) which was eluted with acetonitrile buffered with 25 mM aqueous potassium dihydrogen phosphate (60:40 v/v) at a flow rate of 1.5 mL/min. Radioactivity was detected using an on-line flow detector (liquid cell) and with UV absorption at 230 nm.

Data evaluation

Data were processed using the Debra (Version 4.1) computerised acquisition and processing system. The limit of detection (LOD) of radioactivity measurement during this study was taken as 50 dpm per sample which was twice the liquid scintillation counter's background rate. For the purpose of calculating group mean results, individual values below the LOD are accepted as being equal to the limit of detection. Means which include one or more values which are below the LOD are reported as "<" the mean result and without a standard deviation. The limit of detection obtained for all tissues in this study was 0.004/µg equivalents glyphosate acid/g of tissue (µg equiv/g). This value is based upon a sample size of 200 mg of all

determinations. Organs of less than this weight were analysed as a single sample and hence this figure represent a limiting value.

II. RESULTS AND DISCUSSION

A. EXCRETION AND RETENTION OF RADIOACTIVITY

After repeated application of glyphosate acid excretion of the final phosphonomethyl-labelled glyphosate acid dose was rapid for both sexes with most of the radioactivity being eliminated in the faeces during the first 24 h after dosing (average of 80.6 % for males and 85.8 % for females).

Excretion of radioactivity in the urine during this period accounted for means of 9.2 % and 9.1 % of the administered dose in male and female rats respectively. The total percentage of the administered radioactivity eliminated in excreta 72 h after dosing were means of 97.5 % for males and 101.7 % for females. Please refer to table given below.

Comparison of the cumulative excretion data showed that there were no marked differences in the rates of excretion of radioactivity in the urine or faeces for male and female rats.

Table 5.1-18: Glyphosate acid: Excretion and Tissue Retention of a Single Oral Dose (10 mg/kg) in the Rat Following Repeat Dosing ([redacted], 1996): Excretion of radioactivity (in mean % of applied dose) in urine and faeces in male and female rats treated with 10 mg/kg bw/day for 14 days

	Excretion of radioactivity [%]			
	Males			
Time after dosing (hours)	Urine		Faeces	
	Mean[a]	SD	Mean	SD
0 - 6	3.1	0.8	N/A	N/A
6 - 12	2.7	0.7	N/A	N/A
0 - 12	N/A	N/A	50.2	15.5
12 - 24	3.4	1.6	30.3	9.0
24 - 36	0.9	0.3	3.6	1.5
36 - 48	0.3	<0.1	1.3	0.7
48 - 72	0.2	<0.1	1.1	0.6
0 - 72	10.6	3.0	86.6	5.2
	Mean	**SD**	N/A	N/A
Cage wash at 72 h	0.2	<0.1	N/A	N/A
Total excreted*	97.5	2.7	N/A	N/A
	Females			
Time after dosing (hours)	Urine		Faeces	
	Mean[b]	SD	Mean	SD
0 - 6	3.3	0.6	N/A	N/A
6 - 12	2.5	0.3	N/A	N/A
0 - 12	N/A	N/A	44.7	34.1
12 - 24	3.2	0.6	41.0	31.1
24 - 36	0.9	0.3	2.7	1.1
36 - 48	0.4	0.2	1.2	0.2
48 - 72	0.3	0.1	1.1	0.7
0 - 72	10.7	1.1	90.7	4.2
	Mean	**SD**	N/A	N/A
Cage wash at 72 h*	0.2	<0.1	N/A	N/A
Total excreted*	101.7	4.0	N/A	N/A

N/A not applicable
[a] Mean of 4 animals
[b] Mean of 5 animals
* Minor numerical deviations may occur due to rounding

B. DISTRIBUTION OF RADIOACTIVITY IN TISSUE

The highest tissue concentration of radioactivity was found in bone with a mean concentration of 0.36 µg equiv./g for males and 0.35 µg equiv./g for females, followed by the intestinal tract (GIT) plus contents with 0.11 and 0.12 µg equiv./g for males and females respectively. Lower mean concentrations between 0.02 and 0.06 µg equiv./g were found in kidneys, liver, lungs, salivary glands, and ovaries.

Mean concentrations of 0.05 µg equiv./g were found in the residual carcass of either sex which also includes the remaining skeletal bone.

All other concentrations were either similar to or lower than the corresponding blood concentrations.

The mean total percentage of administered radioactivity present in all of the tissues examined and the residual carcass was 0.5 % for males and 0.4 % for females. The amounts present in the intestinal tract plus contents were 0.1 % for males and females. Please refer to table given below.

Table 5.1-19: Glyphosate acid: Excretion and Tissue Retention of a Single Oral Dose (10 mg/kg) in the Rat Following Repeat Dosing ([redacted] 1996): Tissue and carcass residues of radioactivity in male and female rats

Tissue	Residue of radioactivity			
	Males			
	% radioactivity of dose		µg equivalents/g	
	Mean [a]	SD	Mean	SD
Brain	0.001	<0.001	0.010	0.002
Testes	0.001	<0.001	0.007	0.001
Heart	<0.001	<0.001	0.011	0.002
Kidneys	0.005	0.002	0.061	0.015
Liver	0.031	0.009	0.055	0.014
Lungs	0.001	<0.001	0.026	0.004
Spleen	0.001	<0.001	0.022	0.003
Salivary glands	<0.001	<0.001	0.019	0.004
Bone (femur)	N/A	N/A	0.358	0.177
Fat (abdominal)	N/A	N/A	0.008	0.001
Muscle (femoral)	N/A	N/A	0.008	0.001
Blood	N/A	N/A	0.014	0.006
Plasma	N/A	N/A	<0.004	-
Residual carcass	0.423	0.090	0.050	0.011
Total	0.463	0.101	N/A	N/A
Intestinal tract plus contents	0.108	0.040	0.109	0.041

Tissue	Females			
	% radioactivity of dose		µg equivalents/g	
	Mean [b]	SD	Mean	SD
Brain	0.001	<0.001	0.010	0.002
Ovaries	<0.001	<0.001	0.026	0.006
Heart	<0.001	<0.001	0.012	0.004
Kidneys	0.004	0.001	0.049	0.011
Liver	0.021	0.005	0.045	0.010
Lungs	0.001	<0.001	0.029	0.006
Spleen	0.001	<0.001	0.025	0.006
Salivary glands	<0.001	<0.001	0.027	0.006
Bone (femur)	N/A	N/A	0.345	0.081
Fat (abdominal)	N/A	N/A	0.006	0.002
Muscle (femoral)	N/A	N/A	0.007	0.002
Blood	N/A	N/A	0.010	0.002

Plasma	N/A	N/A	<0.005	-
Residual carcass	0.382	0.067	0.046	0.008
Total	0.411	0.073	N/A	N/A
Intestinal tract plus contents	0.115	0.014	0.117	0.015

N/A not applicable
Residual carcass values include partial tissue percentages
[a] Mean of 4 animals
[b] Mean of 5 animals

C. RECOVERY OF RADIOACTIVITY

The total mean percentage recoveries, including excreta, tissues and residual carcass was 98.0 % for male rats and 102.2 % for females.

III. CONCLUSIONS

Oral doses of glyphosate acid were excreted rapidly and predominantly in the faeces. The remaining radioactivity was excreted with the urine. Elimination was essentially complete within 72 h. Negligible traces of radioactivity (<0.6 %) were still present in the tissues and residual carcass at 72 h, with bone having the highest tissue radioactivity.

3. Assessment and conclusion

Assessment and conclusion by applicant:

After repeated oral administration of 10 mg/kg bw glyphosate to male and female rats for 14 consecutive days, about 11 % was absorbed (based on the amount excreted *via* urine including cage wash). Urinary excretion is mainly completed within 24 h. About 90 % of the dose was excreted *via* faeces, whereby again main portions were excreted within 24 h. No gender difference was noted.

Determination of concentrations in tissue 72 h after the last application revealed overall low levels each below 0.5 % of the dose (residual carcass) or 0.35 to 0.36 µg equiv./g (bone) for female and male rats.

Comparison of the results with those obtained at the same dose level but without pre-administration of unlabelled test substance (see CA 5.1.1/003) showed no significant differences on either the routes or rates of elimination after oral dosing. In both studies the test substance was excreted rapidly and predominantly in the faeces by rats of both sex and low amounts of radioactivity were detected in all the tissue examined.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.1.1/006
Report author	[redacted]
Report year	1996
Report title	Glyphosate acid: Whole body autoradiography in the rat (10 mg/kg)
Report No	[redacted]/P/4943
Document No	Not reported
Guidelines followed in study	Japanese MAFF, 59 NohSan, Notification No. 4200 (1985), OECD 417 (1984), US-EPA FIFRA 85-1, EEC B.36 (1987)
Deviations from current test guideline	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive summary

Two male and two female rats were administered a single oral dose of 10 mg [^{14}C]-glyphosate acid/kg bw. One male and one female was sacrificed 24 h after dosing and the other pair were sacrificed 48 h after dosing. Qualitative whole body autoradiogram was performed on all animals. In addition, radioactivity was measured in urine, faeces and exhaled air.

24 h after dosing, excreted means of the administered dose in the urine and faeces amounted to 22.3 % and 55.5 % in males and 11.9 % and 83.8 % in females, respectively, whereas less than 0.2 % was detected in exhaled air. 48 h after dosing, excretion of the administered dose in urine and faeces increased to 34.0 % and 60.5 % in males and 12.5 % and 91.2 % in females, respectively. The whole body autoradiograms showed no marked differences in the tissue distribution of radioactivity between male and female rats. The greatest intensity of radioactivity was present in the bone for both sexes, followed by the intestinal tract and the kidneys 24 h after dosing with lesser to negligible amounts being present after 48 h.

In conclusion, glyphosate acid was excreted rapidly and predominantly in the faeces. 24 and 48 h after dosing the greatest intensity of radiolabelling were found in the bone and intestinal tract and contents.

I. MATERIALS AND METHODS

A. MATERIALS

1. Non-labelled test material:

Identification: Glyphosate acid (N-phosphonomethyl glycine)
Description: White solid
Lot/Batch #: Y04707/045
Purity: 99.2 % w/w

Stability of test compound:	Not reported

2. Radiolabelled test material:

Identification:	[^{14}C]-Glyphosate acid
Position of radiolabel:	N-(phosphono[^{14}C]methyl)glycine
Lot/Batch #:	Y04707/047
Radiochemical purity:	>96 %
Specific activity :	1.580 GBq/mMol
Stability of test compound:	Stable in the vehicle for longer than a period of use during the study

3. Vehicle and/ or positive control: Deionised water

4. Test animals:

Species:	Rat
Strain:	Alpk:AP$_f$SD
Source:	[redacted]
Age:	Not reported, but at least 6 weeks according to body weight
Sex:	Males and females
Weight at dosing:	215 - 271 g
Acclimation period:	At least 5 days in stock rat cages and 24 h prior to dosing in metabolism cages
Diet/Food:	Pelleted PCD rat diet (Special Diets Services Ltd., Stepfield, Witham, Essex, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	During acclimatisation: Groups of the same sex, in stock rat cages, 24 h prior to dosing transfer individually into metabolism cages After administration of [^{14}C]-phosphonomethyl-labelled glyphosate acid: Housed individually in glass metabolism cages
Environmental conditions:	Temperature: 21 ± 2°C Humidity: 40 - 70 % Air changes: At least 12/hour 12-hour light/dark cycle

B. STUDY DESIGN

In life dates: 1995-10-25 to 1996-04-04

Animal assignment and treatment

Two male and two female non-fasted rats were administered with a single oral dose of 10 mg [^{14}C]-glyphosate acid/kg bw by gavage. 24 and 48 h after dosing, a heterosexual pair was sacrificed and a qualitative whole body autoradiogram was performed. In addition, radioactivity was measured in urine, faeces and exhaled air.

Dosing Formulation Analysis

The radiochemical concentration of the dosing preparation was determined by liquid scintillation counting. The (radiochemical) purity of the [^{14}C]-labelled test substance prior to and following formulation in the vehicle was determined by thin layer chromatography (TLC) high performance liquid chromatography (HPLC).

Collection of excreta

Urine was collected at 0 - 6, 6 - 12, 12 - 24 h from all animals and on 24 - 36 and 36 - 48 h after dosing of two animals in receivers cooled with solid CO_2. Faeces were collected at 0 - 12, 12 - 24 h from all animals and on 24 - 36, 36 - 48 h intervals h after dosing of two animals.
Urine collections comprised rinsing of each cage at each time point with water together with a thorough washing at the end of the study using ethanol/water 1:1 (v/v).

Collection of exhaled air

The exhaled air from one heterosexual pair was passed through sodium hydroxide to trap any radioactivity expired as [^{14}C]-carbon dioxide. Subsamples of the contents of each trap were removed for radiochemical analysis at 6, 12, and 24 h after dosing.

Whole body autoradiography

Immediately after scheduled sacrifice, each carcass was frozen rapidly and embedded in blocks of 2 % (w/v) aqueous carboxymethylcellulose. Longitudinal sagittal sections, 30 µm thick, were taken, mounted on adhesive tape and freeze-dried for approximately 48 h. Autoradiograms were prepared by contact with autoradiographic film and exposed for periods of 2, 4 or 6 weeks before fixing and washing.

Measurement of radioactivity

Samples of urine, cage wash and exhaled air were taken, without further processing, for liquid scintillation counting (LSC). Faecal samples were ground with an approximately equal weight of anhydrous magnesium sulphate until homogeneous. Samples were analysed by sample oxidation followed by LSC.

Liquid scintillation counting

Radioactivity was measured by liquid scintillation analysis by means of Packard Tricarb instruments. The results obtained were corrected for background activity and counting efficacy using [^{133}Ba] as the external source. Disintegrations per minute (dpm) values were calculated using the appropriate quench curve data entered into instrument's computer.
Where sample oxidation had to be performed, samples were oxidised in a Packard Tricarb sample oxidiser.

Thin layer chromatography (TLC)

TLC was conducted using a normal phase silica-gel ($60F_{254}$) with the following solvent system: methanol: water: 28 % ammonium hydroxide: 10 % trichloroacetic acid (60:30:15:5 v/v/v/v).
Radioactivity on the TLC plate was measured using a Berthold Tracemaster linear analyser. Unlabelled glyphosate acid was visualised by spraying the TLC plate with a 0.2 % ethanoic ninhydrin solution.

High performance liquid chromatography (HPLC)

To facilitate analysis a mixture of the unlabelled and radiolabelled test substance was derivatised. Sample analysis was performed by a Hichrom S5NH column (250 x 4.6 mm) which was eluted with acetonitrile buffered with 25 mM aqueous potassium dihydrogen phosphate (60:40 v/v) at a flow rate of 1.5 mL/min.

Radioactivity was detected using an on-line flow detector (liquid cell) and with UV absorption at 230 nm.

Data evaluation

Data were processed using the Debra (Version 4.1) computerised acquisition and processing system. The limit of detection (LOD) of radioactivity measurement during this study was taken as 50 dpm per sample, which was twice the liquid scintillation counter's background rate. The LOD for each carbon dioxide trap in this study was 0.01 % of the administered dose.

II. RESULTS AND DISCUSSION

A. EXCRETION OF RADIOACTIVITY

Excretion was rapid for both sexes with most of the radioactivity being eliminated in the faeces during the first 24 h after dosing (average of 55.5 % for males and 83.8 % for females). Excretion of radioactivity in the urine during this period accounted for means of 22.3 % and 11.9 % of the administered dose in male and female rats respectively. Less than 0.2 % of the applied dose was detected in exhaled air.

After 48 h, 60.5 % and 91.2 % of the applied dose were detected in faeces of the male and the female rat, respectively. 34.0 % and 12.5 % were detected in urinary samples of the male and the female rat, respectively. Urinary radioactivity after 48 h accounted for 34 % of the applied dose in one male rat while the urinary radioactivity in the other three animals was below 19 % each.

Individual results of the excreted radioactivity in urine, faeces and exhaled air expressed as percentages of the administered radioactivity, together with the results for cage washings, are listed in the table given below.

Table 5.1-20: Glyphosate acid: Whole body autoradiography in the rat (10 mg/kg) ([redacted], 1996): Excretion of radioactivity in urine, faeces, cage wash and expired air by male and female rats at 10 mg/kg bw

	Time After Dosing (hours)	Males		Females	
		Rat 1	Rat 2	Rat 3	Rat 4
		(% of applied dose)		(% of applied dose)	
Urine	**0 - 6**	5.00	8.21	4.06	3.67
	6 - 12	5.30	8.67	4.32	4.12
	12 - 24	7.57	9.92	4.39	3.23
	24 - 36	N/A	4.30	N/A	0.93
	36 - 48	N/A	2.90	N/A	0.54
	Total	17.86	34.00	12.77	12.48
	Terminal Cage wash	1.12	0.98	1.43	0.41
Total excreted*		18.98	34.98	14.10	12.89
Faeces	**0 - 12**	30.62	0.21	54.27	32.63
	12 - 24	28.69	51.48	26.03	54.76
	24 - 36	N/A	1.65	N/A	2.71
	36 - 48	N/A	7.12	N/A	1.13
	Total	59.32	60.46	80.30	91.23
Exhaled air	**0 - 6**	0.07	N/A	0.08	N/A
	6 - 12	0.02	N/A	0.04	N/A
	12 - 24	0.02	N/A	0.03	N/A
	Total	0.11	N/A	0.14	N/A
Total*		78.40	95.44	94.64	104.12

N/A not applicable
*Minor numerical deviations may occur due to rounding

B. WHOLE BODY AUTORADIOGRAPHY

The whole body autoradiograms showed no marked differences in the distribution of radioactivity between male and female rats. The greatest intensity of radioactivity was present in the bone for both sexes, as well as the intestinal tract and the kidneys 24 h after dosing with lesser to negligible amounts being present after 48 h.

III. CONCLUSIONS

Oral doses of glyphosate acid were excreted rapidly and predominantly in the faeces. The remaining radioactivity was excreted with the urine. Elimination was essentially complete within 72 h. Only low amounts (<0.2 % of the applied dose) were detected in exhaled air indicating only a low metabolism to $^{14}CO_2$ 24 h after dosing the greatest intensity of radioactivity was observed in the bone, the intestinal tract plus contents and kidneys. The intensity decreased within 48 h.

3. Assessment and conclusion

Assessment and conclusion by applicant:

After single oral administration of 10 mg/kg bw glyphosate acid to male and female rats, about 12 % and 22 % were absorbed by female and male rats respectively (based on the amount excreted *via* urine including cage wash). Urinary excretion is mainly completed within 24 h. About 85 % and 60 % of the dose are excreted *via* faeces within 48 h by females and male respectively, whereby again main portions are excreted within 24 h. Urinary excretion of radioactive labelled material in males is higher compared to females. However this is due to the high urinary excretion of one male rat (35 %). Gender specific differences in absorption were not observed in any of the other available studies and given the limited number of animals per sex in this study this is considered incidental.

The highest intensity of radioactivity in the whole body autoradiography was observed in the bone, the intestinal tract plus contents and kidneys. This confirms the tissue distribution observed in other studies. The decrease in radioactivity after 48 h indicates a fast elimination from the body and thus do not indicate an accumulation, which is also supported by observations made within other studies.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.1.1/007
Report author	[redacted]
Report year	1996
Report title	Glyphosate acid: Biotransformation in the rat
Report No	[redacted]/P/5058
Document No	Not reported
Guidelines followed in study	Japanese MAFF, 59 NohSan, Notification No. 4200 (1985), OECD 417 (1984), US-EPA FIFRA 85-1, EEC B.36 (1987)
Deviations from current test guideline	None
Previous evaluation	Yes, accepted in RAR (2015)

GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive summary

[^{14}C]-labelled glyphosate was administered by gavage as a single dose of 1000 mg [^{14}C]-glyphosate acid/kg bw to two male and two female bile duct cannulated rats. Bile was collected at various time intervals for 48 h. Additionally urine and faeces were collected at various intervals and the proportion and ratio of metabolites were investigated.

To further investigate the biotransformation of glyphosate acid, samples of urine and faeces of former studies (CA 5.1.1/003, CA 5.1.1/004, CA 5.1.1/005) were pooled and analysed by chromatography and NMR for metabolites.

The results showed that following a single oral dose of [^{14}C]-glyphosate acid the excretion of radioactivity in bile is negligible, thus indicating that radioactivity recovered in faeces is most likely unabsorbed material. The radioactivity present in urine and faeces from rats given [^{14}C]-glyphosate acid at low or high dose levels or after repeated dosing was characterised as being predominantly glyphosate acid. Trace amounts of aminomethylphosphonic acid (AMPA) were detected in urine samples.

In conclusion, following an oral dose of glyphosate acid to male and female rats approximately 10 - 20 % of the dose was absorbed. The unabsorbed glyphosate acid was excreted unchanged in faeces. The absorbed dose was excreted in urine as glyphosate acid and trace amounts of AMPA.

I. MATERIALS AND METHODS

A. MATERIALS

1. Non-labelled test material:

Identification:	Glyphosate acid (N-(phosphonomethyl) glycine)
Description:	White solid
Lot/Batch #:	Y04707/048
Purity:	99.5 % w/w
Stability of test compound:	Not reported, but used within the stated expiry date.

2. Radiolabelled test material:

Identification:	[^{14}C]-Glyphosate acid
Position of radiolabel:	N-(phosphono[^{14}C]methyl)glycine
Lot/Batch #:	Y04707/047
Radiochemical purity:	97.8 %
Specific activity :	1.580 GBq/mMol
Stability of test compound:	The test substance was shown to be stable in vehicle for longer than the period of use during this study

3. Vehicle and/or positive control: Deionised water

4. Reference substance:

Identification:	Aminomethylphosphonic acid (AMPA)
Description:	Not reported
Lot/Batch #:	Not reported
Purity:	Not reported
Stability of test compound:	Not reported

5. Test animals:

Species:	Rat
Strain:	Alpk:AP$_f$SD
Source:	[redacted]
Age:	Not reported, but at least 6 weeks according to body weight
Sex:	Males and females
Weight at dosing:	260 - 305 g
Acclimation period:	At least 4 days in stock rat cages and 24 h prior to surgery in metabolism cages
Diet/Food:	Pelleted PCD rat diet (Special Diets Services Ltd., Stepfield, Witham, Essex, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	During acclimatisation: Groups of the same sex, in stock rat cages, 24 h prior to dosing transfer individually into metabolism cages After dosing: Housed individually in glass metabolism cages
Environmental conditions:	Temperature: 21 ± 2°C Humidity: 40 - 70 % Air changes: At least 12/hour 12-hour light/dark cycle

B. STUDY DESIGN

In life dates: 1995-11-26 to 1996 May

Animal assignment and treatment

Four non-fasted rats (2 males, 2 females) received single oral doses of 1000 mg [^{14}C]-glyphosate acid/kg bw by gavage after bile duct cannulation. 48 hours after dosing all animals were sacrificed.

Bile duct cannulation

The abdominal cavity was opened after anaesthesia and the bile duct exposed. A fine plastic cannula was inserted into the bile duct and externalised by passing through the abdominal wall and under the skin to an exit point at the back of the neck. The incisions in the abdominal and body walls were saturated and the exposed cannula was protected within a flexible metal sheath anchored to the skin at the back of the neck. Following surgery each animal was returned to its cage and allowed to recover overnight prior dosing.

Collection of excreta and bile

Urine was collected 0 - 6, 6 - 12, 12 - 24, 24 - 36 and 36 - 48 intervals after dosing. Faeces was collected at 0 - 12, 12 - 24, 24 - 36 and 36 - 48 h intervals after dosing.
Bile was collected at 0 - 2, 2 - 4, 4 - 6, 6 - 8, 8 - 12, 12 - 24, 24 - 36, and 36 - 48 h intervals after dosing.

Dosing Formulation Analysis

The radiochemical concentration of the dosing preparation was determined by liquid scintillation counting. The radiochemical purity of the [^{14}C]-labelled test substance was determined by the high performance liquid chromatography (HPLC) following formulation in the dosing.

Quantification of metabolites

Urine and faecal samples obtained from the excretion and tissue distribution studies described in Sections CA 5.1.1/003 - 005 were used for the quantification of metabolites.
Urine samples from each study were combined by taking a fixed percentage by weight to give separate male and female pools for each of the sample collections intervals. Subsamples of these pools were further combined to give pools representing the entire sample collection period. Each pool was analysed by TLC and HPLC. A representative urine sample was analysed by ^{1}H-NMR.
Faecal samples were combined in the same way as described above for urine samples. Subsamples of pooled faecal samples were mixed with distilled water and sonicated for several hours, the samples were filtered through filter paper and the solid material was re-extracted a second time with distilled water and a third time with 10 % aqueous HCl. Extract volumes were measured and aliquots taken for scintillation counting to allow the calculation of extraction efficiencies.

Measurement of radioactivity

Faecal samples were extracted with water twice and the pooled supernatants were analysed by sample oxidation followed by liquid scintillation counting (LSC) whereas samples of urine and bile were analysed without intermediate processing by LSC.

Liquid scintillation counting

Radioactivity was measured by liquid scintillation analysis by means of Packard Tricarb instruments. The results obtained were corrected for background activity and counting efficacy using [^{133}Ba] as the external source. Disintegrations per minute (dpm) values were calculated using the appropriate quench curve data entered into instrument's computer.
Where sample oxidation had to be performed, samples were oxidised in a Packard Tricarb sample oxidiser.

Thin layer chromatography (TLC)

TLC was conducted using a normal phase silica-gel ($60F_{254}$) with the following solvent system: methanol: water: 28 % ammonium hydroxide: 10 % trichloroacetic acid (60:30:15:15 v/v/v/v).
Radioactivity on the TLC plate was measured using a Berthold Tracemaster linear analyser or a Bioscan System 200 imaging scanner. Glyphosate acid and AMPA standards were located by spraying the plates with a solution of 300 mg ninhydrin in 100 mL of butanol and 3 mL of glacial acetic acid.

High performance liquid chromatography (HPLC)

Two different HPLC methods were employed:
HPLC method 1 was used for the analysis of dosing solutions. Prior to analysis samples were derivatised. A Hichrom S5NH column (250 x 4.6 mm) was eluted with acetonitrile buffered with 25 mM aqueous potassium dihydrogen phosphate (60:40 v/v) at a flow rate of 1.5 mL/min.

HPLC method 2 was used for the analysis of urine and faecal extracts and for the quantification of glyphosate acid and AMPA. Prior to analysis samples were filtered. A Biorad's HLRC acid analysis column (250 x 4.6 mm) was eluted with 5 mM aqueous potassium dihydrogen phosphate with 4 % methanol at a flow rat of 0.5 mL/min.
Radioactivity was detected in both methods by liquid cell.

Proton Nuclear Magnetic Resonance Spectroscopy (^{1}H-NMR)

Proton and phosphorus NMR spectra were acquired using a Brucker 400MHz instrument. Samples of glyphosate acid and AMPA were dissolved in D_2O and analysed by both phosphorus and proton NMR. Control urine and urine from a bile duct cannulated rat administered an oral dose of glyphosate acid were analysed by phosphorus NMR. The urine sample from the rat that had been administered glyphosate was subsequently fortified with AMPA then glyphosate acid and reanalysed by phosphorus NMR.

Data evaluation

Dosing and excretion of radioactivity data were processed using the Debra computerised acquisition and processing system. Metabolites were quantified using the Flo_One integration software for HPLC.

II. RESULTS AND DISCUSSION

A. EXCRETION OF RADIOACTIVITY

48 h after dosing, mean recovery of the administered dose in the urine amounted to 20.8 % and 16.3 % in males and females, respectively. In faeces 39.1 % and 30.5 % of the applied dose were detected in males and females, respectively. The total excreted radioactivity after 48 h accounted for 62.5 % and 52.0 % of the applied dose in males and females, respectively.

Biliary excretion of radioactivity was negligible (about 0.06 %). Please refer to table given below.

Table 5.1-21: Glyphosate acid: Biotransformation in the rat [redacted] 1996): Excretion of radioactivity in urine, faeces and bile by male and female bile duct cannuled rats given a single oral dose of 1000 mg [^{14}C]-glyphosate acid/kg bw (mean of two rats expressed as % of applied dose)

Time after dosing (hours)	Males % of applied dose			Females % of applied dose		
	Urine	Faeces	Bile	Urine	Faeces	Bile
0 - 2	N/A	N/A	0.004	N/A	N/A	0.002
2 - 4	N/A	N/A	0.004	N/A	N/A	0.011
4 - 6	N/A	N/A	0.002	N/A	N/A	0.011
0 - 6	2.137	N/A	N/A	8.718	N/A	N/A
6 - 8	N/A	N/A	0.005	N/A	N/A	0.005
0 - 12	N/A	3.776	N/A	N/A	1.392	N/A
6 - 12	6.765	N/A	N/A	2.495	N/A	N/A
8 - 12	N/A	N/A	0.008	N/A	N/A	0.007
12 - 24	5.432	12.333	0.016	3.631	12.115	0.010
24 - 36	3.468	18.079	0.009	1.004	8.712	0.008
36 - 48	3.013	4.946	0.007	0.427	8.325	0.007
0 - 48	**20.185**	**39.134**	**0.055**	**16.275**	**30.544**	**0.062**
Cage wash at 48 hours		**2.534**			**5.097**	
Total urinary excretion	**22.719**	N/A	N/A	**21.372**	N/A	N/A
Total excretion		**62.538**			**51.978**	

Values are expressed as percentages of administered dose and are then mean of two rats
N/A: Not applicable

B. CHARACTERISATION OF RADIOACTIVITY

Due to the low level of radioactivity in bile samples the radioactivity in bile was not further characterised.

From pooled faeces samples of the low dose application (10 mg/kg bw) 84.5 and 62.3 % of the radioactivity for males and female was extracted. 88.5 and 75.6 % of the radioactivity was extracted from the pooled faeces of the study investigating excretion of ^{14}[C]-glyphosate acid at the high dose (1000 mg/kg bw) from males and females. From faeces of the repeated application of ^{14}[C]-glyphosate acid 61.0 and 79.5 % of the radioactivity was extracted.

Analyses by chromatography and phosphorus NMR of urine pools from those former studies (see CA 5.1.1/003; CA 5.1.1/004; CA 5.1.1/005) covering the 0 - 72 h period demonstrated a single peak identified as glyphosate acid by phosphorous NMR and a fortification experiment. Chromatograms of urine collected at earlier intervals demonstrated a second peak that occurred in measurable quantities. The peak was identified as aminomethylphosphonic acid (AMPA) by co-chromatography.

The percentages of dose accounted for glyphosate acid and AMPA following a low, high or repeated dose of glyphosate acid are given in the table below. For glyphosate acid and AMPA the values range from 63.3 - 95.3 % and 0.07 - 0.66 %, respectively.

Table 5.1-22: Glyphosate acid: Biotransformation in the rat ([redacted] 1996): Percentage of administered radioactivity identified as glyphosate acid and AMPA

		Low dose study 10 mg/kg bw		High dose study 1000 mg/kg bw		Repeated dose study 10 mg/kg bw	
		Male	Female	Male	Female	Male	Female
		(% of applied radioactivity)					
Urine	**Glyphosate acid**	12.71	10.51	16.00	16.73	10.46	10.47
	AMPA	0.19	0.11	0.63	0.66	0.07	0.08
Faeces	**Glyphosate acid**	74.80	55.22	79.25	63.88	52.86	72.09
Total	**Glyphosate acid**	87.52	65.73	95.25	80.61	63.33	82.57
	AMPA	0.19	0.11	0.63	0.66	0.07	0.08

3. Assessment and conclusion

Assessment and conclusion by applicant:

Following an oral dose of glyphosate acid to rats approximately 10 - 20 % of the dose was absorbed (see CA 5.1.1/003, CA 5.1.1/004, CA 5.1.1/005). The absorbed dose was excreted in urine as glyphosate and trace amounts of aminomethylphosphonic acid (AMPA). No significant quantities of glyphosate acid were eliminated in bile, which confirms that faecal radioactivity represents unabsorbed dose. The radioactivity excreted via faeces was confirmed to be glyphosate acid.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.1.1/008
Report author	Part 1: [redacted] (*in vivo* part) Part 2: [redacted] (Metabolite analysis)
Report year	1995
Report title	Part 1: Metabolism Study of ^{14}C-labelled glyphosate after single oral and intravenous administration to Sprague-Dawley Rats Part 2: Glyphosate - ADME-Study in Rats
Report No	Part 1: 9202/95 Part 2: [redacted] 038/94
Document No	Not reported
Guidelines followed in study	None reported
Deviations from current test guideline	Yes, reporting and methodological deficieneies
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Part 1: Yes Part 2: Yes, self-certification (Appendices in report missing)
Acceptability/Reliability	Supportive#
Category study in AIR 5 dossier (L docs)	Category 2a

#: Following the feedback from the Assessment Group Glyphosate in March 2020, the Notifier is aware of concerns regarding work conducted at this specific contract laboratory ([redacted]). For the sake of completeness, this study is included in the dossier submission since it was part of the data package of previous EU evaluation processes.

2. Full summary

Executive summary

The objective of this study was the investigation of the absorption, distribution, metabolism and excretion of glyphosate in rats upon low and high dose oral application and low dose intravenous application. The study was performed according to the ADME scheme (Adsorption, Distribution, Metabolism, Excretion). Part 1 (*in vivo* part) was conducted at the [redacted] and Part 2 (analytical part) was conducted at the A & M Labor für Analytik und Metabolismusforschung, Köln, Germany.

Groups of 4 male and 4 female Sprague-Dawley rats were used for Groups I to III. In Group I [^{14}C]-glyphosate was administered by intravenous bolus injection at ~0.2 mg/kg bw. In Groups II and III, [^{14}C]-glyphosate was administered by oral gavage at ~0.2 mg/kg and ~200 mg/kg bw, respectively.

The mean recovery of administered radioactivity in excreta (urine and faeces) exceeded 90 % in all investigations. Seven days after single intravenous dosing, elimination in urine accounted for ca 89.3 % of the dose compared with 6.3 % in faeces. After oral dosing 83.1 % of the administered [^{14}C]-glyphosate were detected in faeces and 10.9 - 15.1 % in urine. Renal elimination was more protracted following oral administration of [^{14}C]-glyphosate continuing for at least 24 h post-dose. A mean of 89 % of the dose was excreted with the urine and faeces within 24 h following administration.

The proportion of radioactive dose absorbed from the gut, estimated by comparison of renal elimination following single intravenous and oral administration at ~0.2 mg/kg bw, was 12 %.

Total radioactivity in organs 7 days after single intravenous administration of ~0.2 mg/kg bw (Group IV, 8 female rats) was low (<0.3 % of dose), however only a limited number of tissues were analysed.

No radiolabelled metabolites of [^{14}C]-glyphosate were detected in urine of the high dose animals collected within 24 h. The identity of the radioactive component as glyphosate was confirmed by HPLC and thin layer chromatography.

I. MATERIALS AND METHODS

A. MATERIALS

1. Non-labelled test material:

Identification:	Glyphosate (N-(phosphonomethyl)glycine)
Description:	White powder
Lot/Batch #:	Part 1: UN-NO: 1759 Part 2: 32140
Source:	Part 1: Not reported Part 2: Riedel-de Haen
Purity:	98 %
Stability of test compound:	Stable for duration of the study

2. Radiolabelled test material:

Identification:	[^{14}C]-Glyphosate
Position of radiolabel:	N-(phosphono[^{14}C]methyl)glycine
Lot/Batch #:	Not reported
Radiochemical purity:	Not reported
Specific activity:	11.9 and 11.7 MBq/mg (2.04 and 2.00 GBq/mmol)
Stability of test compound:	Stable for duration of the study

3. Reference substances:

Identification:	Aminomethylphosphonic acid (AMPA)
Description:	Not reported
Lot/Batch #:	118F3838
Purity:	Not reported
Stability of test compound:	Stable for duration of the study

4. Vehicle and/or positive control:	Groups 1, 2, 4: 250 µL water Group 3: 2.75 mL aqueous tylose + 250 µL glyphosate in water

5. Test animals:

Species:	Rat
Strain:	Sprague-Dawley (Crl:CD®BR)
Source:	[redacted]
Age:	5 - 7 weeks at start of experiment
Number/Sex:	12 males and 20 females
Weight at dosing:	190 - 209 g
Acclimation period:	7 days
Diet/Food:	Altromin 1324; rats were fasted for approximately 16 h prior to administration

Water:	Tap water, *ad libitum*
Housing:	During acclimation: single housing in Makrolon type III cages After administration in metabolism cages
Environmental conditions:	Temperature: 22 ± 3ºC (at room temperature)
	Humidity: 60 ± 20 %
	Air changes: Not reported
	12-hour light/12-hour dark cycle

B. STUDY DESIGN

In life dates: 1995-06-07 to 1995-06-13

Animal assignment and treatment

The test substance glyphosate was administered to four groups of animals. Groups I to III consisted of four males and four females, Group IV comprised eight females. The test substance was administered according to the following scheme:

Group	Route of administration	Amount of test substance (per animal)	Radioactive dose** (per animal)	Dose** (mg/kg bw)	Samples
I	i.v.	0.04 mg [^{14}C]-glyphosate	501 kBq	~0.2	Urine and faeces
II	Oral	0.05 mg [^{14}C]-glyphosate	702 kBq	~0.2	
III	Oral	0.04 mg [^{14}C]-glyphosate plus 40.0 mg unlabelled glyphosate*	501 kBq	~200	
IV	i.v.	0.04 mg [^{14}C]-glyphosate	501 kBq	~0.2	Blood and organs

* The unlabelled glyphosate was mixed with the labelled compound just before application.
** Doses as reported in the study report by [redacted], 1995 (Part 2)

Preparation of the solution of the test substance

18.5 MBq (1.55 mg) of the test substance (specification of the supplier) dissolved in water were made up to volume with water in a 10 mL volumetric flask. The specific radioactivity of the solution was determined to be 2.005 MBq/mL. The unlabelled glyphosate was mixed with the labelled compound just before application.

Sampling

From the animals of Groups I to III, urine and faeces were collected over 7 days in 24 h fractions. The urine volumes per collection fraction and animal were determined. At the end of each collection period the cages were rinsed with 25 mL aqua *ad injectabilia*. The rinsing water volume per collection fraction and animal was determined.
Animals of Group IV were used for blood and tissue sampling. Two females were sacrificed per sampling time point at 1, 4, 24, and 168 h after administration for sampling of blood and the following organs: brain, spinal cord, fat, kidneys, liver, heart, ovaries. Carcasses were also sent to the analytical laboratory.

Measurement of radioactivity

Urine and rinsing water of rat cages
Aliquots of 0.5 - 1 mL of each sample (urine and rinsing water) were mixed with 4 mL of the scintillation cocktail Pico-Fluor 40. The scintillation was measured for 5 - 10 minutes. A quench curve prepared by internal standard measurement was used to calculate the content of radioactivity of the samples in disintegration per minute (dpm).

Faeces
The faeces samples were homogenised. Aliquots of approx. 0.5 g of each faeces sample were incinerated in a Sample Oxidizer Model 307 (Canberra Packard). The instrument combusts the sample material in a continuous flow of oxygen to trap the CO_2 of the incineration gas using a specially designed reaction column filled with 10 mL of 3-methoxypropylamine as carbon dioxide absorbent. 10 mL of a liquid scintillator for ^{14}C-counting is added automatically. The scintillation of the samples was measured for 5 - 10 minutes.

Organs
Following organs were collected from dose Group IV at 1, 4, 24 and 168 h after treatment, weighed and dissolved using tissue solubiliser: Heart, kidneys, ovaries, fat, brain, liver, and spinal cord. Most of the tissues were completely dissolved after 48 h, only the kidneys required 120 h. The solutions were weighed again and aliquots of approx. 1 mL were mixed with 4 mL of the scintillation cocktail Pico-Fluor 40 to measure their scintillation with the beta counter for 5 - 10 minutes.

Blood
The blood samples were homogenised, weighed and aliquots of approx. 1 g were dried and incinerated as described for the faeces samples.

Determination of absorption

Absorption was determined by comparing the renal excretion after intravenous and oral application.

Metabolite pattern of urine samples

The urine of Group III collected 24 h after application was analysed for metabolites and for un-metabolised test substance. The urine samples were analysed by HPLC with radioactivity monitoring using a Sherisorb 5 SAX column with 5 mM phosphate buffer (pH 1.9) /methanol (96:4) as eluent. In addition, the radioactive HPLC-fraction was collected and converted with 9-fluorenylmethyl chloroformate (FMC). The reaction mixture was separated by thin-layer chromatography. And radioactivity was monitored.

II. RESULTS AND DISCUSSION

A. ABSORPTION

Table 5.1-23: Metabolism Study of 14C-labelled glyphosate after single oral and intravenous administration to Sprague-Dawley Rats ([redacted] & [redacted] 1995): Cumulative total excretion of radioactivity (group means) after 7 days as % of dose

Sample	Group I i.v. dose ~0.2 mg/kg bw*	Group II Oral dose ~0.2 mg/kg bw*	Group III Oral dose ~200 mg/kg bw*
Urine incl. rinsing	89.3 ±3.4	10.9 ±2.3	15.1 ±4.0
Faeces	6.3 ±3.5	83.1 ±4.2	83.1 ±9.8
Total recovery	**95.6** ±2.1	**94.0** ±3.0	**98.2** ±11.3

* Doses as reported in the study report by [redacted], 1995 (Part 2)

The renal excretion of total radioactivity based on the administered parent compound plus metabolite(s) was measured both after oral and intravenous application to rats (Groups I to III). Comparing the urinary excretion after intravenous (Group I) and oral applications (Group II), an absolute bioavailability of about 12 % was calculated. In order to investigate the impact of the dose level on the absorption process, an approximately 800 times higher dose was given orally (Group III). The mean of the excretion rate in urine for Group III was determined to be 15 % of the dose. Comparing Group II and Group III, the absorption rate observed for the high orally administered Group III increased insignificantly about 4 % of the given dose, although the dose was 800 times higher.

B. ELIMINATION

Table 5.1-24: Metabolism Study of 14C-labelled glyphosate after single oral and intravenous administration to Sprague-Dawley Rats [redacted] & [redacted] 1995): Means of renally excreted radioactivity (urine and rinsing water) as % of dose

Group	Sex	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Total
I (i.v.)	M	85.2	2.43	1.05	0.55	0.38	0.23	0.2	90.03
	F	84.88	1.78	0.8	0.5	0.33	0.23	0.18	88.68
	M+F	**85.04**	**2.10**	**0.93**	**0.53**	**0.35**	**0.23**	**0.19**	**89.37**
II (oral)	M	11.18	0.78	0.15	0.1	0.05	0	0	12.25
	F	8.7	0.625	0.1	0.1	0	0	0	9.53
	M+F	**9.94**	**0.70**	**0.13**	**0.10**	**0.05**	**0.00**	**0.00**	**10.92**
III (oral)	M	15.5	1.03	0.25	0.1	0.1	0.08	0.03	17.08
	F	11.73	0.98	0.18	0.10	0.08	0.03	0.00	13.08
	M+F	**13.61**	**1.00**	**0.21**	**0.10**	**0.09**	**0.05**	**0.01**	**15.07**

M = males, F = females

Independently of route or dose level, most of the renally excreted radioactivity was found in urine during the first 24 h period (Group I: >93 %, Group II: >88 %, Group III: >83 %). The findings of excretion via faeces can be seen in the table below.

Table 5.1-25: Metabolism Study of 14C-labelled glyphosate after single oral and intravenous administration to Sprague-Dawley Rats [redacted] 1995): Means of radioactivity excreted via faeces as % of dose

Group	Sex	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Total
I (i.v.)	M	4.36	0.74	0.18	0.10	0.05	0.06	0.07	5.55
	F	6.08	0.53	0.19	0.13	0.09	0.08	0.05	7.14
	M+F	**5.22**	**0.64**	**0.18**	**0.12**	**0.07**	**0.07**	**0.06**	**6.36**
II (oral)	M	79.39	3.21	0.20	0.03	0.01	0.01	0.01	82.85
	F	79.56	3.40	0.17	0.10	0.02	0.01	0.01	83.27
	M+F	**79.47**	**3.30**	**0.18**	**0.06**	**0.02**	**0.01**	**0.01**	**83.05**
III (oral)	M	77.98	3.34	0.34	0.08	0.04	0.02	0.01	81.81
	F	81.93	2.20	0.14	0.04	0.03	0.02	0.01	84.36
	M+F	**79.95**	**2.77**	**0.24**	**0.06**	**0.03**	**0.02**	**0.01**	**83.08**

M = males, F = females

The total radioactivity recovered was 96, 94 and 98 % of the applied dose for Groups I, II and III, respectively.

C. DISTRIBUTION

The dispersal of radioactivity into blood and organs was measured at different times after administration. The mean measured radioactivity of organs collected at 1, 4, 24 and 168 h after dosing is provided in the table below.

Table 5.1-26: Metabolism Study of 14C-labelled glyphosate after single oral and intravenous administration to Sprague-Dawley Rats [redacted] 1995): Summary of the organ distribution of radioactivity (means of the groups, spec. radioactivity as % of dose per gram tissue [%/g])

Group	Animal No	Time after administration	Brain	Spinal cord	Fat	Kidneys	Liver	Heart	Ovaries	Blood	Total
IV (i.v.)	25-26	1 h	0.020	0.025	0.027	5.351	0.132	0.063	0.077	0.119	**5.814**
	27-28	4 h	0.006	0.009	0.005	0.851	0.112	0.012	0.026	0.021	**1.042**
	29-30	24 h	0.005	0.009	0.002	0.025	0.058	0.004	0.006	0.008	**0.117**
	31-32	7 d	0.005	0.006	0.000	0.004	0.004	0.001	0.002	0.004	**0.026**

1 h after application, most organs showed a rather low radioactivity: 0.02 % to 0.13 % of the dose per gram of brain, spinal cord, fat, liver, heart, ovaries, and blood. The concentrations of radioactivity in kidneys was remarkably higher. It was determined to be 5.4 % of the dose/gram. 4 h after application, the radioactivity of the organs has declined, compared to the previous sampling. Radioactivity in the kidney was approx. 0.9 % of the dose per gram 4 h after intravenous application. The remaining organs were containing <0.1 % of the dose/gram of tissue. After 24 h, only liver and kidney exhibited a noticeable amount of approx. 0.06 % and 0.03 % radioactivity of the dose. After 7 days, none of the tissue samples showed a significant radioactivity (below 0.01 % of the dose/gram).

D. METABOLISM

The metabolism of glyphosate was studied by examination of the urine fractions of Group III animals. One aspect was the partition of the total urine radioactivity into the parent compound and its metabolites. When one or more metabolites were observed, an attempt to identify the main metabolite(s) was made. No metabolites were detected in the urine samples of Group III (0 - 24 h) analysed by HPLC. [^{14}C]-Glyphosate was identified by co-chromatography of the labelled reference substance under the same HPLC conditions. Upon conversion of the HPLC-fraction with 9-fluorenylmethyl chloroformate (FMC) and separation by thin-layer chromatography the FMC-derivative of [^{14}C]-glyphosate accounted for 94 % of the total counts.

III. CONCLUSIONS

Absorption, metabolism and elimination were independent of the oral dose in the range of 0.2 to 200 mg/kg bw. The radioactivity of the given test substance was eliminated renally and via faeces with a ratio of 15:1 after intravenous application. In the case of oral administration, the ratio was determined to be 1:8 after giving a low dose of approximately 0.2 mg/kg bw and 1:6 after a high dose of approximately 200 mg/kg bw. The systemic availability was found to be 11 % and 15 %, respectively. The absolute bioavailability was found to be 12 % comparing the low oral and intravenous application. The distribution of the radioactivity to several organs showed high concentrations in the kidneys as excretory organs in the first 24 h after application. In urine no other radioactive compounds than the test substance could be detected. Unchanged glyphosate is very rapidly excreted.

3. Assessment and conclusion

Assessment and conclusion by applicant:

An ADME (absorption, distribution, metabolism and excretion) study in Sprague-Dawley rats was conducted. A single intravenous or oral dose of [^{14}C]-glyphosate at approximately 0.2 mg/kg bw or a single oral dose of 200 mg/kg bw were administered to Sprague-Dawley rats.

At 7 days after single i.v. dose at ~0.2 mg/kg bw the mean urinary excretion of glyphosate was 90.0 % in males and 88.7 % of the applied dose in females; in faeces, excretion was 5.6 % and 7.1 % of the applied dose. After single oral dose at ~0.2 mg/kg bw the mean urinary excretion of glyphosate over the collection period of 7 days was 12.3 % in males and 9.5 % of the applied dose in females; in faeces, excretion was 82.9 % (males) and 83.3 % (females) of the applied dose.
After single oral dose at ~200 mg/kg bw the mean urinary excretion of unchanged glyphosate was 17.1 % in males and 13.1 % of the applied dose in females; in faeces excretion was 81.8 % and 84.4 % of the applied dose. No metabolites were identified in urine collected within 24 h after application

The selection of tissues sampled for evaluation after single i.v. dose at ~0.2 mg/kg bw was limited. Highest tissue levels were detected after 1 h in the kidneys. 4 h after application, radioactivity in the organs had already started to decline. After 24 h, only the kidneys and the liver exhibited a noticeable amount of approximately 0.06 % or 0.03 % of the total radioactivity administered. After 7 days, only trace amounts of radioactive residues were detected, with highest concentration of 0.006 % of the dose detected in spinal cord.

Thus, elimination from the body was rapid after intravenous application and did not indicate a potential for bioaccumulation of glyphosate.

Urinary radioactivity collected within 24 h of the high dose animals was identified as glyphosate by HPLC and TLC. No metabolites were detected in urine.

In conclusion, absorption of glyphosate after oral application is rapid and independent of dose level. Absorption is low (approximately 12 - 15 % of the applied dose was absorbed). The majority of glyphosate is excreted unchanged within the first 24 h after application.

Dosing was done per animal, but individual body weights were not provided. In Part 1 (the report by [redacted]) body weights were indicated to range between 190 - 209 mg and the low dose was assigned as ~0.2 mg/kg bw. In Part 2 (the report by [redacted]) the low dose was assigned as ~0.3 mg/kg bw, however this laboratory received only samples for analyses and may not have received information on individual body weights.

The report does not contain any information on the limits of detection or limits of quantitation. Metabolites were only assessed in urine samples from the oral high dose group. The low dose of only ~0.2 mg/kg bw used in this study was rather low, but the use of this concentration might reflect real exposure conditions. Mass balance cannot be calculated as only urinary and faecal excretions were measured, however total recovery rates of radioactivity were at least 94 % in all three groups tested for excretion. The results of this study can be considered as supplementary data.

Assessment and conclusion by RMS:

For **CA 5.1.1/009** please refer above to CA 5.1.1/008.

1. Information on the study

Data point	CA 5.1.1/010
Report author	[redacted]
Report year	1995
Report title	HR-001: Metabolism in the rat
Report No	SNY 332/951256
Document No	Not reported
Guidelines followed in study	Japanese MAFF, 59 NohSan, Notification No. 4200 (1985) OECD 417 US-EPA FIFRA 85-1
Deviations from current test guideline	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive summary

A single oral dose of [^{14}C]-glyphosate (>98 % radiochemical purity) was administered by gavage, as aqueous solution (with addition of $NaHCO_3$ to achieve dissolution) at 10 or 600 mg/kg bw to male and female Sprague-Dawley rats to determine [^{14}C] plasma concentrations and plasma pharmacokinetic parameters (9 animals per sex), tissue distribution (6 animals per sex) and excretion (5 animals per sex) of the radio-labelled compound. Additionally, the magnitude and nature of metabolites has been investigated in urine and faeces.

The results showed that at least about a quarter (19 – 30 %) of the administered dose is absorbed from the gut. Absorption was similar for both sexes and at both dose levels, with peak plasma [^{14}C] concentrations observed around 3 - 6 h after dosing. The decline in plasma [^{14}C] concentration was monophasic with a half-life of about 8 h at 10 mg/kg bw and 5.9 h at 600 mg/kg bw dosing, indicating a rapid clearance. The areas under the plasma concentrations versus time curves (AUC_t) after the high dose level were approximately 100 to 120 fold higher compared to the low dose level. Neither dose- nor gender differences in excretion were noted. More than 90 % of the radioactivity was excreted with the urine and faeces within 48 h. About 65 – 77 % of the administered [^{14}C] are detected in faeces and 18 – 27 % was excreted via urine. Within 168 h more than 97 % of the administered dose was excreted via urine and faeces. About 74 – 84 % of the administered [^{14}C] are detected in faeces and 19 – 30 % is excreted via urine. No significant radioactivity was detected in exhaled air (<0.2 % of dose). The mean total radioactivity recovered within 168 h exceeded 95 %. The distribution of radioactivity in tissues was similar for males and females at both dose levels with maximum tissue level being detected within 3 h (high dose) or 6 h (low dose) and decreasing time-dependently afterwards. Highest concentrations were detected in the gastrointestinal tract and its content as well as stomach and its content. Concentrations in kidney, muscles and bones accounted for up to 0.79 % , 0.24 % and 0.12 % of the applied dose at the low dose and up to 1.00 %, 0.48 % and 0.09 % of the applied dose at the high dose, respectively at T_{max}. Concentrations in carcass accounted for up to 3.03 % of the applied dose at the low dose after 18 h and up to 2.85 % of the applied dose at the high

dose after 3 h, respectively. Concentrations of radioactivity retained in the tissue 7 days post application were generally low (<0.4 % in carcass). Major urinary (18 – 27 %) and faecal (65 – 78 %) component was the parent compound. A minor compound (0.1 - 2.0 %) was identified as aminomethylphosphonic acid by TLC and HPLC co-chromatography. The nature of the other components (<2 %) was not investigated further.

I. MATERIALS AND METHODS

A. MATERIALS

1. Non-labelled test material:

Identification:	HR-001 (N-(phosphonomethyl)glycine)
Description:	solid
Lot/Batch #:	061221
Purity:	98.9 %
Stability of test compound:	Expiry date 1996-12-20

2. Radiolabelled test material:

Identification:	[^{14}C]-HR001
Position of radiolabel:	N-(phosphono[^{14}C]methyl)glycine
Lot/Batch #:	Not reported
Radiochemical purity:	>98 % (TLC, followed by radioscanning)
Specific activity:	327.7 µCi/mg, 56 mCi/mmol
Stability of test compound:	Not reported

3. Reference substance:

Identification:	Aminomethylphosphonic acid (AMPA)
Description:	solid
Lot/Batch #:	09203L2
Purity:	99 %
Stability of test compound:	Not reported

4. Radiolabelled reference substance:

Identification:	[^{14}C]-Aminomethylphosphonic acid ([^{14}C]-AMPA)
Position of radiolabel:	Amino[^{14}C]methylphosphonic acid
Lot/Batch #:	Not reported
Radiochemical purity:	97.4 %
Specific activity:	2.0 GBq/mmol, 54 mCi/mmol
Stability of test compound:	Not reported

5. Vehicle and/or positive control: Water, solubility was increased by addition of sodium hydrogen carbonate

6. Test animals:

Species:	Rat
Strain:	Sprague-Dawley (CD)
Source:	[redacted]
Age:	6 - 8 weeks (males), 7 - 9 weeks (females)
Sex:	Males and females

Weight at dosing:	approximately 200 g
Acclimation period:	At least 5 days
Diet/Food:	Standard Laboratory Diet LAD 1 (Special Diet Services, Witham, Essex, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	During acclimatisation: Individual housing in suspended, wire bottom, stainless steel cages After dosing: Excretion-balance experiments - individually in glass metabolism cages Blood/plasma kinetics - in stainless steel battery cages Tissue distribution - in stainless steel battery cages
Environmental conditions:	Temperature: 21 ± 2°C Humidity: 40 – 60 % Air changes: not reported 12-hour light/dark cycle

B. STUDY DESIGN

In life dates: not reported

Animal assignment and treatment: Preliminary excretion studies

In two independent experiments two rats (1 male, 1 female) received single oral doses of either 10 or 600 mg/kg bw by gavage and were placed in glass metabolism cages immediately thereafter. Urine was collected at 0 - 6, 6 - 24 h, and every 24 h for 7 days in receivers cooled with solid CO_2. Faeces were collected every 24 h for 7 days. Expired air was passed through traps containing an ethanolamine/2-ethoxyethanol mixture (1:3, v/v). These traps were changed every 24 h for 7 days after dosing. The interior of the cages were washed with water at sacrifice after 7 days. Samples were analysed immediately or were stored at -20°C until taken for analysis.

Animal assignment and treatment: Excretion studies

In two independent experiments 10 rats (5 male, 5 female) received single oral doses of either 10 or 600 mg/kg bw by gavage and were placed in glass metabolism cages immediately thereafter. Urine and faeces were collected as described in the preliminary study. Blood was drawn by cardiac puncture (following light halothane anaesthesia) prior to sacrifice by cervical dislocation and plasma was obtained by centrifugation. The following tissues/organs were taken or sampled for radioactivity measurement:
Adrenals, bone, bone marrow (femur), brain, eyes, fat (abdominal), gastrointestinal tract, heart, kidneys, liver, lungs, lymph nodes (mesenteric), muscle (skeletal), ovaries, pancreas, pituitary gland, plasma, skin, spleen, stomach, testes, thymus, thyroid with parathyroid, urinary bladder, uterus and residual carcass. The contents of the gastrointestinal tract and stomach were analysed separately.

Animal assignment and treatment: Plasma concentrations

In two independent experiments 18 rats (9 male, 9 female) received single oral doses of either 10 or 600 mg/kg bw by gavage. The animals were divided into three groups of six (3 per sex) and blood samples (0.5 mL) were taken from the tail vein into heparinised tubes at the following times from each group.
Group 1: prior to administration, 1, 4, 24 and 96 h
Group 2: 0.25, 2, 6, 48 and 120 h
Group 3: 0.5, 3, 12, 72 and 168 h

Each group was sacrificed upon completion of the specified sampling schedule.

Animal assignment and treatment: Quantitative tissue distribution

In two independent experiments 12 rats (6 male, 6 female) received single oral doses of 10 or 600 mg/kg /day by gavage. The animals were divided into two groups of six (3 per sex) and sacrificed by cervical dislocation 6 and 18 h (low dose) or 3 and 9 h (high dose) after dosing, depending on the peak plasma concentrations and half the plasma concentration derived in the blood/plasma kinetics experiments. Data for 168 h (7 days) was provided by the excretion studies. Blood samples were taken by cardiac puncture (following light halothane anaesthesia) prior to sacrifice by cervical dislocation and plasma was obtained by centrifugation. The following tissues/organs were taken or sampled for radioactivity measurement: adrenals, bone, bone marrow (femur), brain, eyes, fat (abdominal), gastrointestinal tract, heart, kidneys, liver, lungs, lymph nodes (mesenteric), muscle (skeletal), ovaries, pancreas, pituitary gland, plasma, skin, spleen, stomach, testes, thymus, thyroid with parathyroid, urinary bladder, uterus and residual carcass. The contents of the gastrointestinal tract and stomach were analysed separately.

Dosing Formulation Analysis

The radiochemical concentration of the dosing preparation following formulation was determined by thin layer chromatography (TLC) and liquid scintillation counting.

Measurement of radioactivity

Faeces were initially extracted by homogenisation with chloroform:1N HCl (1:1, v/v) followed by further extracts with 1N HCl. After centrifugation radioactivity was measured in both extracts and residues. Samples of urine, plasma, solvent extracts contents of expired air traps, cage washings and other liquid samples were mixed with Special Scintillator MI-31 (Packard Instrument Co. Ltd, Reading, UK) for measurement of radioactivity by liquid scintillation counting (LSC). Samples of faecal residues, gastro-intestinal tract, liver, spleen and whole blood were combusted, absorbed, mixed with scintillation cocktail and analysed thereafter. Carcasses were digested for 48 h at 55°C in a solution of 2M NaOH in 30 % methanol containing Triton X-405 (10 % v/v). Tissue samples suitable for solubilisation were incubated at around 50°C for 18 - 24 h with solubiliser and mixed with scintillation cocktail and analysed thereafter. Radioactivity with less than twice background counts was considered to be below the limit of accurate quantification when performing LSC.

Isolation of the major urinary and faecal metabolites

Samples of urine and faecal extracts from male and female rats were pooled and analysed directly by TLC or HPLC. Radiolabelled metabolites formed in the rat were identified by co-chromatographic comparison using different systems with the reference compound aminomethylphosphonic acid (AMPA) or [^{14}C] AMPA.

Thin layer chromatography (TLC)

TLC was carried out on pre-layered Merck cellulose F plates (0.2 mm, BDH Chemicals Ltd., Poole, UK) using the following development systems:

System 1: Ethanol : water : ammonium hydroxide : trichloroacetic acid : acetic acid
(55:35:5:3.5:2, v/v/v/w/v)
System 2: Ethanol : water : ammonium hydroxide : trichloroacetic acid : acetic acid
(65:35:2.5:3.5:2, v/v/v/w/v)
System 5: Methanol : water : acetic acid
(67:33:1, v/v/v)

Radioactivity was detected with a Berthold Linear Analyser controlled by a computer system (Berthold

Instruments (UK)) and proportions of radioactive components were measured by integrating the areas under the peaks on the radio chromatogram following subtraction of background levels. Alternatively, components were detected and quantified using a Fuji BAS 2000 Bioimage Analyser. The produced images of radioactive TLC plates were processed to generate quantitative data.

High performance liquid chromatography (HPLC)

Two HPLC methods were used. HPLC system 1 (gradient elution method; column: Sperisorp SAX HPLC column (Hichrom, UK) and guard column, eluent A: water, eluent B: 0.75 M KH_2PO_4, pH 3.35) and HPLC system 2 (isocratic method; column: glyphosate analytical column (BioRad, USA), eluent: 0.005 M KH_2PO_4 + 4 % methanol v/v, pH 2.1) were both linked to an UV- and a radio-detector. A Compaq Prolinea computer with Labchrom software was used to collect and process data from the UV and radio detectors. Samples were co-injected with a mixture of the reference standards. Fractions were collected and radio assayed by LSC.

Data evaluation

Data were quantified by LSC using models with automatic external Standard quench correction. After choosing the optimal channel setting, quench correction curves were prepared from radiochemical standards ([^{14}C]-hexadecane, Amersham International plc). The validity of the calibration curves was checked throughout the experiments. Samples with low levels of radioactivity were normally counted for 10 minutes or until the accumulated counts totalled 4 x 10^4.

II. RESULTS AND DISCUSSION

A. EXCRETION AND RETENTION OF RADIOACTIVITY

The preliminary study on two rats per dose (male/female) indicated that more than 90 % of the administered radioactivity was excreted within 7 days at the low and the high dose group after a single application of the test substance. Almost no radioactivity could be detected in expired air (about 0.15 %).

The main study with 10 rats per dose confirmed the initial observation, please refer to table given below. During the 7 days observation period 23 % and 19 % (male/female) were excreted in urine of the low dose group. Slightly higher percentages, 30 % and 29 % (male/female) of total administered radioactivity were detected in urine of the high dose group. The main portion of the radioactivity was detected at both dose levels within the first 48 h in males and females (21 % and 18 %, 10 mg/kg bw; 28 % and 27 %, 600 mg/kg bw). In both dose groups about 75 % of the administered radioactivity could be detected in the faeces of males and females within 7 days (75 % and 84 %, 10 mg/kg bw; 75 % and 74 %, 600 mg/kg bw). Again most of the radioactivity was detected within 48 h after dosing (72 % and 82 %, 10 mg/kg bw; 72 % and 69 %, 600 mg/kg bw). About 0.3 % of the radioactivity remained in the carcasses of the sacrificed animals after 7 days. Thus, in male and female rats almost all the administered radioactivity was excreted *via* the urine and faeces within 7 days (97 % and 104 % at 10 mg/kg bw; 105 % and 104 % at 600 mg/kg bw).

Table 5.1-27: HR-001: Metabolism in the rat ([redacted] 1995): Excretion balance (in mean % of applied dose) at 168 h post dosing

Balance/Excretion	10 mg/kg bw		600 mg/kg bw	
	Males	Females	Males	Females
Urine 0 - 6	2.63	3.25	11.55	9.08
Urine 6 - 24	15.85	12.69	13.85	13.36
Urine 24 - 48	2.82	2.41	2.33	4.40
Urine 48 - 72	0.54	0.44	0.59	1.07
Urine 72 - 96	0.24	0.19	0.30	0.40
Urine 96 - 120	0.15	0.13	0.21	0.24

Table 5.1-27: HR-001: Metabolism in the rat ([redacted] 1995): Excretion balance (in mean % of applied dose) at 168 h post dosing

Balance/Excretion	10 mg/kg bw		600 mg/kg bw	
	Males	Females	Males	Females
Urine 120 - 144	0.09	0.07	0.17	0.17
Urine 144 - 168	0.07	0.05	0.13	0.18
Cage wash	0.12	0.14	1.13	0.60
Subtotal urine + cage wash	**22.51**	**19.37**	**30.26**	**29.50**
Faeces 0 - 24	60.28	74.59	58.94	46.28
Faeces 24 - 48	11.72	7.56	13.41	22.87
Faeces 48 - 72	1.18	1.34	1.36	3.83
Faeces 72 - 96	0.29	0.36	0.35	0.47
Faeces 96 - 120	0.17	0.27	0.36	0.23
Faeces 120 - 144	0.35	0.08	0.08	0.12
Faeces 144 - 168	0.64	0.10	0.15	0.35
Subtotal faeces	**74.63**	**84.30**	**74.65**	**74.15**
Carcass	0.33	0.27	0.31	0.39
Total	**97.47**	**103.94**	**105.22**	**104.04**

B. CONCENTRATION OF RADIOACTIVITY IN THE PLASMA

After a single oral dose of 10 mg/kg bw [^{14}C]-HR001 to rats mean peak concentrations of radioactivity in plasma occurred at 6 h and 2 h in males (0.22 µg equiv./mL) and females (0.28 µg equiv./mL), respectively. The absorption rate constants were 0.2963 h^{-1} in males and 0.4239 h^{-1} in females. Concentrations declined with an approximate half-life of 8.3 h in males and 7.8 h in females. The area under the concentration *versus* time curve (AUC_t) was 3.2 and 3.7 µg equiv./mL*h in males and females, respectively (please refer to the table below).

After a single oral dose of 600 mg/kg bw [^{14}C]- HR001 to rats mean peak concentrations of radioactivity in plasma occurred at 3 h in males (26 µg equiv./mL) and females (29 µg equiv./mL), respectively (please refer to the table below). The absorption rate constants were 0.2845 h^{-1} in males and 0.4477 h^{-1} in females. Thus absorption rate constants did not increase with dose. Concentrations declined with an approximate half-life of 5.9 h in males. The terminal half-life could not be calculated for females of the high dose group due to rapid clearance from plasma. The area under the concentration *versus* time curve (AUC_t) was calculated at 400 and 355 µg equiv./mL*h in males and females, respectively. These values were around 100 to 120 fold higher than the AUC_t obtained in the low dose group.
Measurements in whole blood in general lead to the same result.

Table 5.1-28: HR-001: Metabolism in the rat ([redacted] 1995): Kinetic parameters in plasma after single oral dose of 10 or 600 mg/kg bw

	10 mg/kg bw		600 mg/kg bw	
	Males	Females	Males	Females
C_{max} [µg equiv./mL]	0.2219	0.2789	25.97	28.84
T_{max} [h]	6.00	2.00	3.00	3.00
AUC_t [µg equiv./mL×h]	3.20	3.70	399.90	355.30
AUC [µg equiv./mL×h]	3.80	4.20	419.00	*
Terminal rate constant [h^{-1}]	0.0840	0.0887	0.1174	*
Terminal halflife [h]	8.30	7.80	5.90	*
Absorption rate constant [h^{-1}]	0.2963	0.4239	0.2845	0.4477

* could not be calculated
AUC_t is the area under the plasma curve calculated up to the last detectable sample (up to 24 h within the present study)
AUC is the area under the plasma curve calculated to infinity

C. DISTRIBUTION OF RADIOACTIVITY IN TISSUE

Radioactivity in tissues was low at all times (please refer to tables below) and the relative tissue concentrations were comparable between sexes and dose groups. A time-dependent decrease of radioactivity was observed for all investigated tissues, except carcass where the highest concentration was detected after 18 h. The time dependent decrease and the overall low tissue concentration after 7 days do not indicate a potential of accumulation. Only the gastrointestinal tract (GIT), the stomach, the kidneys (the organs of excretion) the muscles and bones contained higher concentrations of radioactivity than the plasma. High levels of radioactivity were detected in the content of stomach and GIT. At 7 days p.a. the radioactivity in most tissues had decreased to around the limit of detection. Highest remaining concentrations were detected in carcass (<0.4 %).

Table 5.1-29: HR-001: Metabolism in the rat ([redacted] 1995): Radioactivity in tissues after single oral dose of 10 mg/kg bw (in mean % of applied dose, except bone and skin expressed as % of applied dose/g)

Tissue	Males			Females		
	6 h (n=3)	18 h (n=3)	168 h (n=5)	6 h (n=3)	18 h (n=3)	168 h (n=5)
Adrenal glands	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Bone	0.12	0.10	0.02	0.10	0.09	0.03
Bone marrow	0.01	0.01	<0.01	0.01	0.01	0.01
Brain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carcass	2.00	2.69	0.33	1.69	3.03	0.27
Eyes	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Fat (abdominal)	0.06	0.04	0.01	0.04	0.03	0.01
Gastrointestinal tract	19.05	10.04	0.01	16.47	5.41	0.01
GIT contents	31.56	4.89	0.01	34.54	14.30	0.01
Heart	0.01	<0.01	<0.01	0.01	<0.01	<0.01
Kidneys	0.79	0.36	<0.01	0.67	0.26	<0.01
Liver	0.07	0.09	0.01	0.06	0.07	0.01
Lungs	0.01	0.01	<0.01	0.01	0.01	<0.01
Lymph nodes	0.09	0.05	<0.01	0.04	0.04	<0.01
Muscle (skeletal)	0.23	0.13	0.04	0.24	0.11	<0.03
Ovaries	-	-	-	<0.01	<0.01	<0.01
Pancreas	0.02	<0.01	<0.01	0.01	<0.01	<0.01
Pituitary gland	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Plasma	0.12	0.03	<0.01	0.13	0.03	<0.01
Skin	0.01	0.01	<0.01	0.01	0.01	<0.01
Spleen	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Stomach	3.47	0.60	0.60	2.56	0.62	<0.01
Stomach contents	25.16	5.05	0.01	22.90	6.96	0.01
Testes	0.01	0.01	<0.01	-	-	-
Thymus	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Thyroid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Urinary bladder	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Uterus	-	-	-	0.01	<0.01	<0.01
Whole blood	0.20	0.04	<0.03	0.15	0.05	<0.03

Table 5.1-30: HR-001: Metabolism in the rat ([redacted] 1995): Radioactivity in tissues after single oral dose of 600 mg/kg bw (in mean % of applied dose, except bone and skin expressed as mean % of applied dose/g)

Tissue	Males			Females		
	3 h (n=3)	9 h (n=3)	168 h (n=5)	3 h (n=3)	9 h (n=3)	168 h (n=5)
Adrenal glands	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Bone	0.09	0.10	0.02	0.09	0.05	0.02
Bone marrow	0.01	0.01	<0.01	0.01	0.01	<0.01
Brain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carcass	1.87	1.70	0.31	2.85	2.41	0.39
Eyes	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Fat (abdominal)	0.09	0.05	<0.01	0.09	0.02	<0.01
Gastrointestinal tract	19.71	9.99	0.01	20.90	9.33	0.01
GIT contents	30.48	13.19	0.02	22.65	12.86	0.03
Heart	0.01	<0.01	<0.01	0.01	<0.01	<0.01
Kidneys	1.00	0.55	<0.01	0.82	0.21	<0.01
Liver	0.06	0.14	0.01	0.07	0.06	0.02
Lungs	0.02	0.01	<0.01	0.02	0.01	<0.01
Lymph nodes	0.07	0.02	<0.01	0.04	0.01	<0.01
Muscle (skeletal)	0.38	0.19	<0.05	0.48	0.18	<0.05
Ovaries	-	-	-	<0.01	<0.01	<0.01
Pancreas	0.01	0.01	<0.01	0.01	0.01	<0.01
Pituitary gland	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Plasma	0.26	0.07	<0.01	0.30	<0.01	<0.01
Skin	0.01	<0.01	<0.01	0.01	<0.01	<0.01
Spleen	0.01	0.01	<0.01	<0.01	<0.01	<0.01
Stomach	3.53	3.36	<0.01	4.33	3.14	<0.01
Stomach contents	28.73	32.70	0.02	34.20	45.01	0.02
Testes	0.01	0.01	<0.01	-	-	-
Thymus	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Thyroid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Urinary bladder	0.01	0.01	<0.01	<0.01	<0.01	<0.01
Uterus	-	-	-	0.01	<0.01	<0.01
Whole blood	0.28	0.08	0.01	0.30	0.05	<0.01

D. PROPORTION OF RADIOACTIVE COMPONENTS IN URINE

Major urinary (18 - 27 %) and faecal (65 - 78 %) component was the parent compound. One further minor component was also observed in urine (0.1 - 0.3 %) and identified as aminomethylphosphonic acid by TLC and HPLC co-chromatography. In faeces two further minor components were detected (1 - 2 %, low dose; 0.3 - 0.6 %, high dose) one of them could be identified as aminomethylphosphonic acid by TLC and HPLC co-chromatography. The nature of the other component (<2 %) was not investigated further.

E. RECOVERY OF RADIOACTIVITY

The total mean radioactivity recovered within 168 h exceeded 95 % and was below 106 %.

III. CONCLUSIONS

After oral administration of glyphosate at least 20 % are absorbed as indicated by urinary excretion. Absorption was independent of sex but the areas under the plasma concentration vs time curves (AUC_i) after the high dose were increased by 100 to 120 fold compared to the low dose. Only the gastrointestinal tract, the stomach, the kidneys (the organs of excretion) the muscles and bones contained higher concentrations of radioactivity than the plasma. About 75 % and 25 % of the parent compound was excreted via faeces and urine, respectively. Additionally, aminomethylphosphonic acid was detected in faeces and urine accounting for ≤1.6 % of the dose. The nature of the other compounds in faeces (<2 %) was not investigated further. Elimination from the body was fast with half-life´s of 6 - 8 h at the low and high dose, respectively. After 168 h the highest remaining concentrations was detected in carcass (<0.4 % of applied dose). The time dependent decrease and the overall low tissue concentration after 7 days do not indicate a potential of accumulation.

3. Assessment and conclusion

Assessment and conclusion by applicant:

After single oral administration of 10 or 600 mg/kg bw glyphosate to both male and female rats at least 20 % was absorbed (based on the amount excreted *via* urine). About 75 % and 25 % of the dose was excreted *via* faeces and urine, respectively, mainly as the unchanged parent compound within 7 days after administration. Additionally, aminomethylphosphonic acid was detected in faeces and urine accounting for ≤1.6 % of the dose. The nature of the other components in faeces (<2 %) was not investigated further.

Investigation of pharmacokinetics in plasma revealed that plasma peak levels were reached 6 h (males) and 2 h (females) after administration of the low dose and 3 h (males and females) after administration of the high dose. No further significant gender- or dose-dependent differences were noted.

Tissue concentrations were overall low at all investigated sampling time points and dose levels, except the GI tract, stomach and contents. Further, muscles, bones and the kidneys showed higher levels than plasma accounting for up to 0.79 % (low dose) and 1.00 % (high dose) at T_{max}. Thereafter, radioactivity levels decreased time-dependently. Albeit repeated dosing was not investigated within the present study, the findings do not indicate an accumulation, which is also supported by observations made within other studies.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.1.1/011
Report author	[redacted]
Report year	1992
Report title	[^{14}C]-Glyphosate: Absorption and distribution in the rat – preliminary study
Report No	6365-676/1
Document No	Not reported
Guidelines followed in study	US-EPA FIFRA 85-1
Deviations from current	Yes, limited focus

test guideline	
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive summary

The objectives of this preliminary study was to determine the pharmacokinetics and tissue distribution of radioactivity in three male rats after a single oral dose of [^{14}C]-glyphosate using blood sampling and whole-body autoradiography, respectively. The aim was to ascertain appropriate blood sampling times and identify the distribution of radioactivity for a definitive adsorption, distribution, metabolism, and excretion study.

Overnight fasted male rats were orally administered with [^{14}C]-glyphosate at a nominal dose level of 30 mg/kg bw. Following administration to one group of three rats, blood samples were collected at regular intervals up to 48 hour post-dose and radioactivity determined in plasma. After administration to a second group of 3 rats, whole-body autoradiography was performed on one animal at 4, 10 and 24 h post-dosing and target tissues identified.

Low levels of radioactivity were detected in plasma. Maximum plasma concentrations (C_{max}) reached within 4 h was 1.769, 1.137, and 0.705 µg equiv/mL for three animals. Thereafter plasma levels decreased exponentially to non-detectable levels at 12 h post-dose. The elimination half-lives were 6.196 h and 12.35 h for two animals. A value could not be obtained for the third animal.

Whole-body autoradiography showed that distribution of radioactivity into tissues is limited. The greatest concentrations of radioactivity were detected 10 h after application. The highest concentrations of radioactivity were associated with bone and bone marrow, cartilage, some parts of the gastro-intestinal tract, kidney and urinary tract and the nasal mucosa. Except for radioactivity in bone and bone marrow, which appear to be possible target tissues for this compound, there were negligible tissue concentrations 24 h post-dose.

I. MATERIALS AND METHODS

A. MATERIALS

1. Non-labelled test material:

Identification:	Glyphosate (N-(phosphonomethyl)glycine)
Description:	White crystalline solid
Lot/Batch #:	206-JaK-25-1
Purity:	98.6 %
Stability of test compound:	Non-sensitive to sunlight; No degradation observed in 14 days accelerated storage stability test at 54 ± 2° C (CIPAC method MT 46.1.1)

2. Radiolabelled test material:

Identification:	[^{14}C]-Glyphosate
Position of radiolabel:	N-(phosphono[^{14}C]methyl)glycine

Lot/Batch #:	CFA 745 C4
Radiochemical purity:	The stated radiochemical purity was 97.4 %, however, subsequent to this investigation this was amended to 94.3 %
Specific activity:	307.7 µCi/mg (11.38 MBq/mg)
Stability of test compound:	Stored at -20° C and stability in formulation assessed

3. Vehicle and/or positive control: 0.9 % w/v sodium chloride

4. Test animals:

Species:	Rat
Strain:	Sprague-Dawley (Crl:CD BR)
Source:	[redacted]
Age:	Approximatively 9 weeks old on arrival
Sex:	Male
Weight at dosing:	249 - 275 g at the start of treatment for the pharmacokinetic study and 267 - 296 g at the start of the autoradiography study
Acclimation period:	Approximately 10 days
Diet/Food:	During acclimatisation: Commercial pellet diet, SQC Rat and Mouse Maintenance Diet No 1, Expanded(Special Diet Services, Stepfield, Witham, Essex), *ad libitum* Before experiments: The diet was removed for approximately 14 h before and 4 h after administration of the radiolabelled test article.
Water:	Tap water, *ad libitum*
Housing:	During acclimatisation: Groups of up to five per cage, in wire floor polypropylene cages suspended over polypropylene dirt trays containing soft white wood flakes. Experimental conditions: Following administration of [^{14}C]-glyphosate the three animals from dose Group A were transferred to grid floor cage.
Environmental conditions:	Temperature: 22 ± 3°C Humidity: 40 - 70 % Air changes: 15/h 12-hour light/dark cycle

B. STUDY DESIGN

In life dates: not reported

Animal assignment and treatment: Pharmacokinetic study

Rats were randomly allocated unique numbers using ear tags and/or tail marking as shown below:

Study type	Animal identification	Group name
Pharmacokinetic	118M, 119M, 121M	A

Each animal received a single oral administration of [^{14}C]-glyphosate at a dose level of 30 mg/kg bw corresponding to a nominal radioactive dose of 15 µCi per animal. Following administration of [^{14}C]-

glyphosate to three rats (Group A), blood samples (approximately 100 µL) were collected from the lateral tail vein pre-dose and at 0.5, 1.0, 1.5, 2, 2.5, 3, 4, 6, 9, 12, 24 and 48 h after treatment and the radioactivity determined in plasma. The animals were sacrificed in a rising concentration of carbon dioxide.

Animal assignment and treatment: Distribution study

Rats were randomly allocated unique numbers using ear tags and/or tail marking as shown below:

Study type	Animal identification	Group name
Whole-body autoradiography	2M, 3M, 4M	B

Each animal received a single oral administration of [^{14}C]-glyphosate at a dose level of 30 mg/kg bw corresponding to a nominal radioactive dose of 30 µCi per animal. Following administration of [^{14}C]-glyphosate to three male rats, one male per time point was sacrificed 4, 10 and 24 h after treatment for whole-body autoradiography.
After sacrifice, each carcass was pinned out on a board and rapidly and embedded in a block of aqueous carboxymethylcellulose (2 % w/v) and mounted onto the stage of a PMV 450MP cryo-microtome maintained at about –20 °C. Sagittal sections (about 25 µm) were then obtained at 6 levels through the carcass:

Level A; Exorbital lachrymal gland
Level B: Intra-orbital lachrymal gland
Level C: Harderian gland
Level D: Adrenal gland
Level E: Thyroid
Level F: Brain and spinal cord

Autoradiograms were prepared by contact with autoradiographic film for 108 days before fixing and washing.

Dosing Formulation Analysis

The stability of the formulation was not determined due to difficulties with the reproduction of the Thin Layer Chromatography.

Measurement of radioactivity

Portions of plasma were added directly to liquid scintillant and subjected to Liquid Scintillation Counting (LSC). All radioassays were performed in duplicate. Radioactivity was measured for 10 min or for 2 sigma % using a Beckman or Packard Tri-Carb liquid scintillation counter with facilities for computing quench-corrected disintegrations per minute (dpm).

The limit of detection for each batch of samples analysed by direct counting is taken as 2 times the mean background disintegration rate obtained from vials containing an equivalent volume of an appropriate solvent in liquid scintillant.

Thin layer chromatography (TLC)

TLC was carried out on cellulose plates (500 µm) using the following development systems to assess the stability of the formulated [^{14}C]-glyphosate upon preparation and after storage at room temperature for 4 h:
System 1: Methanol : water (67:33 v/v)
System 2: Ethanol : water : 15N ammonium hydroxide : trichloroacetic acid : acetic acid (55:35:2.5:35:2 , v/v/v/w/v)
The plates were developed for at least 18 cm and visualised by 0.5 % ninhydrin in butanol then heated at 100°C for 5 minutes. Radioactivity was quantified with a linear analyser.

Data evaluation

Liquid scintillation counts and weighing data were captured on-line using a validated data acquisition system. The limit of detection for each batch of samples analysed by direct LSC is taken as 2 times the mean background disintegration rate obtained from vials containing an equivalent volume of an appropriate solvent in liquid scintillant.

II. RESULTS AND DISCUSSION

A. PHARMACOKINETIC STUDY

Low levels of radioactivity were detected in plasma. Maximum plasma concentrations of 1.769, 1.137 and 0.705 µg equivalent of [^{14}C]-glyphosate/mL were reached within 3 - 4 h. Thereafter plasma levels declined exponentially to non-detectable levels 12 h after treatment. The elimination half-lives were 6.2 and 12.4 h for two animals. A value could not be obtained for the third animal. Please refer to the tables below.

Table 5.1-31: [^{14}C]-Glyphosate: Absorption and distribution in the rat – preliminary study ([redacted] 1992): Pharmacokinetic parameters for plasma radioactivity in rats following a single oral administration of [^{14}C]-glyphosate at a nominal dose level of 30 mg/kg bw

Animal no. and sex	Cmax (µg eq/mL)	Tmax (h)	AUC (µg eq.h/mL)	$t_{1/2}$ (h)
118M	1.769	3	18.62	6.2
119M	1.137	4	23.09	12.4
121M	0.705	4	N/A	N/A

N/A not applicable

Table 5.1-32: [^{14}C]-Glyphosate: Absorption and distribution in the rat – preliminary study ([redacted] 1992): Plasma concentrations of radioactivity in male rats following a single oral administration of [^{14}C]-glyphosate at a nominal dose level of 30 mg/kg bw

Time (h)	µg equivalents of [^{14}C]-glyphosate/mL of plasma		
	118M	119M	121M
Pre-dose	ND	ND	ND
0.5	ND	ND	ND
1	0.640	0.626	ND
1.5	1.137	0.823	ND
2	1.356	1.032	0.515
2.5	1.671	0.887	0.580
3	1.769	1.118	0.695
4	1.565	1.137	0.705
6	1.170	0.928	0.612
9	0.910	0.849	0.649
12	ND	ND	ND
24	ND	ND	ND
48	ND	ND	ND

ND not detected

B. DISTRIBUTION OF RADIOACTIVITY IN TISSUE

Whole-body autoradiography showed that distribution of radioactivity into tissues was limited. The greatest concentrations of radioactivity were detected 10 h after treatment. The highest concentrations of radioactivity were associated with bone and bone marrow, cartilage, some parts of the gastrointestinal tract, kidney and urinary tract and the nasal mucosa. Except for radioactivity in bone and bone marrow, there were negligible tissue concentrations 24 h post-dose. Please refer to the table below.

Table 5.1-33: [^{14}C]-Glyphosate: Absorption and distribution in the rat – preliminary study ([redacted] 1992): Distribution of relative levels of radioactivity in the tissues of male rats as revealed by whole-body autoradiography following a single oral administration of [^{14}C]-glycosate at a nominal dose level of 30 mg/kg bw

Tissue type	Tissue	Animal number and time of sacrifice		
		4M	2M	3M
		4 h	10 h	24 h
Bone	Bone marrow	2	2	2
	Bone	2	2	2
	Ischium	2	2	2
	Ileum	2	2	2
	Jaw bone	2	2	2
	Rib	2	2	2
	Sternum	2	2	2
	Vertebrae	2	2	2
Connective	Cartilage	1	2	0
	Epimysium	0	1	0
	Fascia	0	1	0
	Xiphoid cartilage	NP	2	NP
Endocrine	Adrenal	0	1	0
	Pituitary	0	1	0
	Thymus	0	1	0
	Thyroid	1	1	1
Secretory	Exorbital lachrymal gland	0	1	0
	Intra-orbital lachrymal gland	0	1	0
	Salivary glands	1	1	1
Gastrointestinal	Caecal contents	3	3	3
	Caecal mucosa	3	3	3
	Large intestine contents	3	3	3
	Large intestine mucosa	3	1	3
	Small intestine contents	2	3	1
	Small intestine mucosa	3	3	3
	Stomach contents	0	1	0
	Stomach mucosa	2	3	2
Gonads	Prostate	0	1	0
	Seminal vesicles	0	1	0
	Testis	1	1	0
Muscular	Myocardium	0	1	0
	Tongue	1	1	0
Urinary	Bladder	3	3	2
	Kidney cortex	3	3	2
	Kidney medulla	1	2	1
Others	Blood	1	1	0
	Liver	1	1	1
	Lung	1	1	1
	Nasal mucosa	2	3	1
	Skin	1	1	1
	Spleen	1	1	0
	Trachea	1	1	0

NP Organ/tissue not present on autoradiogram.
The assessment of the relative levels of radioactivity in the autoradiograms was made by visual inspection of the autoradiograms whilst viewing them on a standard X-ray viewing box. The relative levels of radioactivity in the tissues were described as 3 =“high”, 2 = “moderate”, 1 = “low”, 0 = radioactivity not detected.

III. CONCLUSIONS

Following oral administration of [^{14}C]-glyphosate, systemic absorption of radioactivity was poor and tissue distribution of [^{14}C]-glyphosate and/or its radiolabelled metabolites were limited.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The plasma profiles and distribution of radioactivity have been studied in a pilot absorption and distribution study using three male rats with the aim to obtain preliminary data for a subsequently following full-range ADME study.

Following a single oral administration of [^{14}C]-glyphosate at a nominal dose level of 30 mg/kg bw low levels of radioactivity were detected in plasma. Maximum plasma concentrations (C_{max}) between 0.7 and 1.8 µg equiv/mL were reached within 4 hours (T_{max}) No radioactivity was detectable in plasma 12 h after treatment. The elimination half-lives were 6.2 and 12.4 h for the two animals for which these values could be obtained.

Whole-body autoradiography revealed a limited tissue distribution of radioactivity. Highest levels were observed 10 h post-dose with a specific accumulation in bone, especially in the growing regions, i.e. the epiphyses. This affinity may be attributed to the phosphonomethyl moiety of glyphosate. Except for radioactivity in bone and bone marrow, negligible tissue concentrations were noted 24 h post dose.

The results of this study can be considered as supplemental data only.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.1.1/012
Report author	[redacted]
Report year	1992
Report title	[^{14}C]-Glyphosate: Absorption, distribution, metabolism and excretion in the rat
Report No	7006-676/2
Document No	Not reported
Guidelines followed in study	US-EPA FIFRA 85-1
Deviations from current test guideline	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive summary

The absorption, distribution, metabolism and excretion of oral and intravenously administered [^{14}C]-glyphosate have been investigated in male and female Sprague Dawley rats after a single intravenous and oral dose of 30 mg/kg bw, a single oral dose of 1000 mg/kg bw and after 15 daily oral doses of 30 mg/kg bw/day. Samples were collected for a period of 7 days after (last) administration. Radioactive tissue residues were determined after at least 90 % of the dose had been excreted. Radioactivity in excreta was characterised by chromatographic spectroscopic methods.

The recovery of administered radioactivity in excreta exceeded 90 % in all investigations. The proportion of radioactive dose absorbed from the gut, estimated by comparison of renal elimination following single intravenous or oral administration at 30 mg/kg bw, was 34 % (males) and 36 % (females) respectively. Absorption was similar for both sexes and at all tested doses.

The radioactive tissue residues present after >90 % of the dose had been eliminated were low (<1.5 % of dose) irrespective of dosing regimen. Highest residues were present in bone. The distribution of radioactivity in tissues was similar for males and females after both dose levels.

No radiolabelled metabolites of [^{14}C]-glyphosate were found in any of the investigations. The identity of the radioactive component as glyphosate was confirmed by FT-IR spectrometer.

After intravenous dosing, elimination in urine accounted for ca. 85 % of the dose compared with <4 % in faeces. After oral dosing about 47 - 60 % of the administered [^{14}C]-glyphosate were detected in faeces and 23 - 35 % in urine. Renal elimination was more protracted following oral administration of [^{14}C]-glyphosate continuing for at least 24 h post-dose. At least 80 % of the radioactivity was excreted with the urine, faeces and exhaled air within 48 h. No significant radioactivity was detected in exhaled air (<0.1 % of dose). There was neither a noticeable difference in excretion in males and females nor at different dose levels.

I. MATERIALS AND METHODS

A. MATERIALS

1. Non-labelled test material:

Identification:	Glyphosate (N-(phosphonomethyl)glycine)
Description:	White crystalline solid
Lot/Batch #:	206-JaK-25-1
Purity:	98.6 %
Stability of test compound:	Non-sensitive to sunlight; No degradation observed in 14 days accelerated storage stability test at 54 ± 2°C (CIPAC method MT 46.1.1)

2. Radiolabelled test material:

Identification:	[^{14}C]-Glyphosate
Position of radiolabel:	N-(phosphono[^{14}C]methyl)glycine
Lot/Batch #:	CFQ.6228
Radiochemical purity:	>98 % by two TLC systems (97 % by HPLC)
Specific activity:	52 mCi/mmol, 1.0 mCi/mL
Stability of test compound:	The stability was assessed by two TLC systems.

3. Reference substance:

Identification:	Aminomethylphosphonic acid (AMPA)

Description:	Not reported
Lot/Batch #:	Not reported
Purity:	Not reported
Stability of test compound:	Not reported
4. Vehicle and/or positive control:	0.9 % w/v sodium chloride
5. Test animals:	
Species:	Rat
Strain:	Sprague-Dawley (Crl:CD BR)
Source:	[redacted]
Age:	6 - 10 weeks on arrival
Sex:	Males and females, nulliparous and non-pregnant
Weight at dosing:	150 - 200 g on arrival in May 1990
Acclimation period:	At least 7 days
Diet/Food:	Commercial pellet diet , SQC Rat and Mouse Maintenance Diet No 1, Expanded (Special Diet Services, Stepfield, Witham, Essex), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	During acclimatisation: Groups of five animals per cage according to sex, in wire floor polypropylene cages suspended over polypropylene dirt trays containing soft white wood sawdust. After dosing: Individually in glass metabolism cages suitable for the separate collection of urine, faeces and expired air.
Environmental conditions:	Temperature: 22 ± 3°C Humidity: 40 - 70 % Air changes: At least 10/hour 12-hour light/dark cycle

B. STUDY DESIGN

In life dates: not reported

Animal assignment and treatment

Group	Route of dosing	Frequency of dosing	Dose level (mg/kg bw)		Radioactive dose (µCi)
A	Intravenous	Single	30	Low	25
B	Oral	Single	30	Low	50
C	Oral	Multiple*	30	Low	25
D	Oral	Single	1000	High	25

*14 consecutive daily doses of glyphosate followed approximately 24 h later by radiolabelled glyphosate

Following administration of [^{14}C]-glyphosate (1st treatment in May 1990), rats from each dose group (5 male, 5 female,) were placed in individual glass metabolism cages. Cage side observations were performed in the morning.

Urine and faeces were collected at the following time intervals: 0 - 4, 4 - 8, 8 - 12, 12 - 24, 24 - 36, 36 - 48, 48 - 72, 72 - 96, 120 - 144, 144 - 168 h after treatment.

Expired air was trapped in 2-ethoxyethanol : ethanol amine (3:1 by volume) and was sampled as per urine collections until it was determined that less than 1 % of the administered radioactivity was present.

After each collection of excreta, cage debris was removed and cages washed with water. At the end of the collection period, cages were washed thoroughly with water and then methanol. The radioactivity present in urine, faeces, cage washings, cage debris and expired air was determined. As soon as a mean of ~90 % of the administered radioactivity was excreted or after a maximum of 168 h, the rats were exsanguinated under halothane anaesthesia.

The following tissues were removed or sampled and assayed for radioactivity: blood, bone (femur), bone marrow, brain, fat (abdominal), heart, kidney, 1iver, lungs, muscle (skeletal), ovaries/testes, plasma, salivary gland, spleen, uterus, residual carcass.

Dosing Formulation Analysis

The stability of the formulated [^{14}C]-glyphosate dissolved in 0.9 % saline (at approx. 6 mg/mL) was assessed by two Thin layer chromatography (TLC) systems at 0, 1, 2, 10 and 16 days post-formulation. Comparison of the respective chromatograms showed no significant degradation of [^{14}C]-glyphosate over this time. The specific radioactivity of the [^{14}C]-labelled test substance prior to formulation and the homogeneity of the dosing preparation was determined by liquid scintillation counting.

Measurement of radioactivity

Portions of faeces, cage debris, bone, blood, spleen and liver were combusted. Combusted products were absorbed in Carbo-Sorb™ mixed with Permafluor® V and the radioactivity determined by liquid scintillation counting. Combustion and trapping efficiencies were found to be in excess of 96 %. All other samples were solubilised with Soluene-350™ or Soluene-100™ and incubated as necessary. Hionic-Fluor was admixed and, following dark adaption overnight, the samples were assayed for radioactivity using a liquid scintillation counter. All radioassays were performed in duplicate. Radioactivity was measured for 10 min or for 2 sigma % using a Beckman (Beckman, High Wycombe, Bucks) or Packard Tri-Carb liquid scintillation counter (Canberra Packard, Pangbourne, Berks) with facilities for computing quench-corrected disintegrations per minute (dpm).

Isolation of the major urinary and faecal metabolites

The metabolite profiles of urine and faeces samples from dose Groups A to D were investigated using one HPLC and two TLC systems. They were quantitatively examined for glyphosate and the potential metabolite, AMPA.

Thin layer chromatography (TLC)

TLC was carried out on Merck cellulose plates (0.5 mm) using an 18 cm development system and the following solvent systems:

System 1: Methanol : water (67:33 by volume);
System 2: Ethanol : trichloroacetic acid (10 % aqueous solution) : ammonium hydroxide (20 M) : acetic acid (17M), (55:35:2.5:2 by volume).

Glyphosate and aminomethyl phosphonic acid (AMPA) reference standards were visualized with ninhydrin spray reagent.

Radioactivity was detected with a Raytest RITA Linear Analyser (Lablogic systems Ltd.) and autoradiography Hyperfilm ß-max (Amersham International plc). Regions of interest were located by reference to an autoradiogram. They were assigned manually and the percent radioactivity associated with each region determined using the RITA data system.

High performance liquid chromatography (HPLC) and Spectrometry

HPLC system (gradient elution method; column: Merck Lichrosorb RP-18, Solvent A: 95 % HPLC phosphate buffer, 5 % acetonitrile, Solvent B: 15 % HPLC phosphate buffer, 85 % acetonitrile linked to a Fluorescence detector and on-line radioactivity monitor. Reference standards were glyphosate and aminomethyl phosphonic acid (AMPA).

Extracts from dose Group D, pooled male urine (4 - 8 h post dose) and pooled male faeces (12 - 24 h post dose) were analysed using TLC solvent System 1. Each sample was applied to the origin of a separate TLC plate as a thin band (~ 18 cm long). Immediately after development, the location of the major region of radioactivity was determined using a RITA linear analyser to scan several positions across each plate. The cellulose layer containing this region was scraped from the plate and radioactivity extracted into methanol. Solutions of non-labelled and [^{14}C]-glyphosate standard in methanol (~ 0.1 mg/mL) were prepared. The standards and extracted samples were analysed using a Mattson 2020 Galaxy FT- IR spectrometer (Mattson Instruments Ltd).

Data evaluation

Liquid scintillation counts and weighing data were either entered manually or captured on-line using a validated data acquisition system. The limit of detection for the analysis sample from the excretion balance study was taken as twice the mean background from measured blank samples of the same type.

II. RESULTS AND DISCUSSION

A. CAGE SIDE OBSERVATIONS

No overt pharmacological or toxicological signs were observed in the test animals which could have been attributed to the administration of [^{14}C]-glyphosate.

B. EXCRETION AND RETENTION OF RADIOACTIVITY

More than 90 % of the administered radioactivity was recovered within 7 days by the low and the high dose groups after a single oral or intravenous application of the test substance. After multiple oral dosing 90 % of the administered radioactivity was recovered.

Single bolus intravenous dose at 30 mg/kg bw:
The major proportion of radioactivity excreted in urine (86.0 % male; 84.2 % female) was within the first 4 h (72.1 % male; 71.9 % female). Considering cage wash to calculate the total urinary excretion 92.9 % and 97.4 % were excreted in urine by males and females, respectively.

Faecal excretion of radioactivity was 3.4 % in male and 1.5 % in females. Less than 0.03 % was eliminated in expired air. At termination (168h), radioactive residues were low (1.35 % male; 1.09 % female). Of the tissues sampled at termination, bone contained the highest concentration (4.2 µg equiv/g, male; 4.4 µg equiv/g, female), all remaining tissues possessing <0.5 µg equiv/g except for female bone marrow (1.3 µg equiv/g). Fat, muscle and testes were devoid of quantifiable quantities of radioactivity.

Single oral dose at 30 mg/kg bw:
The experiment was terminated after 120 h since the total excreted exceeded 90 % at that time point. The major proportion of radioactivity excreted in faeces (58.8 % male; 56.5 %, female) was within 24 h post dose (45.8 %, males; 40.7 %, females). Urine accounted for 29.0 % in males and 30.7 % in females with significant amounts still excreted at 24 h post-dose (7.1 %, males; 7.2 %, females). Less than 0.1 % was eliminated in expired air. Residual radioactivity in tissues and carcass at 120 h post-dose accounted for about 0.6 % (males and females). Concentrations in bone were 2.3 µg equiv/g in males and 2.6 µg equiv/g in females. The remaining tissues excised contained <0.6 µg equiv/g; fat (males only), muscle and testes were devoid of quantifiable quantities of radioactivity.

Single oral dose at 1000 mg/kg bw:
The major proportion of radioactivity excreted in faeces (53.3 % male; 60.4 % female) was within 24 h post-dose (38.6 %, males; 48.3 %, females). Urine accounted for 30.6 % in males and 22.4 % in females. Pulmonary elimination was negligible. At 168 h post-dose, radioactive residues in tissues and carcass were low (0.5 %, males; 0.4 %, females). Bone contained the highest concentrations of radioactivity (56.3 µg equiv/g, males; 40.7 µg equiv/g, females), some 25- and 15-fold the levels observed after the single oral dose for a 33-fold increase in dose. The organs of metabolism and excretion contained ca. 5 - 6 µg equiv/g in male animals, whereas in females, though the level in the kidney was ca. 4 µg equiv/g, the liver contained no detectable radioactivity. With the exception of the lungs and bone marrow, no quantifiable radioactivity was determined in the remaining tissues.

14 days repeated oral doses at 30 mg/kg bw:
The experiment was terminated after 72 h since the total excreted exceeded 90 % at that time point. Most radioactivity was excreted in faeces (49.6 %, males; 46.7 %, females), the majority within 24 h post-dose. Urine accounted for 34.28 % (males) and 34.63 % (females) of the dose. The elimination was slower than after the intravenous administration, with 5.1 % (males) and 6.2 % (females) still excreted at 12 - 24 h post-dose. In both sexes, expired air accounted for <0.1 % of the administered dose. The investigation was terminated ca. 72 h post-dose as a mean of ca 90 % of dose had been recovered. At this time, residual radioactivity in tissues and the remaining carcass accounted for 0.96 % (males) and 0.83 % (females) of the dose. As with the single doses, bone exhibited the highest concentration of radioactivity (3.1 µg equiv/g, males; 2.5 µg equiv/g, females), all other tissues contained <0.6 µg equiv/g. Fat, muscle, testes and brain (females only) contained no quantifiable radioactivity.

Table 5.1-34: [^{14}C]-Glyphosate: Absorption, distribution, metabolism and excretion in the rat ([redacted] 1992): Mean (±SD) recovery of radioactivity (% of applied dose) following administration of [^{14}C]-glyphosate to the rat (at termination)

Sample	Intravenous dose* (30 mg/kg bw)		Oral dose* (30 mg/kg bw)		Oral dose** (30 mg/kg bw)		Oral dose* (1000 mg/kg bw)	
	M	F	M	F	M	F	M	F
Urine	85.98	84.18	29.04	30.71	34.28	34.63	30.55	22.41
	(6.286)	(8.127)	(8.280)	(2.772)	(10.37)	(5.994)	(5.369)	(6.612)
Cage wash	6.948	13.25	6.884	7.453	5.145	7.324	12.08	16.66
	(4.549)	(6.399)	(2.914)	(3.165)	(2.025)	(1.832)	(7.297)	(8.328)
Subtotal urine + cage wash	92.928	97.43	35.924	38.163	39.425	39.775	42.63	39.07
Faeces	3.422	1.484	58.84	56.53	49.64	46.73	53.27	60.37
	(3.150)	(0.811)	(7.989)	(4.635)	(10.99)	(5.558)	(8.928)	(10.30)
Expired air (CO_2 Trap 1)	0.014	0.023	0.075	0.065	0.085	0.055	0.064	0.067
	(0.013)	(0.013)	(0.027)	(0.022)	(0.064)	(0.015)	(0.012)	(0.018)
Expired air (CO_2 Trap 2)	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.006
	(0.013)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.013)
Cage debris	0.025	0.021	1.160	1.315	0.033	0.389	3.268	0.519
	(0.036)	(0.029)	(1.188)	(1.192)	(0.061)	(0.519)	(5.628)	(0.682)
Subtotal	**96.397**	**98.907**	**96.011**	**96.075**	**89.185**	**89.125**	**99.231**	**100.0**
Tissues***	1.353	1.093	0.619	0.635	0.955	0.825	0.469	0.400
	(0.604)	(0.169)	(0.132)	(0.108)	(0.210)	(0.171)	(0.052)	(0.076)
Total (Mass Balance)	**97.75**	**100.0**	**96.63**	**96.71**	**90.14**	**89.95**	**99.70**	**100.4**

* Single dose
** Multiple dose (non-radiolabelled glyphosate for 14 consecutive days followed ~24 h later by [^{14}C]-glyphosate)
*** Carcass plus whole tissues

Table 5.1-35: [^{14}C]-Glyphosate: Absorption, distribution, metabolism and excretion in the rat ([redacted] 1992): Excretion balance (in mean % of applied dose) at termination

Balance/Excretion	Intravenous dose* (30 mg/kg bw)		Oral dose* (30 mg/kg bw)		Oral dose** (30 mg/kg bw)		Oral dose* (1000 mg/kg bw)	
	M	F	M	F	M	F	M	F
Urine 0 - 4	72.100	71.89	3.215	3.150	8.992	5.515	5.561	2.078
Urine 4 - 8	7.344	6.397	9.638	11.91	11.790	15.210	14.21	13.32
Urine 8 - 12	2.442	2.056	4.239	4.489	4.950	5.006	2.918	2.386
Urine 12 - 24	1.694	1.429	7.137	7.202	5.091	6.184	3.819	2.622
Urine 24 - 36	0.813	0.778	3.163	2.266	2.387	1.520	1.991	0.966
Urine 36 - 48	0.369	0.364	0.752	0.775	0.622	0.427	0.806	0.378
Urine 0 - 48h	**84.762**	**82.914**	**28.144**	**29.792**	**33.832**	**33.862**	**29.305**	**21.750**
Urine 48 - 72	0.458	0.497	0.588	0.551	0.447	0.766	0.687	0.384
Urine 72 - 96	0.320	0.309	0.197	0.238	NS	NS	0.247	0.165
Urine 96 - 120	0.187	0.206	0.115	0.134	NS	NS	0.172	0.096
Urine 120 - 144	0.141	0.145	NS	NS	NS	NS	0.096	0.012
Urine 144 - 168	0.112	0.109	NS	NS	NS	NS	0.043	0.000
Cage wash 0 - 4	4.829	10.97	0.767	1.581	1.403	1.065	1.152	4.800
Cage wash 4 - 8	1.279	1.459	1.296	2.618	0.383	1.712	3.531	5.243
Cage wash 8 - 12	0.363	0.298	2.452	1.173	0.293	0.868	4.453	4.262
Cage wash 12 - 24	0.139	0.122	1.557	1.449	0.435	2.383	1.767	1.681
Cage wash 24 - 36	0.071	0.100	0.539	0.284	0.269	0.716	0.549	0.333
Cage wash 36 - 48	0.088	0.092	0.165	0.222	0.109	0.376	0.201	0.155
Cage wash 48 - 72	0.062	0.106	0.055	0.070	0.253	0.203	0.179	0.101
Cage wash 72 - 96	0.037	0.040	0.027	0.030	NS	NS	0.129	0.055
Cage wash 96 - 120	0.035	0.026	0.028	0.027	NS	NS	0.069	0.019
Cage wash 120 - 144	0.022	0.014	NS	NS	NS	NS	0.031	0.006
Cage wash 144 - 168	0.025	0.019	NS	NS	NS	NS	0.021	0.009
Expired air 0 - 4	0.024	0.023	0.039	0.038	0.054	0.055	0.060	0.066
Expired air 4 - 8	0.000	0.000	0.024	0.018	0.027	0.000	0.004	0.006
Expired air 8 - 12	0.000	0.000	0.010	0.005	0.004	0.000	0.000	0.000
Expired air 12 - 24	0.000	0.000	0.002	0.004	0.000	0.000	0.000	0.000
Expired air 0 - 24h	**0.024**	**0.023**	**0.075**	**0.064**	**0.085**	**0.055**	**0.064**	**0.072**
Expired air 24 - 36	NS	NS	0.000	0.000	NS	NS	NS	NS
Expired air 36 - 48	NS	NS	0.000	0.000	NS	NS	NS	NS
Expired air 48 - 72	NS	NS	0.000	0.000	NS	NS	NS	NS
Faeces 0 - 4	N/A	N/A	NS	NS	NS	NS	NS	NS
Faeces 4 - 8	N/A	N/A	NS	NS	NS	NS	NS	NS
Faeces 8 - 12	1.193	0.502	NS	NS	24.61	17.44	NS	NS
Faeces 12 - 24	1.035	0.631	45.84	40.68	15.58	18.25	38.63	48.27
Faeces 24 - 36	0.53	0.145	8.304	10.94	7.404	8.613	9.788	8.524
Faeces 36 - 48	0.258	0.388	3.126	2.455	1.046	1.498	2.385	1.945
Faeces 0 - 48h	**3.016**	**1.666**	**57.270**	**54.075**	**48.640**	**45.801**	**50.803**	**58.739**
Subtotal Urine/Expired air/faeces 0 - 48h	**87.802**	**84.603**	**85.489**	**83.931**	**82.557**	**79.718**	**84.72**	**80.561**
Faeces 48 - 72	0.191	0.178	1.409	2.158	1.007	0.925	1.612	1.086
Faeces 72 - 96	0.158	0.047	0.121	0.235	NS	NS	0.549	0.505
Faeces 96 - 120	0.073	0.020	0.040	0.059	NS	NS	0.081	0.029
Faeces 120 - 144	0.023	0.000	NS	NS	NS	NS	0.165	0.006
Faeces 144 - 168	0.029	0.000	NS	NS	NS	NS	0.058	0.009

* Single dose
** Multiple dose (non-radiolabelled glyphosate for 14 consecutive days followed ~24 h later by [^{14}C]-glyphosate)
N/A not applicable
NS no sample

C. DISTRIBUTION OF RADIOACTIVITY IN TISSUE

Radioactivity concentrations in tissues were very low at all times. Radioactive residues were highest in bone. Highest remaining concentrations were detected in carcass.

Table 5.1-36: [^{14}C]-Glyphosate: Absorption, distribution, metabolism and excretion in the rat ([redacted] 1992): Radioactivity in tissues (in mean % of applied dose)

Tissue	Intravenous dose* (30 mg/kg bw)		Oral dose* (30 mg/kg bw)		Oral dose** (30 mg/kg bw)		Oral dose* (1000 mg/kg bw)	
	M	F	M	F	M	F	M	F
Brain	0.003	0.004	0.001	0.002	0.000	0.000	0.000	0.000
Carcass	1.305	1.045	0.576	0.597	0.888	0.771	0.448	0.397
Heart	0.001	0.000	0.000	0.001	0.000	0.000	0.000	0.000
Kidney	0.009	0.008	0.007	0.005	0.011	0.008	0.004	0.003
Liver	0.030	0.027	0.031	0.027	0.051	0.040	0.017	0.000
Lungs	0.004	0.005	0.002	0.002	0.003	0.003	0.001	0.001
Salivary gland	0.001	0.001	0.000	0.001	0.001	0.001	0.000	0.000
Spleen	0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.000
Testes/Ovaries	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Uterus	N/A	0.002	N/A	0.001	N/A	0.001	N/A	0.000

* Single dose
** Multiple dose (non-radiolabelled glyphosate for 14 consecutive days followed ~24 h later by [^{14}C]-glyphosate)
N/A not applicable

Table 5.1-37: [^{14}C]-Glyphosate: Absorption, distribution, metabolism and excretion in the rat ([redacted] 1992): Radioactivity in tissues (in µg equivalents of [^{14}C]-glyphosate/g)

Tissue	Intravenous dose* (30 mg/kg bw)		Oral dose* (30 mg/kg bw)		Oral dose** (30 mg/kg bw)		Oral dose* (1000 mg/kg bw)	
	M	F	M	F	M	F	M	F
Blood	0.050	0.084	0.011	0.000	0.000	0.000	0.000	0.000
Bone	4.195	4.355	2.246	2.562	3.096	2.505	56.32	40.66
Bone marrow	0.255	1.264	0.322	0.545	0.325	0.144	3.080	0.000
Brain	0.118	0.120	0.056	0.056	0.019	0.000	0.000	0.000
Abdominal fat	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.000
Carcass	0.423	0.335	0.197	0.214	0.339	0.284	5.628	4.476
Heart	0.051	0.025	0.051	0.045	0.000	0.000	0.000	0.000
Kidney	0.304	0.298	0.278	0.205	0.515	0.317	5.170	3.986
Liver	0.241	0.222	0.251	0.254	0.615	0.425	6.144	0.000
Lungs	0.264	0.279	0.124	0.126	0.183	0.173	2.904	1.216
Muscle	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Plasma	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000
Salivary gland	0.082	0.068	0.053	0.079	0.084	0.100	0.000	0.000
Spleen	0.117	0.117	0.140	0.091	0.164	0.153	0.000	0.000
Testes/Ovaries	0.000	0.034	0.000	0.068	0.000	0.028	0.000	0.000
Uterus	N/A	0.248	N/A	0.143	N/A	0.239	N/A	0.000

* Single dose
** Multiple dose (non-radiolabelled glyphosate for 14 consecutive days followed ~24 h later by [^{14}C]-glyphosate)
N/A not applicable

D. URINE AND FAECAL METABOLITES

The profiles of radioactivity for each analytical method were similar for all samples, with no apparent differences between sample type, collection time or sex. Likewise, no differences were observed between dose route, level or frequency.

<u>HPLC analysis</u>
Only one major region of radioactivity was detected (90 to 100 % of total radioactivity) in pooled samples. This corresponded to the retention time for non-radiolabelled and [^{14}C]-glyphosate standards. A region of radioactivity corresponding to AMPA standard was not detected. However, some peak tailing from the major region was observed in this area which may have prevented the detection of very low levels of radioactivity (ca. <5 %).The remainder of the radioactivity was located in diffuse areas.

<u>TLC solvent System 1</u>
One major region of radioactivity (90 - 100 % of total radioactivity in pooled sample) was detected. This co-chromatographed with non-labelled glyphosate reference standard. Up to three minor regions of radioactivity were observed (Region 001, ca. 0 - 8 %, Retention factor (R_f) ca. 0.00; Region 002, ca. 0 - 5 %, R_f ca 0.50; Region 004, ca. 0 - 6 %, R_f ca 0.85). Region 001 was detected at very low levels (<1 %) in most samples. However, in samples containing low amounts of radioactivity, Region 001 was detected at more substantial levels (up to 8 %) and is probably due to non-specific binding of radioactivity at the origin of the TLC plate. Regions 004 (which co-chromatographed with AMPA reference standard) and 002 were either not detected or were only present at low levels (<2 %) in the majority of samples. The remaining radioactivity was located in diffuse areas.

<u>TLC solvent System 2</u>
One major region of radioactivity (Region 002, ca. 81 - 100 % of total radioactivity in pooled sample, R_f ca. 0.20 to 0.30) was detected. This co-chromatographed with non-labelled glyphosate reference standard. Up to two minor regions of radioactivity were also observed (Region 001, ca. 0 - 18 %, R_f ca. 0.00; Region 003, ca. 0 - 3 %, R_f ca. 0.30 - 0.40). Region 001 was detected at very low levels (<1 %) in most samples. However, in samples containing low amounts of radioactivity, Region 001 was detected at more substantial levels (up to 18 %) and is probably due to non-specific binding of radioactivity at the origin of the TLC plate. Region 003 (which co-chromatographed with AMPA reference standard) was either not detected or was only present at low levels (<2 %) in the majority of samples. The remaining radioactivity was located in diffuse areas.

<u>Identification of glyphosate isolated from urine and faeces extracts</u>
The FT-IR spectra obtained, when the major region of radioactivity isolated from both pooled urine and pooled faecal extracts was analysed, showed virtually identical characteristics to the spectra produced when non-labelled and [^{14}C]-glyphosate standards were analysed.

III. CONCLUSIONS

After a single oral dose of 30 mg/kg bw of [^{14}C]-glyphosate, ca. 34 - 36 % were absorbed. The absorption profile did not change on repeated daily administration of 30 mg/kg bw or at a single higher dose level of 1000 mg/kg bw. Most of the dose was voided in faeces reflecting the fraction not absorbed. After intravenous administration, renal excretion was rapid and predominated. Tissue residues were low, higher concentrations in bone were noted. Concentrations in bones increased with dose but not with frequency of dosing. The pattern of absorption, distribution and excretion of [^{14}C]-glyphosate appeared to be independent of sex, and no radiolabelled metabolites were identified in urine or faecal samples.

3. Assessment and conclusion

<u>Assessment and conclusion by applicant</u>:

A single intravenous or oral dose of [^{14}C]-glyphosate at 30 mg/kg bw, a single oral dose of 1000 mg/kg bw, or multiple doses (14 daily oral doses of glyphosate followed by a single oral dose of [^{14}C]-glyphosate on Day 15) at 30 mg/kg bw were administered to Sprague-Dawley rats.

<u>Excretion:</u>
Seven days after single oral low dose administration (30 mg/kg bw) 57 - 59 % and 29 - 31 % of the

dose was excreted in faeces and urine (excluding cage wash), respectively. After single oral administration of high dose (1000 mg/kg bw) 53 - 60 % and 23 - 31 % of the dose was excreted in faeces and urine (excluding cage wash), respectively. After multiple doses (14 daily oral doses of glyphosate (30 mg/kg bw/d) followed by a single oral dose (30 mg/kg bw) of [^{14}C]-glyphosate on Day 15), the excretion was 47 - 50 % in faeces and 34 - 35 % in urine (excluding cage wash). After single intravenous dosing at 30 mg/kg bw, 1.5 - 3.4 % and 84 - 86 % of the dose was excreted in faeces and urine (excluding cage wash), respectively.

Absorption and Distribution:
Considering cage wash to calculate the total urinary excretion 92.9 and 97.4 % of the applied radioactivity was excreted in urine by males and females upon intravenous application, respectively. Comparison of mean urinary excretion of radioactivity following single intravenous and single oral administration (30 mg/kg bw) resulted in an oral absorption rate of about 35 % in males and females, respectively. Similar absorption was estimated for multiple and high dose oral administration, indicated by similar proportion of faecal elimination after single low/high and multiple dosing. A high faecal elimination after oral dosing suggests that the rat has limited capacity for absorption of glyphosate since only a small proportion was eliminated in faeces after intravenous dosing. Tissue residues were low; however higher tissue concentrations for bone was noted.

Metabolites:
Chromatographic evaluation of rat urine and faeces indicated that no radiolabelled metabolites of [^{14}C]-glyphosate were detected. The identity of the radioactive component as glyphosate was confirmed by spectrometric method; the same single component was observed after single intravenous and oral doses at 30 and 1000 mg/kg bw and after multiple oral doses at 30 mg/kg bw/d, suggesting there was no change in the pharmacokinetics of glyphosate with time, dose or dose route. Only low levels (<2 %) of radioactivity co-chromatographed with the AMPA reference standard.

Conclusion:
After oral administration of [^{14}C]-glyphosate to rats, there were no differences in the absorption and disposition of radioactivity between 30 and 1000 mg/kg bw doses or after multiple daily oral doses of 30 mg/kg bw/d. Faecal excretion was the major route of elimination (probably unabsorbed compound). Approximately 35 % was absorbed from the gut and subsequently eliminated in urine. Absorption was similar in both sexes. Absorption and excretion appeared to be dose level (30 and 1000 mg/kg bw) and dose frequency (15 daily doses) independent. About 46 - 59 % of the orally administered dose(s) are excreted in faeces and 29 - 35 % are excreted in urine. Total tissue residues (carcass plus whole tissues) after 7 days was <1 % of the administered dose after single or multiple dosing. Radioactive residues showed a high affinity for bone. Sex differences in the rat were not apparent. No radiolabelled metabolites were identified in urine or faecal samples. Only low levels (<2 %) of radioactivity co-chromatographed with the AMPA reference standard. After administration by either dose route, dose level or after multiple dosing there were no apparent sex-linked differences in the absorption, distribution, metabolism and excretion of [^{14}C]-glyphosate. There was no indication for bioaccumulation of glyphosate after single or multiple oral dosing. No metabolites of [^{14}C]-glyphosate were detected.

Investigation of pharmacokinetics in plasma was not in scope of this study.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.1.1/013
Report author	[redacted]
Report year	1990
Report title	Report on metabolism (Absorption, tissue distribution & excretion with radio-active compounds – rats) of glyphosate
Report No	1087
Document No	Not reported
Guidelines followed in study	None reported
GLP/Officially recognised testing facilities	No, not conducted under GLP
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations	Two Wistar rats per dose and time point received a single dose by gavage at 1000 and 2000 mg/kg bw [^{3}H]-labelled glyphosate, respectively. The fed animals had free access to food and water. Urine and faeces was collected separately. The animals were sacrificed after 2, 4, 8, 24 and 48 h and 7th and 15th days after feeding and urine, faeces and blood were collected. At necropsy adrenals, brain, heart, kidney, liver and spleen were removed. Radioactivity in extracted samples was detected by Liquid Scintillation Counting.
Short description of results	Highest radioactivity was observed in faeces and urine at both dose levels within 24 h after application. Radioactivity in blood and tissues was highest after 8 h at the low and high dose and decreased over time. Glyphosate is poorly absorbed and levels of glyphosate in different tissues are negligible after 15 days of oral feeding.
Reasons for why the study is not considered relevant/reliable or not considered as key study	The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance" (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL.
Category study in AIR 5 dossier (L docs)	Category 4b

1. Information on the study

Data point	CA 5.1.1/014
Report author	[redacted]
Report year	1988
Report title	The Metabolism of Glyphosate in Sprague Dawley Rats - Part I. Excretion and Tissue Distribution of Glyphosate and Its Metabolites Following Intravenous and Oral Administration
Report No	[redacted]-7215
Document No	Not reported
Guidelines followed in study	None reported

Deviations from current test guideline	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes (without certificate). However, when the study was performed, GLP was not compulsory.
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive summary

The absorption, distribution, metabolism and excretion of oral and intravenously administered [^{14}C]-glyphosate have been investigated in male and female Sprague Dawley rats after a single intravenous and oral dose (10 mg/kg bw), a single oral dose (1000 mg/kg bw) and after 15 daily oral doses (10 mg/kg bw/day). Samples were collected for a period of up to 7 days after (last) administration. At sacrifice excreta, tissues, organs and residual carcasses were analysed for radioactivity. Radioactive tissue residues were determined after at least 90 % of the oral and intravenous dose, respectively had been excreted.

The radioactivity in expired gases trapped for 24 h after single intravenous dosing at 10 mg/kg bw was <0.2 % of the applied dose.

Seven days after intravenous dosing at 10 mg/kg bw, 74.5 - 79.0 % of the dose was excreted in the urine (excluding cage wash) and 4.65 - 8.3 % in faeces; less than 0.1 % of the dose was found in the organs and approximately 1 % of the dose in the residual carcass. The total recovery in this experiment was 86 % for males and 85 % of the applied dose for females.

Faeces was the major elimination route after oral dosing at 10 mg/kg bw (62.4 and 69.4 % of the applied dose for males and females, respectively) and at 1000 mg/kg bw (68.9 and 69.4 % of the applied dose for males and females, respectively); urine accounted for 28.6 %/17.8 % for males and 22.5 %/14.3 % for females of the low/high dose, respectively. Less than 0.05 % of the applied dose appeared in the organs following oral dosing and less than 0.5 % remained in the residual carcass. Multiple dosing at 10 mg/kg bw/day had no significant effect on the routes of excretion of [^{14}C]-glyphosate nor on the percent of dose remaining in the organs, tissues and residual carcass at sacrifice.

Radiochemical analysis of individual tissues demonstrated that bone contained the highest relative concentration of [^{14}C]-glyphosate equivalents (0.3 - 31 ppm). It was estimated that only 0.2 - 0.6 % of the applied dose was associated with this site after oral dosing and ca. 1 % following intravenous dosing. The remaining tissues contained between 0.0003 and 11 ppm of glyphosate equivalents. For the bone and some highly perfused tissues, the males contained statistically higher levels than the females.

The half-life of the alpha elimination phase varied between 2.11 and 7.52 h and the beta phase was 69 to 337 h. The half-life of the high dose males was found to be significantly longer than the low dose males. Multiple dosing at the low dose had no significant effect on the whole body elimination.

Plots of blood concentration versus time were used to calculate the area under the curve and estimate the oral absorption of glyphosate. In this manner the percent absorption was found to be 30.3 % for males and 35.4 % for females. This compared favourably with the percent absorption of glyphosate calculated from the oral and intravenous urine data (36.2 % for males and 30.2 % for females).

I. MATERIALS AND METHODS

A. MATERIALS

1. Non-labelled test material:

Identification:	Glyphosate (N-(phosphonomethyl)glycine)
Description:	Not reported
Lot/Batch #:	Not reported
Purity:	99.8 %
Stability of test compound:	Not reported

2. Radiolabelled test material:

Identification:	[^{14}C]-Glyphosate
Position of radiolabel:	N-(phosphono[^{14}C]methyl)glycine
Lot/Batch #:	Not reported
Radiochemical purity:	>99 %
Specific activity:	5.3 x 10^4 dpm/µg (Groups 1 - 3, 5 - 7) 5.3 x 10^2 dpm/µg (Group 4, high dose group)
Stability of test compound:	Not reported

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley (Crl:CD BR)
Source:	[redacted]
Age:	7 - 14 weeks at the time of dosing
Sex:	Males and females
Weight at dosing:	148 - 336 g
Acclimation period:	At least 7 days
Diet/Food:	Purina Rat Chow pellets from Ralston Purina (St. Louis, MO), *ad libitum*. Groups 2 - 7: fasted overnight prior to dosing with radioactive glyphosate
Water:	Tap water, *ad libitum*
Housing:	Group 1: Roth-type metabolism units in order to measure expired gases. The cage body of the unit was 17 cm in diameter. An inverted glass funnel was suspended above the faecal collector to deflect urine. The feeding tube was separated from the cage unit by a piece of ½ inch screen. A glass wool filter was used to remove particulates from incoming air. The airflow was maintained at 24 to 30 L per hour. Groups 2 - 7: stainless steel suspension metabolism cages with a mesh screen for separating urine from faeces.
Environmental conditions:	Temperature: Not reported Humidity: Not reported Air changes: Not reported 12-hour light/dark cycle

B. STUDY DESIGN

In life dates: not reported

Animal assignment and treatment

Group	Rats per sex	Route of dosing*	Frequency of dosing	Dose level (mg/kg bw)	Mean dose in DPM	Investigations	Sampling period
1	3	Oral	Single	10	1.15×10^8	Expired gases, urine and faeces	24 h after dosing
2	3	Oral	Single	10	1.11×10^8	Blood at frequent intervals	5 d post dosing and at sacrifice after 7 d
3	5	i.v.	Single	10	1.34×10^8	Urine faeces and tissues	6, 12, 24 h after dosing and then at 24 h intervals until necropsy on Day 7
4	5	Oral	Single	1000	1.20×10^8		
5	5	Oral	Single	10	8.99×10^7		
6	5	Oral	Multiple**	10	1.48×10^8		
7	3	i.v.	Single	10	1.34×10^8	Blood at frequent intervals	5 d post dosing and at sacrifice after 7 d

* oral gavage or IV injection to lateral tail vein
** 14 consecutive daily doses of glyphosate followed by radiolabelled glyphosate
DPM disintegrations per minute

Animal assignment and treatment: Intravenous application (Groups 3 and 7)

Depending on the group assigned three or five rats per sex received intravenous applications of 10 mg [^{14}C]-labelled glyphosate/kg bw into the lateral tail vein. The dose was applied in a volume of 0.19 to 0.28 mL and animals were housed in stainless steel metabolism cages. From the five rats per sex of Group 3 urine and faeces were sampled 6, 12, and 24 h after dosing and then at 24 h intervals until necropsy on Day 7. At necropsy following tissues/organs were taken or sampled for radioactivity measurements: Bone, bone marrow, brain, gastrointestinal tract, fat (abdominal; testicular/ovarian), eye, gonads, heart, kidney, liver, lungs, muscle (abdominal/shoulder), nasal mucosa, plasma, spleen, tail, thyroid, uterus, whole blood, red blood cells and residual carcass.

From the three rats per sex of Group 7 blood was collected at 0.25, 0.50, l, 2, 4, 6, 8, 12, 24, 48, 72, 120 h after treatment. At sacrifice, 168 h after dosing, larger blood samples (approximately 5 - 10 mL) were collected from the posterior vena cava of all animals. No urine or faeces were collected from animals of Group 7.

Animal assignment and treatment: Oral application (Groups 1, 2 and 4 - 6)

Depending on the group assigned three or five fasted rats per sex were treated with the prepared testing solutions per gavage. Rats receiving repeated applications for 14 consecutive days (Group 6) received daily doses of glyphosate followed by radiolabelled glyphosate per gavage.

Following treatment with 10 mg [^{14}C]-labelled glyphosate/kg bw, the rats of Group 1 were housed in Roth metabolism cages allowing collection of expired air for 24 h. At 6, 12 and 24 h after dosing, urine, faeces and expired gases were collected. Cage washes were taken after the final collection period.

Following gavage application of 10 mg [^{14}C]-labelled glyphosate/kg bw blood was collected from the three rats per sex of Group 2 at 0.25, 0.50, l, 2, 4, 6, 8, 12, 24, 48, 72, 120 h after dosing. At sacrifice, 168 h after dosing, larger blood samples (approximately 5 - 10 mL) were collected from the posterior vena cava of all

animals. No urine or faeces were collected from animals of Group 2.

Urine and faeces from rats of the dose Groups 4 to 6 were sampled 6, 12, and 24 h after dosing and then at 24 h intervals until necropsy on Day 7. At necropsy following tissues/organs were taken or sampled for radioactivity measurements: Bone, bone marrow, brain, gastrointestinal tract, fat (abdominal; testicular/ovarian), eye, gonads, heart, kidney, liver, lungs, muscle (abdominal/shoulder), nasal mucosa, plasma, spleen, tail, thyroid, uterus, whole Blood, red blood cells and residual carcass.

Dosing Formulation Analysis

The test material for this study was a mixture of unlabelled and [^{14}C]-labelled glyphosate. The dosing solutions were prepared by dissolving the test material in sterile saline. The disintegration per minute per gram of the dose solution was determined by diluting weighed aliquots with saline and then counting weighed amounts of these diluted solutions.

Measurement of radioactivity

Urine was collected and kept chilled by a circulating water bath. Fresh urine samples were prepared for liquid scintillation counting (LSC) by adding approximately 0.03 - 0.21 mL of sample to 15 mL Instagel® prior to counting. 1 - 2 mL of water or 0.5 N ammonium bicarbonate were added to these samples before counting to prevent glyphosate from precipitating out of the counting solution. The faecal samples were combined with approximately an equal volume of distilled water, homogenized and weighed. Portions (0.05 - 0.40 g) of the homogenized faecal material were transferred to combustion cones lined with a Combustopad, capped with a Combustopad and combusted in a Packard Tricarb B306 Sample Oxidizer using a 1 - 2 minute burn cycle. Samples were run in duplicate. A [^{14}C]-calibration standard and an instrument memory check were run after every twenty experimental samples in order to monitor instrument performance. Whole blood, plasma and blood cells were pipetted onto Combustopads, weighed and then capped with another pad and combusted in the Packard sample oxidizer. Tissues collected at necropsy were combusted or solubilized and duplicate samples processed for LSC. Individual carcasses were frozen, freeze-fractured, lyophilized and ground in a blender. Five aliquots of the resulting powder were combusted for LSC.

Liquid Scintillation Counting

All samples were counted with a Mark III Liquid Scintillation Counter (Model 6882, TM Analytic). Counting efficiencies were determined by external standardization and disintegration per minute by the Mark III DPM calculation accessory using a set of Amersham quench standards. Aqueous samples prepared in Instagel® were counted with efficiencies of 77 - 87 %. Solubilized samples prepared in Dimilume® were counted with efficiencies of 84 - 88 %, while combusted samples had efficiencies of 67 - 78 %.

Data Processing and Statistical Procedures

The liquid scintillation counter data were processed by a computer program written by the data processing group at the Environmental Health Laboratory. In these programs each sample is assigned a unique identification that consists of the EHL study number, a treatment group number, excreta or tissue code, a time interval and a replicate number. The data were assembled, stored and processed by the computer system. The standard error of the mean (SEM) and the Student's t-test were performed to determine the significance of differences between sexes and dose levels.

Kinetic Analysis and Measurement of Absorption

The urine and faecal elimination data were analysed using the "sigma-minus" plotting method. This method is a semi-logarithmic plot of the differences between the percent of dose recovered and the cumulative percent eliminated (urine plus faeces) on the logarithmic scale against time on the linear scale. It is used to

characterise the kinetics of whole body elimination. The blood data for Groups 2 and 7 were processed by a computer program written in the Statistical Analysis System (SAS) which utilizes the trapezoidal rule. Straight line segments were drawn between adjacent experimental points of the graph of blood concentration versus time, from time 0 to time t. The area under this curve was obtained by summing the areas of all the trapezoids formed. The extrapolated area under the curve from time t to infinity was also calculated and added to the previous area. The area under the curve (AUC) was the sum of these two areas. The first method of determination of oral absorption was by comparison of AUCs (AUC_{oral} /AUC_{iv} multiplied by $Dose_{iv}/Dose_{oral}$). The second method of determination of oral absorption consisted of division of the extent of urinary excretion of radiolabel following oral administration by the extent of urinary excretion of radiolabel following intravenous administration (% of dose in urine (oral) x 100 divided by % of dose in urine (i.v.)).

II. RESULTS AND DISCUSSION

A. EXCRETION AND RETENTION OF RADIOACTIVITY

Single bolus intravenous dose at 10 mg/kg bw (Group 3):
79.0 and 74.5 % of the applied dose was excreted in the urine of males and females, respectively. 4.65 and 8.3 % of the applied dose was excreted in the faeces of males and females, respectively. Less than 0.1 % of the dose was found in the organs taken at necropsy with approximately one percent of the dose remaining in the residual carcass.

Single oral dose at 10 mg/kg bw (Group 5):
62.4 and 69.4 % of the dose was excreted in faeces of males and females, respectively. 28.6 and 22.5 % in the urine of males and females, respectively. Less than 0.05 % of the dose appeared in the organs and less than 0.5 % remained in the residual carcass.

Single oral dose at 1000 mg/kg bw (Group 4):
68.9 and 69.4 % of the applied dose was excreted in faeces of males and females, respectively. 17.8 and 14.3 % of the applied dose was excreted in the urine of males and females, respectively. Less than 0.05 % of the dose appeared in the organs and less than 0.5 % remained in the residual carcass.

Multiple oral dose at 10 mg/kg bw/day (Group 6):
Repeated dosing at 10 mg/kg bw/day had no significant effect on the routes of excretion of [^{14}C]-glyphosate compared to the single dose application. 61.0 and 70.9 % of the applied dose was excreted in faeces of males and females, respectively and 30.9 and 23.1 % in the urine of males and females, respectively.

Table 5.1-38: The Metabolism of Glyphosate in Sprague Dawley Rats - Part I. Excretion and Tissue Distribution of Glyphosate and Its Metabolites Following Intravenous and Oral Administration ([redacted] 1988): Mean (±SEM) recovery of radioactivity (% of applied dose) following administration of [^{14}C]-glyphosate to the rat

Sample		Group 1 Oral dose* 10 mg/kg bw		Group 3 i.v. dose* 10 mg/kg bw		Group 4 Oral dose* 1000 mg/kg bw		Group 5 Oral dose* 10 mg/kg bw		Group 6 Oral dose** 10 mg/kg bw/d	
		M	F	M	F	M	F	M	F	M	F
		0 - 24h#		*0 - 168h*							
Urine		12.2	11.1	79.0	74.5	17.8	14.3	28.6	22.5	30.9	23.1
	±	3.0	2.6	5.9	3.3	2.2	1.3	1.8	3.0	4.3	4.3
Faeces		68.6	70.4	4.65	8.30	68.9	69.4	62.4	69.4	61.0	70.9
	±	5.9	5.6	0.93	1.4	2.1	3.0	2.8	3.1	3.5	5.6
CO2		0.137	0.154	Not assessed, as <0.2 % of the dose found in group 1							
	±	0.028	0.030								

Table 5.1-38: The Metabolism of Glyphosate in Sprague Dawley Rats - Part I. Excretion and Tissue Distribution of Glyphosate and Its Metabolites Following Intravenous and Oral Administration ([redacted] 1988): Mean (±SEM) recovery of radioactivity (% of applied dose) following administration of [^{14}C]-glyphosate to the rat

Sample		Group 1 Oral dose* 10 mg/kg bw		Group 3 i.v. dose* 10 mg/kg bw		Group 4 Oral dose* 1000 mg/kg bw		Group 5 Oral dose* 10 mg/kg bw		Group 6 Oral dose** 10 mg/kg bw/d	
		M	F	M	F	M	F	M	F	M	F
		0 - 24h#		*0 - 168h*							
Cage wash		0.539	0.526	0.890	1.30	3.86	8.00	1.30	1.96	0.820	1.96
	±	0.21	0.17	0.28	0.29	0.46	1.3[b]	0.23	0.24	0.18	0.79
Organs/Tissues		Not assessed		0.094	0.052[b]	0.036	0.027	0.046	0.0194[b]	0.047	0.031
	±			0.011	0.005	0.003	0.004	0.003	0.0022	0.0044	0.006
Tail				0.172	0.161	Not assessed as oral dosing					
	±			0.043	0.032						
Residual carcass				1.18	1.04	0.248	0.208	0.395	0.286[c]	0.497	0.315
	±			0.096	0.040	0.046	0.024	0.009	0.032	0.064	0.067
Gastrointestinal contents				0.039	0.039	0.026	0.043	0.023	0.015	0.014	0.010
	±			0.010	0.015	0.008	0.028	0.004	0.002	0.003	0.005
Total tissue* **				**1.485**	**1.292**	**0.31**	**0.28**	**0.46**	**0.32**	**0.56**	**0.36**
Total recovery		**81.4**	**82.8**	**86.0**	**85.3**	**90.9**	**92.1**	**92.8**	**94.2**	**93.3**	**96.3**
	±	**8.8**	**4.8**	**5.6**	**3.8**	**1.4**	**1.2**	**0.94**	**0.78**	**1.4**	**1.9**

[#] Sampling period
* Single dose
** Multiple dose (non-radiolabelled glyphosate for 14 days followed by [^{14}C]-glyphosate
*** Carcass plus whole tissues/gastrointestinal contents
SEM = standard error of the mean
[(b)]significantly different from males by Student's t-test, $p < 0.01$ or $< p \leq 0.05$

B. DISTRIBUTION OF RADIOACTIVITY IN TISSUE

Bone contained the highest relative concentration of [^{14}C]-glyphosate equivalents (0.3 - 31 ppm) corresponding to approximately 0.2 - 0.6 % of the applied dose after oral dosing and approximately 1 % after intravenous dosing. The remaining tissues contained between 0.0003 and 11 ppm of glyphosate equivalents. For the bone and some highly perfused tissues, the males contained statistically higher levels than the females. Repeated dose administration had no significant effect on the percent of dose remaining in the organs, tissues and residual carcass.

Table 5.1-39: The Metabolism of Glyphosate in Sprague Dawley Rats - Part I. Excretion and Tissue Distribution of Glyphosate and Its Metabolites Following Intravenous and Oral Administration ([redacted] 1988): Mean radioactivity in tissues and organs (ppm) following administration of [^{14}C]-glyphosate to the rat

Tissue	Group 3 i.v. dose* 10 mg/kg bw		Group 4 Oral dose* 1000 mg/kg bw		Group 5 Oral dose* 10 mg/kg bw		Group 6 Oral dose** 10 mg/kg bw/day	
	M	F	M	F	M	F	M	F
Whole Blood	0.019	0.010[b]	0.328	0.166[b]	0.005	0.003[b]	0.005	0.003[b]
Blood Plasma	0.003	0.003	0.129	0.127	0.002	0.001[c]	0.002	0.002
Red Cells	0.032	0.014[b]	0.517	0.275[b]	0.009	0.004[b]	0.008	0.005[c]
Liver	0.104	0.050[b]	1.91	1.37	0.030	0.014[b]	0.041	0.026
Eye	0.016	0.010	0.655	0.590	0.002	0.0003	0.004	0.003
Brain	0.041	0.036	0.750	0.556	0.007	0.006	0.014	0.011
Kidney	0.106	0.071[c]	1.94	1.35[c]	0.022	0.013[c]	0.033	0.020

Table 5.1-39: The Metabolism of Glyphosate in Sprague Dawley Rats - Part I. Excretion and Tissue Distribution of Glyphosate and Its Metabolites Following Intravenous and Oral Administration ([redacted] 1988): Mean radioactivity in tissues and organs (ppm) following administration of [^{14}C]-glyphosate to the rat

Tissue	Group 3 i.v. dose* 10 mg/kg bw		Group 4 Oral dose* 1000 mg/kg bw		Group 5 Oral dose* 10 mg/kg bw		Group 6 Oral dose** 10 mg/kg bw/day	
	M	F	M	F	M	F	M	F
Spleen	0.044	0.032[c]	2.61	2.98	0.012	0.007	0.016	0.013
Lungs	0.103	0.079	1.54	1.13	0.015	0.012	0.021	0.017
Heart	0.026	0.017[c]	0.590	0.518	0.006	0.004[c]	0.008	0.006
Thyroid	0.022	0.023	1.50	1.24	0.001	0.001	0.007	0.010
Testes/Ovaries	0.018	0.022	0.363	0.572	0.003	0.003	0.005	0.008
Uterus	-	0.038	-	0.618	-	0.005	-	0.019
Nasal mucosa	0.074	0.040	1.71	1.79	0.005	0.023	0.032	0.013
Stomach	0.024	0.018	2.38	2.36	0.008	0.004[b]	0.038	0.024
Small Intestine	0.026	0.016[c]	1.90	1.55	0.022	0.018	0.044	0.026
Colon	0.035	0.018[c]	11.0	9.2	0.034	0.016[b]	0.043	0.030
Bone	1.48	1.59	30.6	19.7[c]	0.552	0.313[b]	0.748	0.462[c]
Bone marrow	0.069	0.030[c]	4.1	12.5	0.029	0.007	0.025	0.023
Abdominal muscle	0.008	0.006	0.262	0.214	0.002	0.002[b]	0.003	0.002
Shoulder muscle	0.011	0.033	0.419	0.423	0.004	0.007	0.008	0.006
Abdominal fat	0.005	0.004[c]	0.418	0.457	0.004	0.003	0.006	0.006
Testicular/ovarian fat	0.008	0.005	0.442	0.450	0.005	0.004	0.007	0.006
Tail	0.699	0.611	NS	NS	NS	NS	NS	NS
Residual carcass	0.344	0.337	8.27	7.74	0.106	0.087	0.157	0.101
Total								

* Single dose
** Multiple dose (non-radiolabelled glyphosate for 14 days followed by [^{14}C]-glyphosate
[(b)]significantly different from males by Student's t-test, $p \leq 0.01$ or [(c)] $p < 0.05$
All values rounded to 3 decimal places.

C. RATES OF ELIMINATION OF RADIOACTIVITY

The urine and faecal data of Groups 3 - 6 were used to estimate the kinetics of whole body elimination. Males and females of Groups 3 - 6 had alpha half-lives of 2.1 - 7.5 and 5.0 - 6.4 h, respectively. The beta half-lives ranged from 69.0 - 189 h for males and 79.9 - 337 h for females. Mean half-life are presented in the table below. The half-life of the high dose males was found to be significantly longer than the low dose males. Pre-treatment with multiple low doses had no significant effect on whole body elimination.

Table 5.1-40: The Metabolism of Glyphosate in Sprague Dawley Rats - Part I. Excretion and Tissue Distribution of Glyphosate and Its Metabolites Following Intravenous and Oral Administration [redacted] 1988): Mean half-life of whole body elimination of radioactivity

PK parameter	Group 2 Oral dose* 10 mg/kg bw		Group 3 i.v. dose* 10 mg/kg bw		Group 4 Oral dose* 1000 mg/kg bw		Group 5 Oral dose* 10 mg/kg bw		Group 6 Oral dose** 10 mg/kg bw/day		Group 7 i.v. dose* 10 mg/kg bw	
	M	F	M	F	M	F	M	F	M	F	M	F
Alpha rate constant [$h^{-1} \times 10^{-2}$]	-	-	35.0	15.9	13.9	12.6	12.3	13.6	9.60	11.6	-	-
Alpha half-life [h]	-	-	2.11	5.00	5.26	6.44	5.87	6.22	7.52	6.14	-	-
Beta rate constant	-	-	10.9	9.54	4.43	2.86	8.88	7.15	9.41	5.78	-	-

Table 5.1-40: The Metabolism of Glyphosate in Sprague Dawley Rats - Part I. Excretion and Tissue Distribution of Glyphosate and Its Metabolites Following Intravenous and Oral Administration ([redacted] 1988): Mean half-life of whole body elimination of radioactivity

PK parameter	Group 2 Oral dose* 10 mg/kg bw		Group 3 i.v. dose* 10 mg/kg bw		Group 4 Oral dose* 1000 mg/kg bw		Group 5 Oral dose* 10 mg/kg bw		Group 6 Oral dose** 10 mg/kg bw/day		Group 7 i.v. dose* 10 mg/kg bw	
	M	F	M	F	M	F	M	F	M	F	M	F
[h^{-1} x 10^{-3}]												
Beta half-life [h]	-	-	69.0	79.9	181	337	79.0	106	75.2	146	-	-
AUC [µg min/mL]	245	226	-	-	-	-	-	-	-	-	849	662

* Single dose
** Multiple dose (non-radiolabelled glyphosate for 14 days followed by [^{14}C]-glyphosate
PK Pharmacokinetics
Data fit to a bi-exponential function

D. DETERMINATION OF ABSORPTION

Whole blood radiochemical concentrations were used to calculate the ppm of glyphosate or its equivalents. Semi-logarithmic plots of ppm glyphosate equivalents versus time were constructed and the AUCs were estimated. Using AUCs for calculation, the oral absorption of glyphosate for the males was 30.4 % and the oral absorption for the females was 35.4 %. The percent absorption of glyphosate calculated from the oral and intravenous urine data was 36.2 % for males and 30.2 % for females.

E. RECOVERY OF RADIOACTIVITY

More than 90 % of the administered radioactivity was recovered within 7 days in the low and the high dose groups (Groups 4 to 6) after single and multiple oral application of the test substance. After single intravenous dosing >85 % of the administered radioactivity was recovered (Group 3) and the total recovery in the low dose experiment terminated after 24 h was about 82 % (Group 1). No total recovery for the kinetic experiments (Groups 2 and 7) was derived.

III. CONCLUSIONS

The results of this study demonstrate that glyphosate is poorly absorbed and rapidly eliminated following a single oral dose at 10 or 1000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:

A single intravenous or oral dose of [^{14}C]-glyphosate at 10 mg/kg bw, a single oral dose of 1000 mg/kg bw, or multiple doses (14 daily oral doses of glyphosate followed by a single oral dose of [^{14}C]-glyphosate on Day 15) of 10 mg/kg bw/day were administered to Sprague-Dawley rats.

Excretion
Seven days after single oral administration, about 62 - 69 % of dose(s) were excreted in faeces and 14 - 29 % were excreted in urine. Seven days after single intravenous dosing about 5 - 8 % of the dose were excreted in faeces and 75 - 79 % of the dose in urine.

Absorption
Two methods were used to calculate the oral absorption rate. For both the oral absorption of glyphosate was between 30 - 36 % after single and multiple administration.

Distribution
Total tissue residues (carcass plus whole tissues and gastrointestinal content) 7 days after oral dosing was <0.6 % after single or multiple low or high dosing. A higher affinity for bone was noted. There was no indication for bioaccumulation of glyphosate after single or multiple oral dosing.

Metabolism
The alpha plasma half-life (rate of decline in plasma) ranged between 5.3 - 7.5 h after oral administration irrespective of dose level and number of dosing. The alpha plasma half-life after i.v. application ranged between 2 to 5 h. The beta half-lives (rate of decline due metabolism/elimination processes) ranged from 75.2 - 79 h for males and 106 - 146 h for females after single and repeated oral dosing of 10 mg/kg bw. Beta half-lives after i.v. application of 10 mg/kg bw were compared to the beta half-lives after oral application (69 and 79 h in males and females, respectively). Beta half-lives at the high dose were 181 and 337 h for males and females, respectively. Metabolites of glyphosate were not assessed in this part of the study. Characterisation of the radioactivity is described in Part II of this study.

Conclusion:
Absorption and excretion of radioactivity in rats after oral administration was neither dose nor gender depended. Irrespective of dose and gender faecal excretion was the major route of elimination and reflected probably unabsorbed compound. Only approximately 30 - 36 % of the applied radioactivity was absorbed from the gut and subsequently eliminated in urine. Absorption and excretion of [^{14}C]-glyphosate was also independent from the application frequency (i.e. comparable between single and repeated daily applications for 15 consecutive days). Overall tissue residues were low and no indication for bioaccumulation of glyphosate after single or multiple oral dosing was observed. Highest radioactivity level was detected in bones.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.1.1/015
Report author	[redacted] *et al*.
Report year	1988
Report title	The Metabolism of Glyphosate in Sprague Dawley Rats - Part II. Identification, Characterization, and Quantitation of Glyphosate and Its Metabolites After Intravenous and Oral Administration
Report No	[redacted]-7206
Document No	Not reported
Guidelines followed in study	US-EPA FIFRA 85-1
Deviations from current test guideline	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes , however, when the study was performed GLP was not compulsory.
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive summary

A full range ADME (absorption, distribution, metabolism and excretion) study conducted in Sprague-Dawley rats was documented in two separate study reports. In Part I (please refer to Section CA 5.1.1/014) animal husbandry, dosing of glyphosate (oral and intravenous), excretion, distribution (measurement of residues in tissues and organs at sacrifice) and oral absorption rates was reported. In this part (Part II) of the study report details on the quantitation, CHARACTERISATION, and identification of glyphosate and its metabolites were provided.

[^{14}C]-Glyphosate was administered to male and female Sprague Dawley rats as single intravenous and oral dose at 10 mg/kg bw, as single oral dose at 1000 mg/kg bw and at 15 daily oral doses at 10 mg/kg bw/day. Samples were collected for a period of up to 7 days after (last) administration. At sacrifice excreta, tissues, organs and residual carcasses were analysed for radioactivity. Radioactive tissue residues were determined after at least 90 % of the oral dose had been excreted. Orally administered glyphosate was absorbed to the extent of 30 - 36 %.

[^{14}C]-Glyphosate was the predominant material found in the rat excreta as revealed by two different methods of HPLC analyses, cation exchange HPLC and ion pair HPLC. Glyphosate was isolated from urine (overall recovery of 81.3 %) and from faeces (overall recovery of 99.2 %) and was positively identified by different analytical methods. At least 97.5 % of the urine or faecal extract-contained radioactivity was [^{14}C]-glyphosate, in all of the individual rat excreta samples. The HPLC analyses indicated that glyphosate in the excreta accounted for >98.5 - 99.3 % of the administered dose. There was evidence for formation of 0.2 - 0.4 % aminomethylphosphonic acid (AMPA) from *in vivo* metabolism after oral administration of glyphosate at single or multiple low dose level. The remainder of the excreta-contained radioactivity was due to low-level impurities present in the dosing material or due to materials that formed during storage of the excreta samples by reaction of glyphosate with endogenous excreta components.

I. MATERIALS AND METHODS

A. MATERIALS

1. Non-labelled test material:

Identification:	Glyphosate (N-(phosphonomethyl)glycine)
Description:	Not reported
Lot/Batch #:	Not reported
Purity:	99.9 % (determined by strong anion exchange (SAX) HPLC assay) 99.81 % with 0.19 % AMPA present as impurity (determined by silylation of the glyphosate with N-(trimethylsilyl)diethylamine (TMSDEA) and GC analysis on a 3 % DC-200 GC column)
Stability of test compound:	Not reported

2. Radiolabelled test material:

Identification:	[^{14}C]-Glyphosate
Position of radiolabel:	N-(phosphono[^{14}C]methyl)glycine
Lot/Batch #:	Not reported
Radiochemical purity:	>99 %
Specific activity:	5.3 x 10^4 dpm/µg (Groups 1 - 3, 5 - 7) 5.3 x 10^2 dpm/µg (Group 4, high dose group)
Stability of test compound:	Not reported

3. Reference substances:

Identification:	[^{14}C]-Aminomethylphosphonic acid (AMPA)
Description:	Not reported
Lot/Batch #:	Not reported
Purity:	Not reported
Stability of test compound:	Not reported
Identification:	[^{14}C]-N-methylaminophosphonic acid (MAMPA)
Description:	Not reported
Lot/Batch #:	Not reported
Purity:	97.3 % (CX HPLC analysis)
Stability of test compound:	Not reported
Identification:	N-nitroso-glyphosate
Description:	Not reported
Lot/Batch #:	Not reported
Purity:	87 % (IPC analysis)
Stability of test compound:	Not reported

4. Test animals:

Species:	Rat
Strain:	Sprague-Dawley (Crl:CD BR)
Source:	[redacted]
Age:	7 - 14 weeks at the time of dosing
Sex:	Males and females
Weight at dosing:	148 - 336 g
Acclimation period:	At least 7 days
Diet/Food:	Purina Rat Chow pellets from Ralston Purina (St. Louis, MO), *ad libitum.* Groups 2 - 7: fasted overnight prior to dosing with radioactive glyphosate
Water:	Tap water, *ad libitum*
Housing:	Group 1: Roth-type metabolism units in order to measure expired gases. The cage body of the unit was 17 cm in diameter. An inverted glass funnel was suspended above the faecal collector to deflect urine. The feeding tube was separated from the cage unit by a piece of ½ inch screen. A glass wool filter was used to remove particulates from incoming air. The airflow was maintained at 24 to 30 L per hour. Groups 2 - 7: stainless steel suspension metabolism cages with a mesh screen for separating urine from faeces.
Environmental conditions:	Temperature: Not reported Humidity: Not reported Air changes: Not reported 12-hour light/dark cycle

B. STUDY DESIGN

In life dates: not reported

Animal assignment and treatment

A full range ADME (absorption, distribution, metabolism and excretion) study conducted in Sprague-Dawley rats was documented in two separate study reports. Details of animal husbandry and treatment, dosing of glyphosate (oral and intravenous), excretion, distribution (measurement of residues in tissues and organs at sacrifice) and oral absorption rates are provided in Section CA5.1.1/014). The study design is summarised below.

Group	Rats per sex	Route of dosing*	Frequency of dosing	Dose level (mg/kg bw)	Mean dose in DPM	Investigations	Sampling period
1	3	Oral	Single	10	1.15×10^8	Expired gases, urine and faeces	24 h after dosing
2	3	Oral	Single	10	1.11×10^8	Blood at frequent intervals	5 d post dosing and at sacrifice after 7 d
3	5	i.v.	Single	10	1.34×10^8	Urine faeces and tissues	6, 12, 24 h after dosing and then at 24 h intervals until necropsy on Day 7
4	5	Oral	Single	1000	1.20×10^8		
5	5	Oral	Single	10	8.99×10^7		
6	5	Oral	Multiple**	10	1.48×10^8		
7	3	i.v.	Single	10	1.34×10^8	Blood at frequent intervals	5 d post dosing and at sacrifice after 7 d

* oral gavage or IV injection to lateral tail vein
** 14 consecutive daily doses of glyphosate followed by radiolabelled glyphosate
DPM disintegrations per minute

Sampling

The identity of the radioactivity was characterised of urine and faeces sampled 6, 12, and 24 h after dosing and then at 24 h intervals until necropsy on Day 7 from rats of Groups 3 - 6.

Pooling of Rat Excreta for Analyses

For each rat in Group 3 (intravenous) and Groups 4 - 6 (oral), aliquots (10 % each) of the urine samples collected sequentially after dosing that contained together >94 % of the total radioactivity excreted during the entire collection period were pooled. Similar pooling of faecal samples was done. This gave pooled urine and pooled faecal samples for each rat of Groups 3 - 6. In addition, aliquots (10 % each) of the pooled urine samples prepared for each of the males in Group 3 were combined, giving a pooled male Group 3 urine sample. In identical fashion, pooled urine samples for females and males of Groups 3 to 6 were prepared.

Sample Preparation

Urine samples were analysed by HPLC without prior treatment.
Faecal samples were extracted with water. The extracts were cleaned up using C_{18} solid phase extraction cartridges, concentrated by rotary evaporation, and re-dissolved in the HPLC buffer prior to analysis. The percent of faecal-contained radioactivity that was extracted (normalized) was 83 - 91 % for Groups 3, 5, and 6. For Group 4, the high dose group, the extractabilities (normalized) were 96 - 98 %. Recoveries for the clean-up and concentration step were typically 96 - 100 %. Extraction of two Group 3 faecal samples with 1 N HC1 resulted in 99.9 %, extractability compared with 84.6 % extractability with just water, but

adjustment of the pH of the extract (after concentration and re-dissolution in HPLC mobile phase) to higher values appropriate for HPLC analyses resulted in precipitation of 90 % of the radioactivity. The precipitation of radioactivity was decreased to 50 % by incorporation of EDTA in the solvent. These results suggest that complexation of glyphosate with metal ions (or other endogenous materials that were not proteins, since proteins would not be soluble in 1 N HC1) occurs to a minor extent in faeces and that acid extraction dissolves the complexation agents. To avoid losses of radioactivity due to precipitation as the pH of the acid extract was raised by the HPLC mobile phase and to avoid metal poisoning of the cation exchange column employed for HPLC analyses as much as possible (a problem that occurred even with nonacidic extracts of faeces), water was employed for extractions of faecal samples.

HPLC Analyses of Excreta

Two different HPLC methods, CX HPLC and IPC, were employed for analysis of urine and faecal samples. Fraction collection of the HPLC effluent and liquid scintillation counting of the fractions (HPLC/ LSC) were employed in order to maximize sensitivity of detection of low level radioactive components. Column performance was monitored with radiolabelled standards and a radioactivity flow detector (RAD). CX HPLC on a Bio-Rad Aminex A-9 column was employed to analyse the pooled urine samples and pooled faecal extracts for each rat in Groups 3 - 6 and the pooled urine samples for each sex in Groups 3 - 6. IPC was used to analyse the pooled urine and pooled faecal extracts for each sex in Groups 3 - 6.

Isolation and Identification of Glyphosate from Urine and Faeces

Glyphosate was isolated from a rat urine sample collected 0 - 6 h after dosing by ion exchange chromatography. The isolated glyphosate (overall recovery of 81.3 %) was characterised by HPLC, negative ion FAB mass spectrometry, [^{1}H] and [^{31}P]-NMR, and by GC/MS analysis of the trifluoroacetic anhydride/trifluoroethanol (TFAA/TFE) derivative.
Glyphosate was isolated from an extract of a rat faecal sample collected 6 - 12 h after dosing by ion exchange chromatography. The isolated glyphosate (overall recovery of 99.2 %) was characterised by HPLC, negative ion FAB mass spectrometry, [^{1}H] and [^{31}P]-NMR, and GC/MS analyses of the TFAA/ TFE derivative.

Sample Stability Studies

Stability studies were conducted to determine the stability of (1) a mixture of glyphosate and a small amount of AMPA, a potential metabolite, in urine; of (2) glyphosate in urine and faeces; and of (3) glyphosate-derived metabolites in a urine sample collected from a dosed rat. Stability samples were analysed with CX HPLC and/or IPC. The stability of glyphosate, the test substance, was determined by spiking samples of control rat urine and faeces, with subsequent handling and storage as for the un-spiked samples.

HPLC setup

The HPLC instrumentation employed in this study consisted of various combinations of Waters Model U6K injectors, Model 440 UV detectors employed at 254 nm, a Waters Model 490 programmable multiwavelength detector, Waters Model 6000A HPLC pumps, Waters Model 510 hPLC pumps, Waters Model 680 automated gradient controllers, and a Waters Model 720 systems controller.

The dosing solutions used for the treatment of the rats of Groups 1 and 3 to 6 were analysed for AMPA and glyphosate content at the time of preparation by strong anion exchange (SAX) HPLC. Subsequently, better HPLC methods of analysis were developed; the unused dosing solutions for Groups 1 and 3 - 6 were analysed for several compounds by cation exchange (CX) HPLC and by ion pair HPLC (IPC). These HPLC methods allowed analysis for glyphosate (1), AMPA (2), MAMPA (3), N-formylglyphosate (4), N-acetylglyphosate (5), N-nitrosoglyphosate (6), N-methylglyphosate (7), methylphosphonic acid (8), hydroxymethylphosphonic acid (9), phosphonoformic acid (10), and an unknown compound (11).

Liquid Scintillation Counting

All samples were counted with a Mark III Liquid Scintillation Counter (Model 6881, TM Analytic).

Gas Chromatograph

A Varian Model 3700 gas chromatograph with a flame ionization detector was employed for analysis of the trifluoroacetic anhydride/trifluoroethanol derivatization reactions.

Combustor (Sample Oxidizer)

Samples were combusted using a Packard Tri-Carb sample oxidizer (Model 306).

Mass Spectrometers

Chemical ionization (Cl) and electron impact (El) mass spectra were obtained on a Finnigan 4535 quadrupole mass spectrometer with an INCOS data system. Fast atom bombardment (FAB) mass spectra were recorded on a VG ZAB-HF double-focusing mass spectrometer and processed with a Digital PDP 11/24 data system.

Nuclear Magnetic Resonance Spectrometer

NMR spectra ([^{1}H] and [^{31}P]) were obtained on a Varian XL-300 NMR spectrometer.

Data Processing and Statistical Procedures

In HPLC/LSC analyses of urine samples, the injected dpm value typically was 50,000. For a background count per minute (cpm) of 14, the threshold was ca. 20. Thus, ca. 8 dpm of sample (ca. 0.02 % of 50,000 injected dpm) in one sample vial could be detected. If the dpm for a metabolite were spread among two or more vials, the sensitivity would be less.
In CX HPLC/RAD analyses employing the 0.5 mL RAD cell the detection limits (at 2 times background) were 110 dpm for nonbasic compounds, 144 dpm for N-methylglyphosate, 201 dpm for MAMPA, and 228 dpm for AMPA (optimized MACS peak width parameter = 500).

II. RESULTS AND DISCUSSION

A. ANALYSES OF DOSING SOLUTIONS

The composition of dosing solutions were analysed by three different methods as shown in the table below. In all study groups, administered doses consisted of at least 98.2 % glyphosate. The main impurity in the dosing solution was AMPA.

Table 5.1-41: Metabolism of Glyphosate in Sprague Dawley Rats - Part II. Identification, CHARACTERISATION, and Quantitation of Glyphosate and Its Metabolites After Intravenous and Oral Administration ([redacted] 1988): HPLC analyses of dosing solutions

Sample	Method	Distribution [%]							HPLC recovery [%]
		Gly	AMPA	MAMPA	N-Ac-gly	N-F-gly	N-NO-gly	Unk.	
Group 1	SAX	>99.10	0.10		N/A				97.6
Oral dose*	CX	98.42	0.63	0.26	0.56				99.6
10 mg/kg bw	IPC	98.21	0.89		≤0.04	0.48	≤0.05	≤0.06	96.6
Group 3	SAX	>98.90	0.28		N/A				101.3
IV dose*	CX	99.33	0.36	0.00	0.32				99.2
10 mg/kg bw	IPC	99.14	0.39		≤0.02	0.36	≤0.01	0.03	99.2
Group 4	SAX	98.62	0.74		N/A				98.2

Oral dose*	CX	99.00	0.57	0.31	0.13				98.6
1000 mg/kg bw	IPC	98.88	0.83		≤0.03	0.14	≤0.02	0.04	99.7
Group 5	SAX	>9940	0.10		N/A				103.9
Oral dose*	CX	99.61	0.17	0.00	0.15				98.1
10 mg/kg bw	IPC	99.41	0.20		≤0.03	0.18	≤0.03	≤0.03	95.0
Group 6	SAX	>99.3	0.10		N/A				96.1
Oral dose**	CX	99.50	0.19	0.07	0.14				101.9
10 mg/kg bw/day	IPC	99.36	0.27		≤0.03	0.21	≤0.02	≤0.02	98.0

* Single dose
** Multiple dose (non-radiolabelled glyphosate for 14 days followed by [^{14}C]-glyphosate
Gly glyphosate
N-Ac N-acetyl
N-F N-formyl
N-NO N-nitroso
Unk. unknown
N/A not analysed
SAX strong anion exchange HPLC
CX cation exchange HPLC
IPC gradient ion pair HPLC

B. HPLC ANALYSES OF EXCRETA

Retention times of the radioactive components assigned as glyphosate and AMPA matched those of authentic synthetic standards both in the CX and IPC-HPLC analyses.

CX HPLC Analyses

All the pooled samples for the individual rats were analysed by CX HPLC. The analysis of dosing solutions, the metabolite levels as percentages of distribution and recovery rates are given in the table below.

Table 5.1-42: Metabolism of Glyphosate in Sprague Dawley Rats - Part II. Identification, CHARACTERISATION, and Quantitation of Glyphosate and Its Metabolites After Intravenous and Oral Administration ([redacted] 1988): Mean metabolite concentration in % of urine/faeces-contained radioactivity (CX HPLC)

	Sample		Distribution [%]				HPLC recovery of injected dpm [%]
			Gly	AMPA	MAMPA	Non-basic compounds	
Group 3 i.v. dose* 10 mg/kg bw	*Dose*		*99.33*	*0.36*	*ND*	*0.32*	*99.2*
	Urine	M	99.74	0.17	ND	0.08	97.3
		F	99.56	0.23	ND	0.19	96.9
	Faeces	M	98.78	0.66	ND	0.47	94.6
		F	99.05	0.50	ND	0.27	96.3
Group 4 Oral dose* 1000 mg/kg bw	*Dose*		*99.00*	*0.57*	*0.31*	*0.13*	*98.6*
	Urine	M	98.12	0.76	0.30	0.69	97.2
		F	97.98	0.78	0.30	0.77	97.2
	Faeces	M	98.86	0.55	0.32	0.17	97.8
		F	98.78	0.61	0.31	0.20	98.5
Group 5 Oral dose* 10 mg/kg bw	*Dose*		*99.61*	*0.17*	*ND*	*0.15*	*98.1*
	Urine	M	99.33	0.26	ND	0.32	99.0
		F	99.21	0.25	ND	0.42	99.0
	Faeces	M	99.20	0.42	ND	0.25	99.8
		F	99.09	0.52	ND	0.26	97.7
Group 6 Oral dose** 10 mg/kg bw/day	*Dose*		*99.50*	*0.19*	*0.07*	*0.14*	*101.9*
	Urine	M	99.46	0.25	0.01	0.24	98.9
		F	99.14	0.27	0.02	0.44	99.1
	Faeces	M	98.78	0.74	ND	0.35	97.6
		F	98.76	0.74	ND	0.35	98.0

* Single dose

Table 5.1-42: Metabolism of Glyphosate in Sprague Dawley Rats - Part II. Identification, CHARACTERISATION, and Quantitation of Glyphosate and Its Metabolites After Intravenous and Oral Administration ([redacted] 1988): Mean metabolite concentration in % of urine/faeces-contained radioactivity (CX HPLC)

** Multiple dose (non-radiolabelled glyphosate for 14 days followed by [^{14}C]-glyphosate
Gly glyphosate
ND not detected
CX cation exchange HPLC
dpm disintegrations per minute
Analyses of the dosing solutions in italics

For calculations of the factors for conversion of percent of distribution to percent of dose, the radioactivity in the cage washes for Groups 3 and 5 - 6 (0.82 - 1.96 % of the dose) was assigned to urine and faeces in the same proportion as the radioactivity levels found in the urine and faeces in these groups. In Group 4, the radioactivity levels in the cage wash (4 - 8 % of the dose) were higher than in the other groups and appeared to be associated mainly with faecal contamination of the cage due to some minor diarrhoea in these high dose animals. Therefore, the radioactivity in the cage wash for this group was attributed to the faeces for the calculations.

Metabolite levels as percentages of administered radioactive dose are given in the table below.

Table 5.1-43: Metabolism of Glyphosate in Sprague Dawley Rats - Part II. Identification, CHARACTERISATION, and Quantitation of Glyphosate and Its Metabolites After Intravenous and Oral Administration ([redacted] 1988): Mean metabolite concentration as % of administered radioactivity (CX HPLC)

	Sample		Administered ^{14}C [%]			
			Gly	AMPA	MAMPA	Non-basic compounds
Group 3 i.v. dose* 10 mg/kg bw	*Dose*		*99.33*	*0.36*	*ND*	*0.32*
	Urine	M	92.44	0.16	ND	0.08
		F	88.08	0.20	ND	0.19
	Faeces	M	5.39	0.04	ND	0.47
		F	9.77	0.05	ND	0.27
Group 4 Oral dose* 1000 mg/kg bw	*Dose*		*99.00*	*0.57*	*0.31*	*0.13*
	Urine	M	19.21	0.15	0.06	0.14
		F	15.22	0.12	0.05	0.12
	Faeces	M	79.13	0.44	0.26	0.14
		F	83.01	0.51	0.26	0.17
Group 5 Oral dose* 10 mg/kg bw	*Dose*		*99.61*	*0.17*	*ND*	*0.15*
	Urine	M	31.01	0.08	ND	0.12
		F	24.16	0.06	ND	0.02
	Faeces	M	67.58	0.29	ND	0.17
		F	74.44	0.39	ND	0.20
Group 6 Oral dose* 10 mg/kg bw/day	*Dose*		*99.50*	*0.19*	*0.07*	*0.14*
	Urine	M	33.21	0.08	ND	0.08
		F	24.25	0.07	ND	0.11
	Faeces	M	65.12	0.49	ND	0.23
		F	74.14	0.56	ND	0.26

* Single dose
** Multiple dose (non-radiolabelled glyphosate for 14 days followed by [^{14}C]-glyphosate
Gly glyphosate
ND not detected
CX cation exchange HPLC
Analyses of the dosing solutions in italics

IPC analyses of the pooled male and pooled female urine and faecal samples for Groups 3 - 6 expressed as

distribution values, as well as the analytical values for the dosing solutions are given in the table below.

Table 5.1-44: Metabolism of Glyphosate in Sprague Dawley Rats - Part II. Identification, CHARACTERISATION, and Quantitation of Glyphosate and Its Metabolites After Intravenous and Oral Administration ([redacted] 1988): Mean metabolite concentrations in excreta (IPC HPLC) as % radioactivity counted in HPLC effluent

	Sample		Distribution [%]						HPLC recovery of injected dpm [%]
			Gly	AMPA	N-Ac-gly	N-F-gly	N-NO-gly	Unk.	
Group 3 i.v. dose* 10 mg/kg bw	*Dose*		*99.14*	*0.39*	*≤0.02*	*0.36*	*≤0.01*	*0.03*	*99.2*
	Urine	M	99.49	0.19	≤0.03	0.07	0.06	0.14	99.4
		F	99.18	0.22	0.07	0.07	0.06	0.40	99.9
	Faeces	M	98.57	0.59	0.00	0.00	0.32	0.44	99.8
		F	99.28	0.46	0.00	0.00	0.10	0.15	97.2
Group 4 Oral dose* 1000 mg/kg bw	*Dose*		*98.88*	*0.83*	*≤0.03*	*0.14*	*≤0.02*	*0.04*	*99.7*
	Urine	M	97.76	1.25	0.10	0.20	0.09	0.46	99.2
		F	97.71	1.39	≤0.05	0.25	0.09	0.33	100.6
	Faeces	M	98.64	0.82	≤0.03	≤0.04	0.13	0.16	98.6
		F	98.68	0.88	≤0.04	≤0.04	0.11	0.17	98.7
Group 5 Oral dose* 10 mg/kg bw	*Dose*		*99.41*	*0.20*	*≤0.03*	*0.18*	*≤0.03*	*≤0.03*	*95.0*
	Urine	M	99.05	0.32	≤0.05	0.12	0.11	0.31	105.1
		F	98.65	0.30	≤0.06	0.25	0.11	0.58	99.9
	Faeces	M	98.78	0.56	≤0.06	≤0.10	0.21	0.16	99.0
		F	98.23	0.64	≤0.05	≤0.09	0.22	0.16	98.2
Group 6 Oral dose** 10 mg/kg bw/day	*Dose*		*99.36*	*0.27*	*≤0.03*	*0.21*	*≤0.0.2*	*≤0.02*	*98.0*
	Urine	M	99.24	0.29	≤0.05	0.11	0.08	0.18	100.1
		F	98.84	0.26	≤0.04	0.12	0.15	0.51	100.3
	Faeces	M	98.31	0.90	≤0.06	≤0.10	0.24	0.17	99.0
		F	98.27	0.93	≤0.05	≤0.10	0.22	0.23	98.8

*single; **multiple; gly = glyphosate; N-Ac = N-acetyl; N-F = N-formyl; N-NO = n-nitroso; Unk. = unknown; IPC = gradiant ion pair HPLC, dpm = disintegrations per minute
Analyses of the dosing solutions in italics
* Single dose
** Multiple dose (non-radiolabelled glyphosate for 14 days followed by [^{14}C]-glyphosate
Gly glyphosate
N-Ac N-acetyl
N-F N-formyl
N-NO N-nitroso
Unk. unknown
IPC gradient ion pair HPLC
dpm disintegrations per minute
Analyses of the dosing solutions in italics

N-nitroso-glyphosate was present at 0.06 - 0.15 % of the urine-contained radioactivity and at 0.10 - 0.32 % of the faecal contained radioactivity. N-formyl-glyphosate was detected in urine at 0.07 - 0.25 % of the urinary contained radioactivity but not in faeces. AMPA concentrations in urine ranged from 0.26 - 1.39 % of the urine-contained radioactivity and from 0.56 - 0.93 % in faeces upon oral application.

Yields of the radiolabelled excreta components as percentages of administered radioactivity are given in the table below. The factors for conversion of distribution values to percent of dose values were the same as employed in CX HPLC.

Table 5.1-45: Metabolism of Glyphosate in Sprague Dawley Rats - Part II. Identification, CHARACTERISATION, and Quantitation of Glyphosate and Its Metabolites After Intravenous and Oral Administration [redacted] 1988): Mean metabolite concentrations as % of administered radioactivity (IPC HPLC)

	Sample		Administered ^{14}C [%]					
			Gly	AMPA	N-Ac-gly	N-F-gly	N-NO-gly	Unk.
Group 3 i.v. dose* 10 mg/kg bw	*Dose*		*99.14*	*0.39*	*≤0.02*	*0.36*	*≤0.01*	*0.03*
	Urine	M	92.21	0.18	≤0.03	0.06	0.06	0.13
		F	87.74	0.19	0.06	0.06	0.05	0.35
	Faeces	M	5.38	0.03	0.00	0.00	0.02	0.02
		F	9.79	0.05	0.00	0.00	0.01	0.01
Group 4 Oral dose* 1000 mg/kg bw	*Dose*		*98.88*	*0.83*	*≤0.03*	*0.14*	*≤0.02*	*0.04*
	Urine	M	19.14	0.24	0.02	0.04	0.02	0.09
		F	15.17	0.22	≤0.01	0.04	0.01	0.05
	Faeces	M	78.95	0.66	≤0.02	≤0.03	0.10	0.13
		F	82.93	0.74	≤0.03	≤0.03	0.09	0.14
Group 5 Oral dose* 10 mg/kg bw	*Dose*		*99.41*	*0.20*	*≤0.03*	*0.18*	*≤0.03*	*≤0.03*
	Urine	M	30.92	0.10	≤0.02	0.04	0.03	0.10
		F	24.02	0.07	≤0.01	0.06	0.03	0.14
	Faeces	M	67.29	0.38	≤0.04	≤0.07	0.14	0.11
		F	73.79	0.48	≤0.04	≤0.07	0.17	0.12
Group 6 Oral dose** 10 mg/kg bw/day	*Dose*		*99.36*	*0.27*	*≤0.03*	*0.21*	*≤0.0.2*	*≤0.02*
	Urine	M	33.14	0.10	≤0.02	0.04	0.03	0.06
		F	24.18	0.06	≤0.01	0.03	0.04	0.12
	Faeces	M	64.81	0.59	≤0.04	≤0.07	0.16	0.11
		F	73.77	0.70	≤0.04	≤0.08	0.17	0.17

* Single dose
** Multiple dose (non-radiolabelled glyphosate for 14 days followed by [^{14}C]-glyphosate
Gly glyphosate
N-Ac N-acetyl
N-F N-formyl
N-NO N-nitroso
Unk. unknown
IPC gradient ion pair HPLC
Analyses of the dosing solutions in italics

Total yields of N-nitroso-glyphosate in rat excreta ranged from 0.01 - 0.2 % of the administered dose. In the oral dose groups, the yields of N-nitroso-glyphosate were always greater in the faeces than in the urine. Less than 0.1 % N-acetylglyphosate was formed. No detectable amounts of hydroxylmethylphosphonic acid or methylphosphonic acid were found in any of the samples. Slight increases in amounts of AMPA were noted in the excreta of rats of Groups 5 and 6 but not of Groups 3 and 4, in agreement with the results found by CX HPLC.

C. STABILITY ANALYSES

In all of the stability samples, the variation of amounts of glyphosate with time was minor and appeared to be within the range of measurement errors. The recovery ranged from 97.94 % - 99.8 % of the spikes glyphosate.
The excreta stability analyses showed formation of 0.05 - 0.13 % N-nitrosoglyphosate in urine. Faeces spiked with glyphosate (which contained <0.03 % N-nitrosoglyphosate) showed formation of 0.15 % N-nitrosoglyphosate, which can be a result of reaction of glyphosate with nitrite naturally occurring in the urine and faeces. The sample stability studies also showed formation of up to 0.22 - 0.28 % unknown compound, that eluted last in the IPC analyses.

D. CHARACTERISATION OF UNKNOWN

Efforts to characterise the unknown compound indicated that this material was non-basic (it eluted in the void volume upon CX HPLC) and generated glyphosate and perhaps AMPA upon hydrolysis in pH 2.1 phosphate buffer. The latest eluting radioactive component in IPC of rat excreta did not correspond in retention time to any standard on hand. Its structure was not determined.

III. CONCLUSIONS

Metabolism of glyphosate occurs only to a minor extent in Sprague-Dawley rats, regardless of whether the route of administration is intravenous or oral. Traces of AMPA (0.2 - 0.4 %) appeared to form in rats administered single or multiple oral doses of glyphosate at 10 mg/kg bw. Since the amount of AMPA was so small (<1 %), the identification of AMPA was based solely on the HPLC retention times in the two radically different methods of HPLC. The results of the stability studies and the finding of the majority of the N-nitroso glyphosate (total yields of 0.06 - 0.50 %) in the faeces suggest strongly that the observed N-nitroso glyphosate is not a result of metabolism in the rat but rather is a result of chemical reaction of nitrite naturally occurring in the excreta with glyphosate.

The overall results of this study show that orally-administered glyphosate is absorbed to the extent of 30 - 36 %, metabolism of glyphosate in the rat is very minor, and AMPA, the sole metabolite, appears to be formed at <1 % by *in vivo* metabolism of glyphosate in rats dosed orally at 10 mg/kg bw, either in single or multiple doses.

3. Assessment and conclusion

Assessment and conclusion by applicant:

A full range ADME (absorption, distribution, metabolism and excretion) study in Sprague-Dawley rats was documented in two separate study reports. In this study report the results on metabolites were provided.

The predominant radioactive component with at least 97.5 % of the urine or faecal extract-contained radioactivity was [^{14}C]-glyphosate. The HPLC analyses of dosing solutions and excreta samples indicated that glyphosate in the excreta accounted for >98.5 - 99.3 % of the administered dose. Only small amounts (<1 %) of aminomethylphosphonic acid (AMPA) were found in rats administered single or multiple oral doses of [^{14}C]-glyphosate. Amounts of glyphosate and AMPA in excreta were at similar extents when comparing the results of two different HPLC methods (cation exchange and ion pair HPLC).

Total yields of N-nitroso-glyphosate in rat excreta ranged from 0.06 - 0.20 % of the administered dose and less than 0.1 % N-acetylglyphosate was formed. The fact that the majority of the N-nitroso glyphosate (total yields of 0.06 - 0.50 %) was excreted in faeces suggest that N-nitroso glyphosate is not a result of metabolism in the rat but rather is a result of chemical reaction of nitrite naturally occurring in the excreta with glyphosate.

No detectable amounts of hydroxymethylphosphonic acid or methylphosphonic acid were found. The remainder of the radioactivity detected in the excreta was attributed to low-level impurities present in the dosing material or to materials that had formed during storage of the excreta samples.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.1.1/016
Report author	[redacted]
Report year	1973
Report title	CP 67573 residue and metabolism. Part 13: The dynamics of accumulation and depletion of orally ingested N-phosphonomethylglycine-^{14}C
Report No	309
Document No	Not reported
Guidelines followed in study	None reported
Deviations from current test guideline	No batch number and no purity of the applied glyphosate was given. Dose levels appear too low for meaningful and reliable residue analysis. No analytical description provided.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, not conducted under GLP (not compulsory at time of study conduct)
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

Full summary
Executive summary

[^{14}C]-Glyphosate (no purity given) was administered as dietary preparation for 14 consecutive days to 120 Wistar rats (16 male and 16 female per dose groups as well as 12 male and 12 female in the control group) to investigate the accumulation and depletion of N-phosphonomethylglycine-^{14}C. The dietary dose levels were 1, 10 and 100 ppm. Thereafter, animals received dietary administration of glyphosate for 14 days followed by a recovery period of up to 10 days on normal basal diet.

Faeces and urine were analysed daily for radioactivity. Two animals per sex and dose were sacrificed on treatment Days 2, 6, 10 and 14 and on Days 1, 3, 6 and 10 after withdrawal of dosed feed. No control animals were killed at the second day of administration and on the first day of the recovery period since the number of control rats was too small. Following sacrifice, tissues were examined for radioactive residues.

I. MATERIALS AND METHODS

A. MATERIALS

1. Radiolabelled test material:	N-phosphonomethylglycine-^{14}C
Identification:	N-phosphonomethylglycine-^{14}C
Position of radiolabel:	Not stated
Lot/Batch #:	Not reported
Purity:	Not reported
Specific activity:	Not reported
Stability of test compound:	Not reported

2. Test animals:

Species:	Rat
Strain:	Wistar-strain, SPF albino rats
Source:	[redacted]
Age:	Not stated, but less than 6 weeks considering the body weight
Sex:	Male and Female
Weight at dosing:	Males approx.. 125 g, females approx. 120 g
Acclimation period:	7 days
Diet/Food during the acclimation period:	A weighed portion of feed was provided at 0900 in the tared feed cup and removed at 1600
Water:	*ad libitum*
Housing:	Four animals per cage (Hanging Rodent Metabolism Study Cages)
Environmental conditions:	Temperature: not reported Humidity: not reported Air changes: not reported

In life dates: 2019-07-08 to 2019-08-22

Animal assignment and treatment

After a seven-day acclimation period all animals were weighed and assigned to treatment groups. During the twenty-four day experimental period, feed consumption was recorded on a daily basis in order to calculate the daily intake of N-phosphonomethylglycine-^{14}C.

Thirty-two rats (16 male, 16 female) received repeated oral target doses of either 1 ppm, 10 ppm or 100 ppm by dietary administration for 14 consecutive days. Thereafter, animals received basal diet for up to 10 days during which toxicokinetic samples were taken. Individual weights were determined for all surviving animals at seven day intervals and terminal weights were taken at the time of sacrifice of each animal. Daily observations were made for gross behavioural changes, moreover urine volumes and faces weights were recorded daily.

Replicate groups of four animals of each sex were distributed into treatment groups, and placed into five cages according to a randomized block design. At each sampling period, two animals of each sex from each treatment group were sacrificed and tissues taken for analysis. Determinations of tissue residues were made after 2, 6, 10 or 14 days of administration, and 1, 3, 6 or 10 day after withdrawal of test substance application. The excretion of ingested CP 67573-^{14}C was determined daily.

Measurement of radioactivity in excreta and tissues

Urine was analysed for ^{14}C-activity by diluting the duplicate of 0.2 mL aliquots with 2 mL of 0.5M NH_4HCO_3. Phosphor solution of 15 mL was added, the mixture shaken vigorously and chilled to form a gel. The samples were then analysed by liquid scintillation counting (Nuclear-Chicago, DesPlaines, Illinois).

Daily individual faces samples were homogenized in 20 ml of aqueous 30% isopropyl alcohol using a Brinkman Polytron (Brinkman Inst., Westbury, N.Y.). The homogenate was frozen, lyophilized, weighed and duplicate 100 mg aliquots were submitted for combustion. The resulting $^{14}CO_2$ was trapped and analysed by liquid scintillation counting.

The collected tissue samples (liver, kidney, heart, spleen, gonads, brain, strained muscles, adipose tissues, gut) were weighed, frozen and lyophilized. The lyophilized samples were weighed, then the aliquots submitted for combustion as well as for subsequent liquid scintillation counting.

Statistical evaluation

The limitation imposed by the number of available metabolism units was taken into account when statistical analyses were made. Statistical comparisons were made only among those samples, which would include control values, i.e., 2-days administration and l-day withdrawal were omitted. A two-way analysis of variance (AOV) was conducted in order to increase the sensitivity for detecting treatment differences in relative organ weight.

In the case of relative tissue residues, i.e., tissue to blood residue ratios, the control animals were omitted from the analysis for the ease of computations. Relative tissue residues were then analysed for sex differences independent of the two-way analysis of variance for treatment differences.

The values reported in the data Table 1 (Table 1a-Table 1i) are the mean of two animals of each sex at each sampling period. The accumulation depletion curves were plotted from the mean of tissues from two males and two females +/- one standard deviation. The percent cumulative intake curves were constructed from the mean of all rata (males + females) surviving at that time, but the µg excreted curves are plotted as the mean of surviving members of each sex +/- one standard deviation.

The calculation of standard deviations was facilitated by use of the Olivetti-Underwood Programme 101. In case in which sex comparisons were indicated, comparisons were made by calculation of the t-statistic and the significance determined from a table of critical values for the "Students" t-statistic for non-directional (two-tailed) test.
The AOV used was a two-way factorial analysis adapted from Bruning and Kintz and programmed into the Corn-Share system by Mr. John T. Moran. Statistical significance was determined from a table of F-distribution of means - square ratios.

II. RESULTS AND DISCUSSION

A. BODY AND ORGAN WEIGHT CHANGES

The animals were weighed at the initiation of the experimental period and weekly thereafter. All groups except the control female rats, which shoved a premature growth plateau, exhibited similar growth patterns. A few animals snowed a slight negative response to the individual housing and rigid feeding schedule during the adaptation period. However, all animals except the control females quickly overcame the temporary lag in growth and grew normally throughout the experimental period.

The weight of the fresh organs of the animals were compared to those of the control animals sacrificed at the same sampling period. To correct for the individual weight differences among rats, the weights of the organs were expressed as a percent of the live body weight of the individuals. The statistical comparisons of relative organ weights of animals receiving CP 67573-^{14}C versus control animals were restricted to 6, 10 and 14 day medication, and 3, 6 and 10 days withdrawal, because no control animals were sacrificed on days 2 medication and 1 withdrawal. Nonetheless, the relative organ weights of the treated animals sacrificed on those days are included for completeness.
The two-way analysis of variance demonstrated that there were no significant changes in the size of the liver, kidney, spleen, heart, brain or gonads associated with the level of CP 67573-^{14}C administered. On the other hand, sex-related differences in organ sizes were quiet apparent. Organ weight changes, liver and kidney in particular, consistently showed statistically significant sex differences. It is however evident that the 14-day administration of CP 67573-^{14}C elicited no gross pathological changes in either sex.

B. CHARACTERIZATION OF THE EXCRETED RADIOACTIVITY

There were no statistical differences between sexes in percent of the daily intake of CP 67573-^{14}C, which was excreted in the urine. The percent daily intake excreted in urine reached a relatively constant level within 24 hours after the experimental period started. The daily excretion ranged from 6-12% of the daily intake of CP 67573-^{14}C and the mean urinary excretion throughout the 14-day medication period was 8.3%, 10.5% and 8.5% of the daily intake for 1 ppm, 10 ppm and 100 ppm treatments, respectively.
The similar percent daily intake excreted in urine indicates that both male and female animals absorb equivalent fractions of the ingested material. However, since there was a difference in the feed consumption between sexes, the absolute excretion of CP 67573-^{14}C was statistically significant during medication ($p<0.001$). Due to the higher intake by males, the µg CP 67573-^{14}C excreted either in urine or faeces was higher than that excreted by female rats.
The plateau of urinary excretion was reached in approximately 72 hours and in approximately 48 hours for faecal clearance.

The combined urinary and faecal clearance very nearly equals the total intake of CP 67573-^{14}C after six days. Expressed as percent of the cumulative intake, the CP 67573-^{14}C ingested was being cleared within 24-48 hours. By day four excretion had exceeded 90% of the cumulative intake and by the end of the 14-day medication period, the excretion of CP 67573-^{14}C was within +- 15% of the cumulative intake of the compounds (96%, 115% and 93% for 1 ppm, 10 ppm and 100 ppm, respectively).

After approximately 4 days of withdrawal, the urinary excretion curves plateau for about 48 hours before resuming characteristic exponential decline. Since the animals are no longer receiving dietary 67573-^{14}C, the appearance of ^{14}C-activity in the urine at 4-days withdrawal must indicate that the small bw load accumulated during CP 67573-^{14}C administration was being mobilized from the tissues and was filtered from the plasma by the kidneys.
The faecal excretion pattern was analogous to that of urine. The µg of CP 67573-^{14}C excreted plateaued at about three days except for the gentle positive slope due to the increase in feed intake with age. There was also the same precipitous drop concomitant with cessation of CP 67573-^{14}C administration, although there was a slight delay attributable to passage time through the gut, i.e., the ^{14}C-activity excreted at 1 day withdrawal was due primarily to the intake at 14 days medicated.

The ^{14}C-activity, which was secreted into the bile must have originated from the small tissue stores attained during the accumulation phase. As with the single dose situation, the biliary excretion of chronically ingested CP 67573 must be a proportionately minor route of excretion of CP 67573. This is evident by the fact that faecal ^{14}C-activity dropped to about 1% of the peak value observed at 14 days medicated before the contribution of biliary secretion became noticeable. That the contribution from biliary excretion did not become noticeable until the total amount of compound to be handled was small,
indicates that the amount in this cycle was small. If the absolute amount being excreted through the bile was constant throughout, i.e., percent excreted changed with dose and body load, then it is consistent with capacity-limited or "zero-order" processes, and was probably transferred to the bile by active secretion.
When the combined excretion through urine and faeces are examined closely, i.e., amount excreted expressed as percent cumulative intake excreted, the indication is clearly that 10% of the total amount ingested can be retained as a body load (< 4% for 1 ppm).

C. DISTRIBUTION OF RADIOACTIVITY FOLLOWING ORAL ADMINISTRATION OF ^{14}C-CP 67573

The continuous administration of a compound, which is eliminated only or primarily by saturable processes, which are describable by Michaelis-Menten kinetics, presents obvious hazards. Unlike substances eliminated by first-order processes, compounds excreted by capacity limited-systems will continue either to accumulate without apparent limit until the insult is removed or the intake is diminished below the threshold value of saturation of the elimination processes.

An early indication that the capacity of the excretory processes has been exceeded would manifest itself in a decreased fraction of the dose eliminated when the dose is increased beyond the saturation limits (K_m). When CP 67573 was sub-acutely administered to rat, the percent of the daily intake of CP 6757 excreted in the urine expressed no differences in treatment groups of 1 ppm, 10 ppm or 100 ppm. The total µg CP 67573 excreted in the urine, did increase during the initial phases of the experiment but plateaued at 4 to 6 days of the administration.
The constant percent of intake cleared by the kidneys and the demonstrated capacity to maintain that percent urinary excretion through several orders of magnitude of dose is a strong indication of first-order elimination kinetics.

Table 5.1.1-46: ^{14}C-residues (µg CP 67573-^{14}C equiv./g fresh tissue) in the liver of rats sub-acutely administered CP 67573-^{14}C

Time (days)	Treatment					
Administration and withdrawal	1 ppm		10 ppm		100 ppm	
	M	F	M	F	M	F
2nd of administration	0.001	0.002	0.021	0.024	0.168	0.136
6th of administration	0.003	0.003	0.033	0.034	0.283	0.226
10th of administration	0.003	0.004	0.041	0.032	0.264	0.272
14th of administration	0.004	0.003	0.037	0.030	0.319	0.291
1st of withdrawal	0.002	0.002	0.027	0.027	0.235	0.229
3rd of withdrawal	0.002	0.001	0.021	0.017	0.186	0.169
6th of withdrawal	0.001	0.001	0.013	0.013	0.106	0.098
10th of withdrawal	0.001	0.001	0.008	0.008	0.069	0.065

Table 5.1.1-47: ^{14}C-residues (µg CP 67573-^{14}C equiv./g fresh tissue) in the kidney of rats sub-acutely administered CP 67573-^{14}C

Time (days)	Treatment					
Administration and withdrawal	1ppm		10 ppm		100 ppm	
	M	F	M	F	M	F
2nd of administration	0.008	0.007	0.102	0.079	0.709	0.560
6th of administration	0.008	0.008	0.112	0.109	0.916	0.779
10th of administration	0.009	0.008	0.136	0.091	0.772	0.637
14th of administration	0.012	0.006	0.096	0.063	0.803	0.564
1st of withdrawal	0.003	0.003	0.049	0.039	0.333	0.319
3rd of withdrawal	0.002	0.002	0.028	0.027	0.242	0.218
6th of withdrawal	0.002	0.001	0.019	0.019	0.161	0.136
10th of withdrawal	0.001	0.001	0.013	0.012	0.102	0.098

Table 5.1.1-48: ^{14}C-residues (µg CP 67573-^{14}C equiv./g fresh tissue) in the muscle of rats sub-acutely administered CP 67573-^{14}C

Time (days)	Treatment					
Administration and withdrawal	1ppm		10 ppm		100 ppm	
	M	F	M	F	M	F
2nd of administration	0.001	<0.001	0.007	0.007	0.051	0.027
6th of administration	0.001	0.001	0.011	0.011	0.101	0.077
10th of administration	0.002	0.001	0.018	0.012	0.126	0.095
14th of administration	0.002	0.001	0.017	0.013	0.195	0.125
1st of withdrawal	0.001	0.001	0.016	0.010	0.120	0.086
3rd of withdrawal	0.001	0.001	0.016	0.012	0.135	0.101
6th of withdrawal	0.001	0.001	0.012	0.011	0.111	0.092

Time (days)	Treatment					
Administration and withdrawal	1ppm		10 ppm		100 ppm	
	M	F	M	F	M	F
10th of withdrawal	0.001	0.001	0.013	0.009	0.097	0.090

Table 5.1.1-49: ^{14}C-residues (µg CP 67573-^{14}C equiv./g fresh tissue) in the fat of rats sub-acutely administered CP 67573-^{14}C

Time (days)	Treatment					
Administration and withdrawal	1ppm		10 ppm		100 ppm	
	M	F	M	F	M	F
2nd of administration	0.002	0.004	0.065	0.044	0.055	0.681
6th of administration	0.003	0.006	0.063	0.062	0.710	0.411
10th of administration	0.002	0.003	0.036	0.026	0.254	0.244
14th of administration	0.003	0.003	0.036	0.030	0.345	0.351
1st of withdrawal	0.002	0.003	0.025	0.021	0.240	0.225
3rd of withdrawal	0.003	0.002	0.034	0.029	0.218	0.188
6th of withdrawal	0.002	0.002	0.023	0.022	0.142	0.136
10th of withdrawal	0.002	0.001	0.016	0.015	0.112	0.128

Table 5.1.1-50: ^{14}C-residues (µg CP 67573-^{14}C equiv./g fresh tissue) in the gut of rats sub-acutely administered CP 67573-^{14}C

Time (days)	Treatment					
Administration and withdrawal	1ppm		10 ppm		100 ppm	
	M	F	M	F	M	F
2nd of administration	0.234	0.181	3.494	5.286	25.360	19.090
6th of administration	0.194	0.206	2.433	4.148	37.305	19.127
10th of administration	0.506	0.370	3.075	3.288	17.605	26.903
14th of administration	0.185	0.113	2.760	2.842	23.174	23.850
1st of withdrawal	0.009	0.010	0.101	0.109	0.659	0.585
3rd of withdrawal	0.002	0.002	0.024	0.022	0.210	0.178
6th of withdrawal	0.001	0.001	0.014	0.013	0.094	0.178
10th of withdrawal	0.001	<0.001	0.008	0.008	0.059	0.076

Table 5.1.1-51: ^{14}C-residues (µg CP 67573-^{14}C equiv./g fresh tissue) in the spleen of rats sub-acutely administered CP 67573-^{14}C

Time (days)	Treatment					
Administration and withdrawal	1ppm		10 ppm		100 ppm	
	M	F	M	F	M	F
2nd of administration	0.004	0.007	0.051	0.098	0.696	0.636
6th of administration	0.007	0.011	0.076	0.122	0.656	0.290
10th of administration	0.004	0.005	0.076	0.080	0.348	0.406
14th of administration	0.005	0.006	0.031	0.036	0.350	0.390
1st of withdrawal	0.001	0.002	0.022	0.016	0.152	0.157
3rd of withdrawal	0.002	0.001	0.017	0.015	0.132	0.129
6th of withdrawal	0.001	<0.001	0.011	0.014	0.088	0.074
10th of withdrawal	0.001	<0.001	0.008	0.008	0.067	0.060

Table 5.1.1-52: ^{14}C-residues (µg CP 67573-^{14}C equiv./g fresh tissue) in the heart of rats sub-acutely administered CP 67573-^{14}C

Time (days) Administration and withdrawal	Treatment					
	1ppm		10 ppm		100 ppm	
	M	F	M	F	M	F
2nd of administration	0.002	0.003	0.020	0.027	0.142	0.173
6th of administration	0.001	0.004	0.024	0.032	0.404	0.095
10th of administration	0.002	0.002	0.038	0.027	0.211	0.209
14th of administration	0.002	0.002	0.019	0.022	0.197	0.194
1st of withdrawal	0.001	0.001	0.016	0.016	0.124	0.116
3rd of withdrawal	0.002	0.001	0.017	0.015	0.116	0.120
6th of withdrawal	0.001	0.001	0.012	0.007	0.098	0.086
10th of withdrawal	0.001	<0.001	0.012	0.009	0.084	0.078

Table 5.1.1-53: ^{14}C-residues (µg CP 67573-^{14}C equiv./g fresh tissue) in the brain of rats sub-acutely administered CP 67573-^{14}C

Time (days) Administration and withdrawal	Treatment					
	1ppm		10 ppm		100 ppm	
	M	F	M	F	M	F
2nd of administration	<0.001	<0.001	0.006	0.009	0.077	0.091
6th of administration	0.001	0.001	0.014	0.010	0.179	0.044
10th of administration	0.001	0.001	0.013	0.013	0.116	0.116
14th of administration	0.001	0.001	0.010	0.009	0.090	0.095
1st of withdrawal	<0.001	<0.001	0.009	0.008	0.065	0.063
3rd of withdrawal	0.001	0.001	0.008	0.008	0.072	0.065
6th of withdrawal	0.001	0.001	0.007	0.006	0.058	0.052
10th of withdrawal	0.001	<0.001	0.006	0.005	0.050	0.043

Table 5.1.1-54: ^{14}C-residues (µg CP 67573-^{14}C equiv./g fresh tissue) in the gonads of rats sub-acutely administered CP 67573-^{14}C

Time (days) Administration and withdrawal	Treatment					
	1ppm		10 ppm		100 ppm	
	M	F	M	F	M	F
2nd of administration	<0.001	0.006	0.007	0.056	0.068	0.590
6th of administration	0.001	0.009	0.010	0.180	0.106	0.555
10th of administration	0.001	0.004	0.014	0.045	0.099	0.333
14th of administration	0.001	0.003	0.011	0.043	0.114	0.449
1st of withdrawal	0.001	0.002	0.011	0.021	0.087	0.160
3rd of withdrawal	0.001	0.002	0.009	0.020	0.088	0.181
6th of withdrawal	0.001	0.001	0.008	0.017	0.069	0.105
10th of withdrawal	0.001	0.000	0.007	0.010	0.057	0.062

Table 5.1.1-55: ^{14}C-residues (µg CP 67573-^{14}C equiv./g fresh tissue) in whole blood of rats sub-acutely administered CP 67573-^{14}C

Time (days) Administration and withdrawal	Treatment					
	1ppm		10 ppm		100 ppm	
	M	F	M	F	M	F
2nd of administration	<0.001	<0.001	0.005	0.012	0.044	0.030
6th of administration	0.001	0.001	0.010	0.013	0.080	0.053
10th of administration	0.001	0.001	0.013	0.012	0.078	0.090
14th of administration	0.001	0.001	0.009	0.009	0.092	0.080
1st of withdrawal	0.001	0.001	0.008	0.006	0.067	0.053
3rd of withdrawal	0.001	0.001	0.007	0.007	0.071	0.058
6th of withdrawal	0.001	<0.001	0.006	0.006	0.062	0.050
10th of withdrawal	0.001	<0.001	0.006	0.006	0.059	0.046

Table 5.1.1-56: Calculated t-Statistics for comparison of ^{14}C-residue (ppm - fresh weight) in male and female tissues

Tissue	1 ppm		10 ppm		100 ppm	
	Administered	Withdrawal	Administered	Withdrawal	Administered	Withdrawal
Liver	0.321	1.227	0.868	0.268	0.106	0.910
Kidney	1.662	0.911	2.179	0.470	2.316	1.124
Muscle	1.016	2.898*	1.257	4.268**	0.937	3.710**
Fat	1.820	1.366	0.938	0.075	0.745	0.886
Gut	0.601	0.144	1.685	0.046	0.918	0.780
Spleen	1.022	1.426	1.321	0.577	1.175	0.786
Heart	1.593	2.229	0.275	1.134	1.335	0.934
Brain	0.948	0.810	0.147	1.428	1.073	1.374
Gonads	4.984**	0.916	2.481*	4.618**	6.871	2.203
***p< 0.05**						
****p< 0.01**						

The sub-acute administration of CP 67573 to rats required approximately six days to reach the plateau level in which the rate of elimination was very nearly equal to the rate of ingestion. Furthermore, within the range described in this experiment, the data do not suggest that the excretory systems have approached their capacity for elimination of CP 67573. It is demonstrated by the facts that there was a log increase in excretion with a log dose increase, the percent of daily intake fluctuated very little and the total excretion at all dose ranges reached a plateau level simultaneously. However, it must also be recognized that even those processes that appear to be apparent first-order may actually be saturable in theory, and are in fact a limiting case of Michaelis-Menten kinetics in which the substrate concentration is very much lower than K_m

Since-L "steady-state" equilibrium level had been established in the animal by 6-8 days, it was, therefore not unexpected that the tissues reached equilibrium residue levels concomitantly with equilibrium in the entire animal. The fact that the tissue plateau is a steady state equilibrium and not a saturation limit was verified by the direct proportionality of the plateau level to the dietary exposure. Furthermore, the equilibrium is very sensitive to the dietary intake of the compound. Removal of the insult from the animal (in this case after 14 days medication) resulted in an immediate depletion phase.

Some re-distribution of residues was observed. It is highly probable the re-distribution by enterohepatic circulation was operative throughout the experiment but at a relatively low level. The reabsorption of residues secreted into the gut through the bile cannot be directly proven from these data, but the faecal ^{14}C-activity observed at 4-day withdrawal was indicative of biliary secretions as demonstrated by acute doses.

Re-distribution was also observed in the peripheral tissues. The residue level of spleen and fat exhibited maxima at two to six day medication, but decreased to establish a plateau at a lower level. There are also indications of re-distribution in other tissues during the withdrawal phase, but the inferences are since they were difficult to demonstrate levels at all three dose levels.

The onset of tissue depletion, coincident with the withdrawal of medicated feed, was evident in every tissue examined. In fact the half-life of depletion (T1/2 d) can he easily determined for most of the tissues. Importantly the T1/2 d, of tissue did not vary with the dose which had been ingested or the plateau level attained. It was, therefore, evident that the capacity to clear the residues from the tissues had not been exceeded. Such facile reversibility of residue concentration would appear to preclude the potential of bio-magnification of CP 67573 in mammals. Accumulation did occur upon chronic feeding of CP 67573, but at a maximum level directly proportional to the dose. Since maintenance of that plateau level was dependent on maintenance of a constant tissue: a blood ratio, any alteration resulted in diffusion of tissue residues into the blood to maintain that ratio. However, during withdrawal the only source of CP 67573 was the tissues and the kidneys continued to filter the CP 67573 from the block. The resultant mobilization from the tissues therefore became evident in the urine in approximately four days withdrawal as. Evidence of tissue residue mobilization was also seen in the faeces at four days withdrawal as a plateau of ^{14}C-activity excreted in the faeces. The immediate source of these residues was probably the biliary system, but it cannot be unequivocally stated that they were exclusively of hepatic origin. Anatomical and physiological considerations make it obvious that contributions to biliary secretions must traverse the liver, but single dose studies of CP 67573 metabolism have demonstrated that 6-14% of an intraperitoneal injection can be subjected to enteral secretion.

The profile of redistribution following chronic administration is identical to that of single-dose oral administration, but the timing is slightly different. Following a single oral dose a redistribution is seen at about 48 hours post administration, whereas, withdrawal following 14 days chronic administration results in a redistribution phase which equals or exceeds excretion at approximately % hours. In both cases the redistribution phase predominates for approximately 48 hours. The more rapid appearance of redistribution under conditions of acute exposure may possibly be due to the more readily accessible compartments compared to chronic or multiple dose experiments.

III. CONCLUSIONS

All data available on the absorption of CP 67573 following oral administration suggest that absorption from the gastrointestinal fluids can be interpreted by a simple model following Ficks law of perfusion (and therefore the uptake of CP 67573 from the gut would follow first-order kinetics. The physiological consequences would then be that as the dietary intake of CP 67573 were increased the amount of "drug" transported into the central compartment would be directly proportional to the intake. The levels in the tissues would also increase, in direct proportion to that concentration in the central compartment, and would maintain a constant ratio of tissue: blood concentration.

3. Assessment and conclusion

Assessment and conclusion by applicant:

By Day 4 of dosing, combined excretion of [14C]-activity in urine and faeces exceeded 90 % of the cumulative intake. After six days, the overall excretion was approximately equal to the total intake of [14C]-glyphosate and this was confirmed for all dietary levels at the end of the 14-day dosing period. Thus, it was established that the body load had plateaued at a level directly proportional to the administered dose. Upon withdrawal from dosed feed, the excretion of radioactivity dropped significantly but plateaued temporarily after four days. This was assumed to be due to mobilization of previously established body loads. Most tissues reached maximum residue levels during the dosing period in 10 days or less. Highest tissue concentrations were found in kidneys and spleen. However, bone was not investigated in this study. The tissue residues were clearly not bound. As soon as the

exogenous supply of [^{14}C]-glyphosate was removed, the residues began to decrease.
The urinary excretion was 8.3 - 10.5 % of the daily intake. The occurrence of faecal excretion during a treatment-free recovery interval after a 14-day dosing period was presumably due to biliary elimination. Nevertheless, in the absence of bile cannulation data, the discussion on biliary excretion is of speculative nature.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.1.1/017
Report author	[redacted]
Report year	1973
Report title	CP 67573 residue and metabolism. Part 9: The gross distribution of n-phosphonomethylglycine-^{14}C in the rabbit
Report No	298
Document No	Not reported
Guidelines followed in study	None reported
Deviations from current test guideline	No batch number and no purity of the applied glyphosate was given.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, not conducted under GLP (not compulsory at time of study conduct)
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

Full summary
Executive summary

These studies were initiated to investigate the metabolic fate of phosphonomethylglycine-^{14}C (CP 67573-^{14}C) by following a single oral dose to male rabbits.

Male New Zealand White rabbits were administered a single oral dose of [^{14}C]-glyphosate (assumed to be manufactured by Monsanto, purity not given) by gavage at dose levels within a range of 5.7 - 8.8 mg/kg bw. Three treatment groups of two or three animals were used each receiving the test compound labelled at one of three different sites. Urine, faeces and expired CO_2 were sampled at 12, 24, 48, 72, 96 and 120 h after dosing and assayed for radioactivity. At sacrifice after 120 h, blood and tissue samples were examined for radioactive residues.

After a single oral gavage dosing to male rabbits 79 - 97 % of the dose was excreted within 120 h. Total urinary clearance was only 7 - 11 % in this period. Less than 1 % was recovered in expired air. The carcass retention at sacrifice was 1.87 - 4.85 % of the dose, however, most (74 - 98 %) could be accounted for by the gut and its content. In the other tissues, highest residues were found in the liver and kidneys; however,

radioactivity in bone was not determined.

The results indicate similarities and differences between species such as a lower urinary clearance rate and higher tissue retention in rabbits as compared to the rat.

I. MATERIALS AND METHODS

A. MATERIALS

1. Radiolabelled test material:	phosphonomethylglycine-^{14}C
Identification:	CP 67573-^{14}C
Position of radiolabel:	Not stated
Lot/Batch #:	Not reported
Purity:	Not reported
Specific activity:	0.08 mCi/mM, 1.33 mCi/mM, 2.2 mCi/mM
Stability of test compound:	Not reported
2. Test animals:	
Species:	Rabbit
Strain:	New Zealand white rabbits
Source:	[redacted]
Age:	Not stated, but about 6 to 8 weeks considering the body weight
Sex:	Male
Weight at dosing:	Approximately 1.3-1.4 kg
Acclimation period:	Not reported
Diet/Food:	*ad libitum,* fasted (3 hours)
Water:	*ad libitum*
Housing:	Individually in Roth metabolism cages (Delmar Scientific Glass Products, Maywood, Illinois)
Environmental conditions:	Temperature: not reported Humidity: not reported Air changes: not reported

B. STUDY DESIGN

Animal assignment and treatment

Male rabbits were fasted for three hours, before application of an aqueous solution containing CP 67573-^{14}C in doses ranging from 5.7 to 8.8 mg/kg bw. Through gastrointubation in the two replicate experiments conducted, a total of three rabbits received CP 67573-methylene-^{14}C (0.08 mCi/mM), two rabbits CP 67573-Gly-1-^{14}C (1.33 mCi/mM) and two other rabbits CP 67573-Gly-2-^{14}C (2.2 mCi/mM).

After dosing, each rabbit was individually housed in Roth metabolism units for 120 hours. During post-administration feed and water were supplied *ad libitum*.

Excreta and CO_2 samples were collected at 12, 24, 48, 72, 96 and 120 hours post-administration. Urine

and CO_2-radioassays were performed, but details not reported. Faeces samples were immediately frozen until processing. The processing included homogenisation in isopropyl alcohol, then lyophilized faeces were taken for combustion and the trapped CO_2 analyzed by liquid scintillation counting.
At termination of the experimental period, the animals were fasted, weighed and anesthetized by injection of 100 mg sodium pentobarbital.
A blood sample was taken by cardiac puncture and the animals sacrificed by ether anesthesia. Tissue samples were removed, weighed, frozen and lyophilized, then following combustion radioassay had been performed.

II. RESULTS AND DISCUSSION

A. DISTRIBUTION OF RADIOACTIVITY FOLLOWING ORAL ADMINISTRATION OF PHOSPHONOMETHYLGLYCINE-^{14}C

The major route of excretion of orally administered CP 67573-^{14}C is via the faeces. More than 50 % of the administered dose was excreted in the faeces within 48 hours and a total of 79-97 % within 120 hours. Total urinary clearance was 7-11% in the same period.
Very little of the absorbed material was catabolised. Less than 1.0 % of the administered ^{14}C-activity was recovered in expired air.

Table 5.1.1-57: Distribution of ^{14}C-activity in male rabbits orally administered phosphonomethylglycine-^{14}C

Treatment[1]		Percent dose recovered[3]					
CP 67573-$^{14}CH_2$	Time (Hour)	Urine	Faeces	CO_2	Washes[2]	Tissues	Cumulative
	12	2.13	7.53	0.01			9.67
	24	1.95	13.18	0.01			24.81
	48	3.34	13.58	0.01			41.74
	72	1.77	24.50	0.01			67.42
	96	1.30	16.64	0.00			85.36
	120	0.20	4.26	0.00			89.82
		10.09	**79.69**	**0.04**	**0.17**	**1.87**	**91.86**
		Percent dose recovered[3]					
CP 67573-^{14}Glycine-1-^{14}C	Time (Hour)	Urine	Faeces	CO_2	Washes[2]	Tissues	Cumulative
	12	1.27	20.74	0.54			22.55
	24	3.14	10.96	0.12			36.77
	48	3.99	10.06	0.08			50.90
	72	2.01	22.36	0.04			75.31
	96	0.66	11.10	0.02			87.09
	120	0.33	3.78	0.01			91.21
		11.40	**79.00**	**0.81**	**0.06**	**5.64**	**96.91**
		Percent dose recovered[3]					
CP 67573-^{14}Glycine-2-^{14}C	Time (Hour)	Urine	Faeces	CO_2	Washes[2]	Tissues	Cumulative
	12	1.64	37.40	0.40			39.44
	24	0.62	11.44	0.16			51.66
	48	2.92	17.88	0.06			72.52
	72	0.83	12.60	0.04			85.99
	96	1.10	14.08	0.02			101.19
	120	0.18	3.88	0.01			105.25
		7.29	**97.28**	**0.68**	**0.03**	**4.85**	**110.13**

[1]Glycine-l- ^{14}C and Glycine-2-^{14}C treatments are averages of two animals; methylene-^{14}C is the average of three animals.
[2]Consists of 0.5M NH_4HCO_3 washes of cage, urine-faeces separator, urine collector, and faeces collector.
[3]Doses were in the range of 5.7-8.8 mg/kg body weight.

The total clearance pattern in the rabbit was somewhat different from that of rats given CP 67573-^{14}C *per os*. The rabbit exhibited an initial burst of ^{14}C-activity excreted in the first 12-24 hours post-administration ranging from 25-52% of the dose, but then the total clearance from the body showed a gradual decline. In the cases of the methylene and Glycine-1 labelled materials, 120 hours were required before more than 90% of the dose was voided from the body.

Total carcass retention by the rabbit not only appeared to be higher than in the rat, but also appeared to have a different propensity for retention, i.e., Gly-l> Gly-2 > methylene. However, in the rabbit the gut and its contents (Table 2) could account for 80-97% of that ^{14}C-activity retained at 120 hours. When the ^{14}C-activity recovered from the gut is separated from the true tissue retention, the order becomes Gly-2> Gly-l> methybene which is the order predicted from previous studies in rats. After adjusting the carcass ^{14}C-levels for the ^{14}C-activity recovered in the gut, the total carcass retention also closely approximated the values observed in rats. Exclusive of the gut there was in the tissues of rabbits 1.2%, 0.7% and 0.1% of the dose following administration of CP 67573-Gly-2-^{14}C, -Gly-1-^{14}C or -methylene-^{14}C, respectively, compared to 0.7%, 0.4% and 0.1% for the corresponding labels in the rat.

Table 5.1.1-58: ^{14}C-Residues in rabbit tissues 120 hours following a single oral dose of CP 67573-^{14}C

CP 67573-$^{14}CH_2$		
Tissue	**% Dose Recovered**	**PPM CP 67573-^{14}C equivalents (µg/g Fresh tissue)**
Liver	0.04	<0.10
Kidney	<0.01	<0.10
Muscle	<0.01	<0.10
Fat	0.00	0.00
Gut	1.83	0.68
Spleen	<0.01	<0.05
Heart	<0.01	<0.05
Testes	<0.01	<0.05
Blood	<0.01	<0.01
CP 67573-^{14}Glycine-1-^{14}C		
Tissue	**% Dose Recovered**	**PPM CP 67573-^{14}C equivalents (µg/g Fresh tissue)**
Liver	0.11	0.06
Kidney	0.04	0.07
Muscle	0.46	0.02
Fat	0.04	<0.01
Gut	4.92	0.55
Spleen	<0.01	0.05
Heart	<0.01	0.03
Testes	<0.01	0.02
Blood	0.07	0.02
CP 67573-^{14}Glycine-2-^{14}C		
Tissue	**% Dose Recovered**	**PPM CP 67573-^{14}C equivalents (µg/g Fresh tissue)**
Liver	0.18	0.30
Kidney	0.04	0.24
Muscle	0.80	0.11
Fat	0.13	0.12
Gut	3.60	0.93

Table 5.1.1-58: ^{14}C-Residues in rabbit tissues 120 hours following a single oral dose of CP 67573-^{14}C

CP 67573-$^{14}CH_2$		
Tissue	**% Dose Recovered**	**PPM CP 67573-^{14}C equivalents (µg/g Fresh tissue)**
Spleen	<0.01	0.23
Heart	<0.01	0.13
Testes	<0.01	0.12
Blood	0.10	0.08

B. CHARACTERIZATION OF THE EXCRETED RADIOACTIVITY

Following the administration of CP 67573-methylene-^{14}C, no tissue other than gut exceeded 0.1 ppm on a fresh weight basis. Even though the liver contained 0.04% of the dose, the specific activity of the material used was too low to quantitate the ppm CP 67573 equivalents retained.
The ^{14}C-residue concentrations (ppm) in each tissue differed by 4-5 fold between the Gly-1-^{14}C and Gly-2-^{14}C treatments, but the ranking was similar in each case, i.e., liver > kidney > spleen > heart, muscle and gonads. It is interesting that only the Gly-2-^{14}C was incorporated to any measurable quantity in the fat. These data clearly suggest that one- or two-carbon fragments from CP 67573 are being incorporated into adipose tissue.

The rabbit appears to be exposed to a single oral dose of CP 67573-^{14}C for a longer period of time than the rat as evidenced by the fact that the rabbit requires four to five days to clear the dose, whereas, the rat typically exhibits 95-98% clearance in 48 hours following a single oral dose. Consequently, slightly higher fractions of the original dose may be recovered from the tissues and less urinary excretion occurs.

III. CONCLUSIONS

Orally administered glyphosate is only moderately absorbed from the gut of the rabbit after single application of 6.7 mg/kg bw. Consequently, the faeces represent the major route of excretion of ingested glyphosate. Approximately 20 % of the material is absorbed and most of that amount is rapidly excreted unchanged in the urine. Of the material absorbed, very small amounts are catabolized as evidenced by the facts that less than 1.0 % of the dose is expired in air.

The rabbit requires a longer period of time to clear a single oral dose of CP 67573-^{14}C than does a rat given approximately the same dose in mg/kg body weight. The species variation may be due to either slower gut passage times or differences in redistribution patterns of absorbed materials.
The primary route of excretion of orally administered CP 67573 is in the faeces. Less than l% of the material is oxidized to CO2 and only the glycine moiety appears as tissue residues. It is the carbon-2 of the glycine moiety which is most likely to appear in tissues, indicating metabolism to one- or two-carbon fragments prior to synthesis of tissue components.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The excreta from male rabbits orally administered CP 67573-^{14}C were collected at various intervals through 120 hours post-administration and analysed for ^{14}C-activity.

More than 90% of the administered ^{14}C-activity cleared in five days or less as well as more than 80% of the ^{14}C-activity appeared in the faeces and 7-11% in the urine. Less than 1% was oxidized and expired as $^{14}CO_2$.
After five days most of the unexcreted ^{14}C-activity was recovered from the colon. The liver, kidney, spleen, heart, muscle, gonads and adipose contained less than 0.1 ppm ^{14}C-residues.

The report does not contain sufficient information on the identity of the active substance, on purity, any description on analytical methods, limits of detection or limits of quantitation. Nonetheless, in agreement with previous evaluations the study is considered valuable as bringing information on species differences.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA **5.1.1/018**
Report author	[redacted]
Report year	1998
Report title	Report on metabolism (absorption, tissue distribution & excretion with radio-active compounds - rats) of glyphosate
Report No	1087
Document No	Not reported
Guidelines followed in study	None reported
GLP/Officially recognised testing facilities	No, not conducted under GLP
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations	Two male Wistar rats per dose were administered unknown doses of [^{14}C]-glyphosate (0.56 x 10^7 and 1.11 x 10^7 Bq radioactivity in low and high dose, respectively) by feed. Urine, faeces and blood were collected at 2, 4, 8, 24 and 48 h and 7 and 15 days after dosing and assayed for radioactivity. At sacrifice after 15 days, the following tissues were radiochemically analysed: Brain, liver, spleen, heart, kidney, adrenals.
Short description of results	Glyphosate is poorly absorbed and levels of glyphosate in different tissues are negligible after 15 days of oral feeding.
Reasons for why the study is not considered relevant/reliable or not considered as key	The description of testing procedures and the reporting of results is very poor. Essential information like the dates of experimental work or the year when the study report was issued is lacking. Furthermore, there is no information on the amount of test substance administered (i.e. only the radioactivity applied is

study	indicated).
Category study in AIR 5 dossier (L docs)	Category 3b

CA 5.1.2 Absorption, distribution, metabolism and excretion by other routes

Table 5.1-59: Overview of ADME studies with glyphosate

Annex Point	Study	Scope of study	Substance(s)	Reference list- related category§	Result
CA 5.1.2/001	[redacted] 2020	Determination of the metabolite profile of glyphosate in human, rat, mouse, dog and rabbit cryo-preserved hepatocytes.	[^{14}C]-Glyphosate (Batch: 6848SXD008-2, Purity: >98.3 %)	Valid, Category 1	Incubations with cryo-preserved hepatocytes of all tested species exhibited a very similar biotransformation pattern of the applied [^{14}C]-glyphosate. No AMPA was detected in hepatocytes of any species. No human unique metabolites were detected.

§ The category describes the acceptability/reliability of the study within the AIR 5 submission. This category does not describe hazard classification. (for details please refer to the Doc ID: 110054-B-GRG_Jun_2020)

1. Information on the study

Data point	CA 5.1.2/001
Report author	[redacted]
Report year	2020
Report title	Metabolic stability and profiling of [^{14}C]-Glyphosate in hepatocytes from human, rat, mouse, dog and rabbit for inter-species comparison
Report No	S19-04081
Document No	Not reported
Guidelines followed in study	Not available, but based on Commission Regulation (EU) No 283/2013, 5.1.1, in accordance with Regulation (EC) No 1107/2009 (ANNEX to SANCO/11802/2010 Rev. 7, as voted by the standing committee in July 2012)
Deviations from current test guideline	N/A
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive summary

The objective of this study was to compare the metabolism of glyphosate after incubation with mixed gender cryo-preserved hepatocytes from human, rat, mouse and dog as well as with female rabbit cryo-preserved hepatocytes. 1 µM and 10 µM [^{14}C]-glyphosate were incubated separately with cryo-preserved hepatocytes and incubation buffer for 60 and 120 min and analysed by HPLC using radio detection.

The metabolic competence of the cryo-preserved hepatocytes was demonstrated using the positive controls testosterone and 7-hydroxycoumarin. 6β-hydroxytestosterone, 7-hydroxycoumarin sulphate and 7-hydroxycoumarin glucuronide were selected as a biological transformation marker compounds. All marker substances were detected after incubation in varying concentrations, proving the metabolic competence of the hepatocytes.

The dependence of the reaction on activated cryo-preserved hepatocytes was shown by the incubation of the test item (10 µM) for 120 min with inactivated cryo-preserved hepatocytes (negative control).

The determined metabolic transformation rates after incubation for 120 min with human, rat, dog and rabbit hepatocytes for [^{14}C]-glyphosate amounted up to 0.0 % for incubations with 1 µM and ≤1.0 % for incubations with 10 µM [^{14}C]-glyphosate, respectively. The rates after incubation for 120 min with mouse mixed cryo-preserved hepatocytes amounted up to 0.0 % for incubations with 1 µM and up to 1.56 % for incubations with 10 µM [^{14}C]-glyphosate, respectively.

Incubations of 1 µM [^{14}C]-glyphosate with human, rat, dog, mouse and rabbit hepatocytes revealed the parent substance and additional three potential metabolites during the incubation period. One unidentified substance (Unknown 1) detected in dog hepatocytes after an incubation time of 0 min showed the highest abundance (2.0 %). No major metabolites (>5 % of applied radioactivity; AR) were detected after an incubation time of 120 min in any species.

Incubations of 10 µM [^{14}C]-glyphosate with human, rat, dog, mouse and rabbit hepatocytes revealed the parent substance and additional seven potential metabolites during the incubation period. “Unknown 1” detected in mouse hepatocytes after an incubation time of 120 min showed the highest abundance (2.8 %). No major metabolites (>5 % AR) were detected after an incubation time of 120 min in any species.

Incubations of 1 and 10 µM [^{14}C]-glyphosate were being investigated for the metabolite AMPA, but no AMPA was detected in any species after an incubation time of 120 min.

No human unique metabolites were found. Therefore, all detected metabolites were only characterised based on their chromatographic behaviour and a metabolic pathway was not derived.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	[^{14}C]-Glyphosate
Position of radiolabel:	N-(phosphono[^{14}C]methyl)glycine
Lot/Batch #:	6848SXD008-2
Radiochemical purity:	98.3 % (HPLC)
Specific activity:	2060 MBq/mmol, 55.7 mCi/mmol
Stability of test compound:	Expiry date 2020-11-27 at ≤-18°C in a dark and dry place

2. Positive controls:

Phase 1:

Identification:	[^{14}C]-Testosterone
Position of radiolabel:	[4-^{14}C]-Testosterone
Lot/Batch #:	2379862
Radiochemical purity:	97 % (HPLC and TLC)
Specific activity:	1880 MBq/mmol, 50.8 mCi/mmol
Stability of test compound:	Expiry date 2020-01-16 at ≤-18°C in a dark and dry place

Phase 2:

Identification:	7-Hydroxycoumarin
Description:	Beige solid
Lot/Batch #:	BCBZ4431
Purity:	99.4 %
Stability of test compound:	Expiry date 2024-05-31 at ≤30°C in a dark and dry place

3. Reference substances:

Identification:	Glyphosate
Description:	White solid
Lot/Batch #:	BCHR 1037-02
Purity:	98.5 %
Stability of test compound:	Expiry date 2023-01-17 at ≤10°C in a dark and dry place

Identification:	Testosterone
Description:	White solid
Lot/Batch #:	SLBV0956
Purity:	100 %
Stability of test compound:	Expiry date 2020-06-30 at 5-30°C in a dark and dry place

Identification:	Aminomethylphosphonic acid (AMPA)
Description:	White solid
Lot/Batch #:	1002969
Purity:	98.7 %
Stability of test compound:	Expiry date 2024-06-05 at 1-10°C in a dark and dry place under inert gas

Identification:	6ß-Hydroxytestosterone
Description:	White solid
Lot/Batch #:	MKCD3716
Purity:	99 %
Stability of test compound:	Expiry date 2024-03-07 at ≤30°C in a dark and dry place

Identification:	7-Hydroxycoumarin sulphate (potassium salt)

Description:	White solid
Lot/Batch #:	0538267-5
Purity:	98.6 %
Stability of test compound:	Expiry date 2021-01-21 at ≤-18°C in a dark and dry place
Identification:	7-Hydroxycoumarin glucuronide (sodium salt)
Description:	White solid
Lot/Batch #:	023M3900V
Purity:	97 %
Stability of test compound:	Expiry date 2021-06-30 at ≤10°C in a dark and dry place

4. **Negative control:** Inactivated hepatocytes
5. **Hepatocytes:**

Species / Strain:	Human / - rat / Sprague Dawley mouse / CD-1 dog / Beagle rabbit / New Zealand White
Source:	XenoTech, USA and Prymacyt Cell Culture Technology GmbH, Germany
Sex:	All species male & female, except rabbit (female only)

B. STUDY DESIGN

Preparation of cryo-preserved hepatocytes

Cryo-preserved cell suspensions of human, rat, dog, mouse or rabbit hepatocytes were thawed and washed with the respective buffer media. After the last cleaning step, hepatocytes were re-suspended with Hepatocytes Plating Medium-Cryo (HPM-Cryo) to achieve 0.5 x 10^6 viable cells/mL.

Incubation of human, rat, mouse, dog and rabbit hepatocytes with the test substance [^{14}C]-glyphosate

The hepatocytes of humans, rats, dogs, mice and rabbits were incubated separately with [^{14}C]-glyphosate at concentration of 1 and 10 µM for 0, 60 and 120 min at 37 ± 2°C, in a final volume of 1000 µL, using a water bath with gentle shaking. [^{14}C]-Glyphosate in HPM-Cryo was incubated under the same conditions for 120 min as control. For each condition, duplicate samples were prepared and analysed at the beginning and after termination of the incubation.

The radioactivity in the supernatants was determined by LSC and investigated qualitatively and quantitatively by HPLC.

Incubation of human, rat, mouse, dog and rabbit hepatocytes with [^{14}C]-testosterone as positive control for Phase 1

The first positive control assay consisted of the evaluation of the ability of the cryo-preserved hepatocytes to metabolise testosterone. For this purpose, 10 µM [^{14}C]-testosterone was incubated with a volume of 500 µL cryo-preserved hepatocytes in HPM-Cryo. Incubations were run for 120 min.

The radioactivity in the supernatants was determined by LSC and investigated qualitatively and quantitatively by HPLC.

Incubation of human, rat, mouse, dog and rabbit hepatocytes with 7-hydroxycoumarin as positive control for Phase 2

The second positive control assay consisted of the evaluation of the ability of the cryo-preserved hepatocytes to metabolise 7-hydroxycoumarin. For this purpose, 3.244 mg/L solution of 7-hydroxycoumarin was incubated with a volume of 500 µL cryo-preserved hepatocytes in HPM-Cryo. Incubations were run for 120 min.

C. ANALYTICAL PROCEDURES

Radioactivity Measurement

Radioassaying of liquid samples was carried out by liquid scintillation counting (LSC) with automatic quench correction.

High Performance Liquid Chromatography (HPLC)

The test item [^{14}C]-glyphosate, the reference items, testosterone/7-hydroxycoumarin and their metabolites were qualitatively and quantitatively determined using HPLC. For all samples HPLC analysis was performed on an Agilent 1260 system with radiometric- and UV-detection.

To ensure the quantitative elution of the injected radioactivity from the HPLC-column, the radioactivity of aliquots was measured before and after injection.

The chromatographic recovery for [^{14}C]-glyphosate accounted for 96.7 - 100.1 % of the injected radio-activity indicating almost quantitative elution of the injected radioactivity.

Evaluation and presentation of the results

The cell vitality is calculated based on the ratio between counted living and total cells multiplied with the trypan blue dilution factor, the volume of the cell suspension and the factor of the Neubauer counting chamber according to following equation:

$$cell\ vitality = \emptyset\ Number\ of\ cells\ (living/total\ cells)\ \times trypan\ blue\ dilution\ factor \times volume\ cell\ suspension\ \times factor\ of\ Neubauer\ counting\ chamber$$

The relative percentages were calculated based on the area values of the HPLC profiles at the different incubation times according to following equation:

$$\%\ Relative\ P_i = \frac{(Area\ P_i)}{\sum Area\ P} \times 100$$

Where Area P_i is the area of a compound peak in the HPLC profile of a test sample, and $\sum$ Area P is the sum of the total radioactive peak areas in the chromatogram.

The metabolic transformation rate of the test item [^{14}C]-glyphosate over time was calculated according to the following equation:

$$\%\ Metabolic\ transformation\ rate$$

$$= 100 - \frac{\%\ Area\ test\ item\ incubation\ 0,\ \ 60\ or\ 120\ min}{\%\ Area\ reference\ item\ incubation\ 0\ min} \times 100\%$$

Where the relative percentages of the peak area of the test item after 0, 60 or 120 min incubation time were

compared to the relative percentage measured at 0 min incubation time of the control incubation without hepatocytes.

The metabolic transformation rate of the positive control compounds [^{14}C]-testosterone and 7-hydroxycoumarin over time was calculated according to following equation:

$$\%\ Metabolic\ transformation\ rate = 100 - \frac{\%\ Area\ positive\ control\ incubation\ 120\ min}{\%\ Area\ positive\ control\ incubation\ 0\ min} \times 100\%$$

Where the relative percentages of the peak area of the positive control after 0, 60 or 120 min incubation time were compared to the relative percentage measured at 0 min incubation time.

High Performance Mass Spectrometry (LC-MS)

HPLC combined with high resolution mass spectrometry was used to confirm the presence of the test item and available references. Samples were analysed by LC-MS using a Thermo Orbitrap QExactive Plus operating at a mass resolution of 70000 in positive and in negative ion mode. MS/MS product ion spectra were obtained using collision induced dissociation (CE 30) at a resolution of 17500.

As the resolution of the reference item AMPA in the analytical standard with UV detection has been insufficient, the presence of AMPA in the analytical standard was confirmed by HPLC combined with MS (full scan and negative ion mode) too. Therefore, the analytical standard of AMPA was measured using HPLC and the fraction where AMPA was assumed to retarded was isolated and then measured.

For the HPLC analysis a quantifiable radioactive peak (LOQ) was regarded as relevant having a signal at least higher than the possible statistical deviation of the background, which was set to 3 Sigma expected for testosterone incubates (6 Sigma). Sigma is the square root of the background part within the integration region. 3 Sigma therefore means 3 x the square root of the background part within the integration region. The limit of detection (LOD) was half this amount. Based on the estimated LOQ of the HPLC-analysis of incubates (0.10 - 1.97 % of the applied radioactivity), the profiling HPLC-methods ensured enough sensitivity for the detection of the test item, the reference item and their metabolites.

For Liquid Scintillation counting the detection limit was predefined on the base of the background radioactivity-counting rate, which was about 24.3 cpm. For the calculations, a reference point of 20 dpm was set as average background radioactivity. The limit of detection (LOD) was established as twice the average background radioactivity (40 dpm). The limit of quantification (LOQ) was established as three times the average background radioactivity (60 dpm). Single background subtraction and a quench and counting efficiency correction for transformation of gross counts (cpm) into net counts (dpm) were automatically performed by the instruments. Samples with individually measured values below 40 dpm (after correction for the background radioactivity) were not quantified and labelled as “n.q.” in the respective tables.

II. RESULTS AND DISCUSSION

A. CELL VITALITY

After reprocessing of the cryo-preserved hepatocytes, the cell vitality as number of living cells in ratio to total counted cells was determined for human, rat, mouse, dog and rabbit hepatocytes. The cell vitality amounted to 87, 83, 90, 92 and 91 % at the test item concentration of 1 µM and 84, 83, 86, 86 and 91 % at the test item concentration of 10 µM, respectively.

B. RECOVERY OF RADIOACTIVITY

The recovery of radioactivity at 0 min of the supernatants of treated hepatocytes ranged between 93.5 - 109.4 % of the applied radioactivity for the test item at 1 and 10 µM. After 60 min incubation the recoveries were between 93.1 - 103.4 % for the test item at 1 and 10 µM. After 120 min incubation the mean recoveries were between 90.7 - 106.4 % for the test item at 1 and 10 µM. The recovery for negative controls (incubation of 10 µM test item and inactivated hepatocyte) was between 90.4 – 106.4 % after 0 min and 90.2 - 101.2 % after 120 min.
The incubation of inactivated cryo-preserved hepatocytes with the test item (10 µM) proved that the biotransformation of the test item is dependent on the presence of activated cryo-preserved hepatocytes.

Table 5.1-60: Metabolic stability and profiling of [^{14}C]-Glyphosate in hepatocytes from human, rat, mouse, dog and rabbit for inter-species comparison ([redacted] 2020): Recovery of radioactivity in supernatants after incubation of 1 and 10 µM [^{14}C]-glyphosate with cryo-preserved hepatocytes from human, rat, mouse, dog and rabbit and inactivated hepatocytes

Sample description	Incubation time [min]	Recovery of applied radioactivity [%]		
		Activated hepatocytes (1 µM)	Activated hepatocytes (10 µM)	Inactivated hepatocytes (10 µM)
Human	0	97.7	97.1	105.1
		100.5	99.0	98.3
Rat		102.9	101.6	100.6
		109.4	100.1	90.4
Mouse		93.5	100.7	106.4
		94.6	99.3	90.6
Dog		102.3	98.2	104.0
		101.2	100.2	104.0
Rabbit		103.3	96.5	98.8
		104.0	96.8	-
Human	60	98.5	96.4	-
		99.5	99.7	-
Rat		102.4	102.2	-
		99.7	101.5	-
Mouse		93.1	98.1	-
		93.7	99.0	-
Dog		102.5	97.7	-
		102.1	96.8	-
Rabbit		98.6	95.2	-
		103.4	93.3	-
Human	120	99.9	96.0	98.3
		102.1	98.0	101.2
Rat		99.1	103.5	90.2
		99.7	101.6	94.7
Mouse		90.7	98.3	99.3
		92.9	96.0	98.3
Dog		99.4	95.6	100.9
		101.9	97.5	100.1
Rabbit		100.8	94.8	94.4
		106.4	96.2	94.3

Table 5.1-61: Metabolic stability and profiling of [^{14}C]-Glyphosate in hepatocytes from human, rat, mouse, dog and rabbit for inter-species comparison ([redacted] 2020): Recovery of radioactivity in supernatants and [^{14}C]-testosterone and 6β-hydroxytestosterone concentrations in percent of total radioactive peak area after incubation of 10 µM [^{14}C]-testosterone with cryo-preserved hepatocytes from human, rat, mouse, dog and rabbit

Sample description	Incubation time [min]	Recovery of applied RA [%]	Area of [^{14}C]-testosterone [%*]	Area of 6β-hydroxy-testosterone [%]
Human	120	104.0	14.80	1.67
		101.9		
Rat		109.6	3.37	10.17
		110.5		
Mouse		106.8	23.80	10.89
		107.7		
Dog		102.4	37.95	6.40
		103.5		
Rabbit		103.6	2.91	ND
		108.9		

RA radioactivity; samples were taken after 120 min of incubation and analysed by LSC
ND not detected
* % peak area

C. BIOTRANSFORMATION OF [^{14}C]-GLYPHOSATE

The highest transformation rate (1.71 %) was observed after 0 minutes of incubation at 10 µM [^{14}C]-glyphosate with mouse cryo-preserved hepatocytes. The results are compiled in the table below.

Table 5.1-62: Metabolic stability and profiling of [^{14}C]-Glyphosate in hepatocytes from human, rat, mouse, dog and rabbit for inter-species comparison ([redacted] 2020): Concentration of [^{14}C]-glyphosate in percent of total radioactive peak area and metabolic transformation [^{14}C]-glyphosate after incubation with rats, mouse, dog, rabbit and human hepatocytes at 1 and 10 µM for 60 and 120 min

Species	Conc. [µM]	Area of [^{14}C]-glyphosate [%]			Metabolic transformation rate [%]*,**		
		Incubation time [min]			Incubation time [min]		
		0	60	120	0	60	120
Rat	1	99.20	100.00	100.00	0.00	0.00	0.00
	10	98.76	98.46	98.65	0.00	≤1.00	≤1.00
Mouse	1	100.0	100.00	99.10	0.00	0.00	0.00
	10	97.03	97.71	97.18	1.71	1.02	1.56
Dog	1	98.03	100.00	100.00	≤1.00	0.00	0.00
	10	99.09	99.84	99.20	≤1.00	≤1.00	≤1.00
Rabbit	1	99.38	100.00	99.76	0.00	0.00	0.00
	10	98.71	98.14	98.41	≤1.00	≤1.00	≤1.00
Human	1	99.77	100.00	100.00	0.00	0.00	0.00
	10	98.87	97.81	97.74	0.00	≤1.00	≤1.00

* The metabolic transformation rate was calculated on the basis of the % area of [^{14}C]-glyphosate of the control incubation without cryo-preserved hepatocytes.
** Metabolic transformation rates between 0 and 1.00 % are reported as ≤1.00 %

D. METABOLITES AFTER BIOTRANSFORMATION OF [^{14}C]-GLYPHOSATE

Incubations of 1 µM [^{14}C]-glyphosate with human, rat, dog, mouse and rabbit hepatocytes revealed the parent substance and three further unknown substances/peaks during the incubation period. One peak "Unknown 1" showed the highest abundance (2.0 %) in dog hepatocytes after an incubation time of 0 min. The highest concentration of another not further identified peak "Unknown 2" was detected in rat hepatocytes after an incubation time of 0 min. No major metabolites (>5 % AR) were detected after incubation of hepatocytes of any species with 1 µM [^{14}C]-glyphosate at any time point.

Incubations of 10 µM [^{14}C]-glyphosate with human, rat, dog, mouse and rabbit cryo-preserved hepatocytes revealed the parent substance and additional six unknown substances during the incubation period. "Unknown 1" detected in mouse hepatocytes after an incubation time of 120 min showed the highest abundance (2.8 %) but was already detected at 2.6 % after 0 min of incubation. Further unknown substances/peaks were detected in low concentrations. Most of those were however already present before incubation, decreased by time or were just observed at one time point. No major metabolites (>5 % AR) were detected after incubation of hepatocytes of any species with 10 µM [^{14}C]-glyphosate at any time point.

All detected unknown substances in human hepatocytes were also detected in rat, mouse, dog or rabbit hepatocytes. Therefore, no human specific metabolite was observed.

All detected unknown substances were only characterised based on their chromatographic behaviour and a metabolic pathway was not derived. The metabolite AMPA which was characterised based on LC-MS/MS was not detected in any sample.

Table 5.1-63: Metabolic stability and profiling of [^{14}C]-Glyphosate in hepatocytes from human, rat, mouse, dog and rabbit for inter-species comparison ([redacted] 2020): Distribution of the applied radioactivity [%] after incubation of rats, mouse, dog, rabbit and human hepatocytes with 1 and 10 µM [^{14}C]-glyphosate after 0, 60 and 120 min

Species	Metabolite	1 µM			10 µM		
		Incubation time [min]			Incubation time [min]		
		0	60	120	0	60	120
Human	Unknown 1	-	-	-	0.8	2.2	2.0
	Unknown 2	0.2	-	-	0.3	-	-
	Unknown 4	-	-	-	-	-	0.3
	Glyphosate	99.8	100.0	100.0	98.9	97.8	97.7
Rat	Unknown 1	-	-	-	0.9	0.9	1.2
	Unknown 2	0.8	-	-	-	-	-
	Unknown 4	-	-	-	0.3	0.3	0.2
	Unknown 5	-	-	-	-	0.4	-
	Glyphosate	**99.2**	**100.0**	**100.0**	**98.8**	**98.5**	**98.7**
Mouse	Unknown 1	-	-	0.3	2.6	1.9	2.8
	Unknown 2	-	-	0.3	-	-	-
	Unknown 3	-	-	-	-	0.2	-
	Unknown 4	-	-	-	0.35	0.2	-
	Unknown 5	-	-	0.3	-	-	-
	Glyphosate	**100.0**	**100.0**	**99.1**	**97.0**	**97.7**	**97.2**

Table 5.1-63: Metabolic stability and profiling of [14C]-Glyphosate in hepatocytes from human, rat, mouse, dog and rabbit for inter-species comparison ([redacted] 2020): Distribution of the applied radioactivity [%] after incubation of rats, mouse, dog, rabbit and human hepatocytes with 1 and 10 µM [14C]-glyphosate after 0, 60 and 120 min

Species	Metabolite	1 µM			10 µM		
		Incubation time [min]			Incubation time [min]		
		0	60	120	0	60	120
Dog	Unknown 1	2.0	-	-	0.3	-	0.3
	Unknown 2	-	-	-	0.4	0.2	0.3
	Unknown 4	-	-	-	0.2	-	-
	Unknown 6	-	-	-	-	-	0.3
	Glyphosate	**98.0**	**100.0**	**100.0**	**99.1**	**99.8**	**99.2**
Rabbit	Unknown 1	0.6	-	-	1.8	1.3	1.4
	Unknown 2	-	-	0.2	-	-	-
	Glyphosate	**99.4**	**100.0**	**99.8**	**98.2**	**98.7**	**98.6**

E. BIOTRANSFORMATION OF POSITIVE CONTROLS

[14C]-Testosterone

6β-Hydroxytestosterone was selected as a biological transformation marker compound and detected after incubation with [14C]-testosterone for 120 min with human, rat, dog and mouse cryo-preserved hepatocytes. After incubation with rabbit cryo-preserved hepatocytes 6β-hydroxytestosterone was not detected. The relative peak area in percent of [14C]-testosterone was low (2.91 %) while the recovery of applied radioactivity of testosterone for rabbits was high. This suggests that [14C]-testosterone was further transformed. The highest metabolic activity was thus detected for rabbit female cryo-preserved hepatocytes. The concentration of [14C]-testosterone was 2.91 % after 120 min. The concentration of [14C]-testosterone in human hepatocytes was 14.80 % after 120 min. The highest [14C]-testosterone concentration after 120 min was 37.95 % in dog hepatocytes.

7-Hydroxycoumarin

7-Hydroxycoumarin sulphate was found after incubation with 7-hydroxycoumarin for 120 min with rat and dog cryo-preserved hepatocytes. 7-Hydroxycoumarin glucuronide was found after incubation with 7-hydroxycoumarin for 120 min with human, rat, mouse, dog and rabbit cryo-preserved hepatocytes.
The lowest concentration of 7-hydroxycoumarin, considered as marker for the highest metabolic activity, was measured for rat cryo-preserved hepatocytes and amounted to 10.01 % of 7-hydroxycoumarin after 120 min. 25.43 % of the total applied radioactivity was identified as 7-hydroxycoumarin after 120 min incubation with human cryo-preserved hepatocytes while 54.44 % of the totally applied radioactivity was identified as the respective glucuronide conjugate.

Table 5.1-64: Metabolic stability and profiling of [^{14}C]-Glyphosate in hepatocytes from human, rat, mouse, dog and rabbit for inter-species comparison ([redacted] 2020): Recovery of radioactivity after incubation of rat, mouse, dog, rabbit and human hepatocytes with 10 µM [^{14}C]-testosterone and hydroxycoumarin, respectively after 120 min

Species	6β-hydroxytestosterone	[^{14}C]-testosterone	7-hydroxy coumarin glucuronide	7-hydroxy coumarin	7-hydroxy coumarin sulphate
	% Relative peak area				
Human	1.67	14.80	54.44	25.43	-
Rat	10.17	3.37	23.11	10.01	6.97
Mouse	10.89	23.80	33.87	12.94	-
Dog	6.40	37.95	49.50	11.33	10.94
Rabbit	-	2.91	47.31	27.37	-

III. CONCLUSIONS

Incubations with cryo-preserved hepatocytes of all tested species exhibited a very similar biotransformation pattern of the applied [^{14}C]-glyphosate. The highest biotransformation rate of [^{14}C]-glyphosate was detected after incubation of 10 µM with cryo-preserved hepatocytes from mouse (1.71 %). The biotransformation rate after incubations with human, rat, dog and rabbit hepatocytes was ≤1.00 %.
All detected unknown substances were characterised based on their chromatographic behaviour. No AMPA was detected in hepatocytes of any species after an incubation time of 120 min with 1 and 10 µM [^{14}C]-glyphosate, respectively. No human unique metabolites were detected.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The *in-vitro* metabolite profile of 1 µM and 10 µM [^{14}C]-glyphosate was determined after incubation with cryo-preserved hepatocytes from human, rat, dog, mouse and rabbit for 0, 60 and 120 min at 37°C and analysis by HPLC and liquid scintillation counting. Based on the estimated limit of quantitation (LOQ) of the HPLC-analysis of incubates, the profiling HPLC methods ensured enough sensitivity for the detection of the test item, the reference item and their metabolites. The metabolic capability of the used cryo-preserved hepatocytes batches was proven by control incubations with 7-hydroxycoumarine and [^{14}C]-testosterone. Negative controls with inactivated hepatocytes confirmed the stability of the test item in the test medium.

After incubation of cryo-preserved hepatocytes of all tested species with 1 and 10 µM [^{14}C]-glyphosate no relevant metabolism was observed in any species. At least 97 % of the applied radioactivity was identified as glyphosate. The remaining proportion of the applied radioactivity was detected as several smaller peaks. The structure of the respective substances was not elucidated. The highest radioactivity of such a unknown substance reached up to 2.8 % of applied radioactivity for "Unknown 1" in mouse hepatocytes after 120 minute incubation. However, this substance was already detected at 2.6 % in mouse hepatocytes without incubation time, indicating that this substance is not a metabolite of mouse hepatocytes. Further unknown substances/peaks were detected in lower concentrations for all species. Most of those were however already present before incubation, decreased by time without appearance of another unknow substance or were just observed at one time point. Thus, most of the identified unknow substances are considered to be not metabolites of glyphosate. No AMPA was detected in hepatocytes of any species after an incubation time of 120 min with 1 and 10 µM [^{14}C]-glyphosate, respectively.
Taken together, no major metabolites (>5 % AR) were detected after incubation of hepatocytes of any species with 10 µM [^{14}C]-glyphosate at any time point and no human unique metabolites were detected.

Assessment and conclusion by RMS:

CA 5.2 Acute toxicity

Glyphosate (glyphosate acid and its salts) is of low acute toxicity independently of the application route. The oral and dermal LD_{50} values were constantly higher than the limit test doses. These limit test doses are different according to the EU (2000 mg/kg bw) and GHS classification (5000 mg/kg bw) systems. Overall, it can be concluded that the endpoint LD_{50} is higher than 5000 mg/kg bw for the acute oral and dermal toxicity. General signs of oral intoxication were breathing difficulties, reduced activity, diarrhoea, ataxia and convulsions.

The acute inhalation toxicity was also low with LC_{50} values above the limit dose of 2 mg/L air or 5 mg/L air per 4 hours obtained for glyphosate acid and glyphosate salts (isopropylamine (IPA) and potassium salt). Achieving inhalation doses up to the limit dose of 5 mg/L was often technically challenging. Clinical signs after inhalation exposure included slight dyspnoea, increased or decreased respiratory rate, slight tremor and a slight decrease in body weight but were not consistently observed throughout the studies.

Regarding primary irritation, glyphosate acid and the salts were found to be non-irritant to intact skin and only slightly irritant to abraded skin. Glyphosate acid was found to be strongly irritating to rabbit eyes requiring classification; **'Irreversible effects on the eye/serious damage to eyes (Category 1)'** under GHS/CLP. There was markedly less eye irritation observed with the salts which are used in formulated products, presumably due to the salts having a more neutral pH than glyphosate acid. Based on the results of available studies, classification according to criteria of the CLP Regulation (EC No. 1272/2008) is considered not needed for the glyphosate salts.

Neither glyphosate acid nor the salts have shown sensitizing effects in guinea pigs. However, only the acid and the IPA salt have been tested under the more stringent conditions of the Magnusson-Kligman test.

A phototoxicity study is not required as glyphosate does not absorb light the range of 290-700 nm. The ultraviolet/visible molar extinction/absorption coefficient of glyphosate is smaller than 10 L/(mol*cm).

Nota bene: In autumn of 2019 NGOs and media published accusations against the German based contract research organization "[redacted]" on animal welfare and GLP. In the frame of this glyphosate renewal dossier GRG is submitting 24 studies performed during the years 1995 – 2010 at [redacted]. All these studies were conducted during the validity periods of GLP certificates routinely renewed and re-confirming the GLP-standard every three years. Studies performed at this laboratory are clearly indicated in the overview table (Table 5.2.1-1, 5.2.2.-1, 5.2.3-1, 5.2.4-1, 5.2.5-1 and 5.2.6-1) as well as in the respective study header. Due to the large number of corroborative studies available within the Glyphosate Renewal Group, the overall endpoint conclusion of glyphosate is not reliant on the results of the studies performed at [redacted].

CA 5.2.1 Oral

Acute oral toxicity of glyphosate was assessed in a total of 39 studies with rats and mice, of which 29 were performed with glyphosate acid and ten with glyphosate salts (IPA, ammonium salt and sodium salt).

A majority of the studies using glyphosate acid as the test substance was performed in rats (25 studies) while four studies were performed in mice. The LD_{50} exceeded 2000 mg/kg bw in all of these studies. In three of the four mice studies the LD_{50} was greater than the applied top dose of 5000, 2000 and 7500 mg/kg

bw, while in one study (CA 5.2.1/036) the LD_{50} was 4000 mg/kg bw. In rats the LD_{50} was greater than the applied top dose of 2000 mg/kg bw in 11 of 11 studies. In studies applying doses greater than 2000 mg/kg bw the LD_{50} was greater than 5000 mg/kg in nine of 12 studies. In one study of the 25 studies performed with glyphosate acid in rats the LD_{50} value was 5600 mg/kg bw (CA 5.2.1/039), while the LD_{50} values in two studies exceeded 7500 mg/kg bw and 8000 mg/kg bw (CA 5.2.1/026 and CA 5.2.1/028). Mortality in rats and mouse occurred in six studies (two rat and four mouse studies) starting from a dose level of 2500 mg/kg bw. Clinical findings, if any, that were repeatedly observed included salivation, piloerection, diarrhea, decreased activity, ruffled fur, sedation and ataxia. Conclusively, glyphosate acid shows a low toxic potential in rats and mice by the oral route and does not need to be classified for acute oral toxicity according to the CLP Regulation (EU) No. 1272/2008.

Acute oral toxicity of glyphosate salts was investigated in three studies in mice and seven studies in rats. The LD_{50} exceeded 2000 mg/kg bw in all of these studies. The LD_{50} of glyphosate salts in mice were 3669, 4373 and >5000 mg/kg bw (CA 5.2.1/035, CA 5.2.1/034 and CA 5.2.1/014, respectively). Thus, the LD_{50} was below 5000 mg/kg bw in two of three studies performed with glyphosate salt in mice. The LD_{50} of glyphosate salts in rats always exceeded the limit dose of 2000 (CA 5.2.1/018, CA 5.2.1/030) and 5000 (CA 5.2.1/012, CA 5.2.1/020 and CA 5.2.1/038) mg/kg bw, except for one study (CA 5.2.1/032) where no mortality occurred at 2222 mg/kg bw but 5/5 female and 1/5 male rats were found dead at 5000 mg/kg bw. The acute oral LD_{50} in males was calculated to be 5904 mg/kg bw and the combined LD_{50} was calculated to be 4613 mg/kg bw in this study. Common clinical signs, if any, included diarrhea and soft feces when administered up to a dose of 5000 mg/kg bw. Generally, the administration of glyphosate acid and salts at doses above 5000 mg/kg bw triggered mortality and further clinical signs. In conclusion, glyphosate salts show a low toxic potential in rats and mice by the oral route and do not need to be classified for acute oral toxicity according to the CLP Regulation (EU) No. 1272/2008.

Overall, the 39 studies presented in the following chapter, demonstrate that glyphosate acid and glyphosate salt possess a low toxic potential when administered orally to rats and mice.

Table 5.2.1-1: Studies on acute oral toxicity with glyphosate

Annex Point	Study	Study type	Substance(s)	Reference list- related category[§]	Result [LD_{50}]
CA 5.2.1/001	[redacted], 2014	*in vivo*, RccHanTM:Wistar rats, ♀ (fixed dose method)	Glyphosate technical (Batch: 04062014, Purity: 85.79 %)	Valid, Category 2a	>2000 mg/kg bw (females)
CA 5.2.1/002	[redacted] 2011	*in vivo:* RjHan:WI rats, ♀ (up and down procedure)	Glyphosate technical (Batch: 569753(BX20070911), Purity: 96.3 %)	Valid, Category 2a	>5000 mg/kg bw (females)
CA 5.2.1/003	[redacted] 2010	*in vivo:* CD / Crl:CD(SD) rats, ♀ (ATC method)	Glyphosate technical (Batch: 2009051501, Purity: 96.4 %)	Valid[#], Category 2a	>2000 mg/kg bw (females)
CA 5.2.1/004	[redacted] 2010	*in vivo:* CD / Crl:CD(SD) rats, ♀ (ATC method)	Glyphosate technical (Batch: 20090506, Purity: 97.3 %)	Valid[#], Category 2a	>2000 mg/kg bw (females)
CA 5.2.1/005	[redacted] 2009	*in vivo:* CD / Crl:CD(SD) rats, ♀ (ATC method)	Glyphosate technical (Batch: 20080801, Purity: 98.8 %)	Valid[#], Category 2a	>2000 mg/kg bw (females)
CA 5.2.1/006	[redacted] 2009	*in vivo:* HanRcc: WIST (SPF) rats, ♀ (ATC method)	Glyphosate technical (Batch: GI-1045, Purity: 96.66 %)	Valid, Category 2a	>2000 mg/kg bw (females)
CA 5.2.1/007	[redacted], 2009	*in vivo:* Sprague-Dawley rats, ♀ (up and down procedure)	Glyphosate tech grade mixed 5-batch (Batch: 080704-1 thru 5, Purity:	Valid, Category 2a	>5000 mg/kg bw (females)

Table 5.2.1-1: Studies on acute oral toxicity with glyphosate

Annex Point	Study	Study type	Substance(s)	Reference list- related category[§]	Result [LD_{50}]
			96.40 %)		
CA 5.2.1/008	[redacted] 2008	*in vivo:* Wistar Hannover rats, ♀ (ATC method)	Glyphosate technical (Batch: 20070606, Purity: 98.05 %)	Valid, Category 2a	>2000 mg/kg bw (females)
CA 5.2.1/009	[redacted] 2007	*in vivo:* HanRcc: WIST (SPF) rats, ♀ (up and down procedure)	Glyphosate technical material (Batch: 0507, Purity: 96.1 %)	Valid, Category 2a	>5000 mg/kg bw (females)
CA 5.2.1/010	[redacted] 2007	*in vivo:* HanRcc: WIST (SPF) rats, ♀ (ATC method)	Glyphosate technical (Batch: 200609062, Purity: 95.1 %)	Valid, Category 2a	>2000 mg/kg bw (females)
CA 5.2.1/011	[redacted] 2005	*in vivo:* Sprague-Dawley derived rats, ♀ (up and down procedure)	Glyphosate acid technical (Batch: 040205, Purity: 97.23 %)	Valid, Category 2a	>5000 mg/kg bw (females)
CA 5.2.1/012	[redacted], 1999	*in vivo:* Sprague-Dawley derived, albino rats, ♂ / ♀	NUP5a99 (Batch: Drum Sample E, Purity: 62 %) IPA salt ##	Supportive Category 2a	>5000 mg/kg bw
CA 5.2.1/013	[redacted], 1996	*in vivo:* Alpk:AP$_f$SD (Wistar-derived) rats, ♂ / ♀	Glyphosate acid, (Batch: P24, Purity: 95.6 %)	Valid, Category 2a	>5000 mg/kg bw
CA 5.2.1/014	[redacted] 1995	*in vivo:* Crj:CD-1(ICR) mice, ♂ / ♀	MON 0139 (Batch: LBRV-11092, Purity: 62.34 %) IPA salt ##	Valid, Category 2a	>5000 mg/kg bw
CA 5.2.1/015	[redacted], 1995	*in vivo:* Sprague-Dawley (Crj:CD), SPF, albino rats, ♂ / ♀	Glyphosate technical, HR-001 (Batch: 940908-1, Purity: 95.68 %)	Valid, Category 2a	>5000 mg/kg bw
CA 5.2.1/016	[redacted], 1995	*in vivo:* ICR (Crj:CD-1), SPF mice, ♂ / ♀	Glyphosate technical, HR-001 (Batch: 940908-1, Purity: 95.68 %)	Valid, Category 2a	>5000 mg/kg bw
CA 5.2.1/017	[redacted] 1995	*in vivo:* rats (limit test)	Glyphosate acid technical (Batch: 1073, Purity: 97.6 %)	Valid, Category 2a	>2000 mg/kg bw
CA 5.2.1/018	[redacted] 1995	*in vivo:* rats (limit test)	Glyphosate (Batch: 940950, Purity: 62 % IPA) ##	Supportive, Category 2a	>2000 mg/kg bw
CA 5.2.1/019	[redacted] 1995	*in vivo:* rats	Not applicable	Valid, Category 4a	Not applicable
CA 5.2.1/020	[redacted] 1994	*in vivo:* Sprague-Dawley rats, ♂ / ♀	Glyphosate Premix (Batch: 290-JaK-146-4, Purity: 46.1 % (Glyphosate), 62.2 % (IPA salt) ##	Supportive, Category 2a	>5000 mg/kg bw
CA 5.2.1/021	[redacted] 1994	*in vivo:* rats (limit test)	Glyphosate	Valid, Category 4a	>2000 mg/kg bw
CA 5.2.1/022	[redacted] 1994	*in vivo:* Wistar rats, ♂ / ♀	Glyphosate Technical (Batch: 36300892, Purity: 99.6 %)	Valid, Category 2a	>5000 mg/kg bw
CA 5.2.1/023	[redacted] *et al.*, 1994	*in vivo:* rats	Glyphosate technical	Valid, Category 4a	>2000 mg/kg bw
CA	[redacted] &	*in vivo:* Sprague-	Glyphosate (Batch:	Valid,	>2000 mg/kg bw

Table 5.2.1-1: Studies on acute oral toxicity with glyphosate

Annex Point	Study	Study type	Substance(s)	Reference list- related category§	Result [LD_{50}]
5.2.1/024	[redacted], 1992	Dawley rats, ♂ / ♀	L3258; purity: not specified)	Category 2a	
CA 5.2.1/025	[redacted] 1991	*in vivo:* Bom:NMRI mice, ♂ / ♀	Glyphosate Technical (PMG) (Batch: 206-JaK-25-1, Purity: 98.6 %)	Valid, Category 2a	>2000 mg/kg bw
CA 5.2.1/026	[redacted], 1991	*in vivo:* Wistar rats, ♂ / ♀	Glyphosate Technical (Batch: 60, Purity: 96.80 %)	Valid, Category 2a	>7500 mg/kg bw
CA 5.2.1/027	[redacted], 1991	*in vivo:* Swiss albino mice, ♂ / ♀	Glyphosate Technical (Batch: 60, Purity: 96.80 %)	Valid, Category 2a	>7500 mg/kg bw
CA 5.2.1/028	[redacted] 1990	*in vivo:* CD rats, ♂ / ♀	Glyphosate Technical (Batch: 0190 A, Purity: 98.1 %)	Valid, Category 2a	>8000 mg/kg bw
CA 5.2.1/029	[redacted] 1989	*in vivo:* Sprague-Dawley rats, ♂ / ♀	Glyphosate Technical (PMG) (Batch: 206-JaK-25-1, Purity: 98.6 %)	Supportive, Category 2a	>5000 mg/kg bw
CA 5.2.1/030	[redacted] *et al.*, 1989	*in vivo:* rats	Glyphosate technical (IPA salt 62 %) ##	Valid, Category 4a	>2000 mg/kg bw
CA 5.2.1/031	[redacted] 1988	*in vivo:* Sprague-Dawley rats, ♂ / ♀	Glyphosate (Batch: XLI-55, Purity: 97.76 %)	Valid, Category 2a	>5000 mg/kg bw
CA 5.2.1/032	[redacted], 1987	*in vivo:* Sprague-Dawley rats, ♂ / ♀	Glyphosate (MON8750) (Batch: XLG-255, Purity: 90.8 %, ammonium salt) ##	Valid, Category 2a	5904 mg/kg bw (males) >2222 mg/kg bw (females) 4613 (males/females) mg/kg bw
CA 5.2.1/033	[redacted] 1987	*in vivo:* Sprague-Dawley rats	Glyphosate (MON8722) (Purity: 80.3 %, sodium salt) ##	Valid, Category 4a	Ataxia, decreased activity, diarrhea, rectal sores
CA 5.2.1/034	[redacted], 1987	*in vivo:* mice	SN750721 (Purity: 64 %) IPA salt ##	Valid, Category 4a	4373 mg/kg bw
CA 5.2.1/035	[redacted] 1987	*in vivo:* mice	SN750721 (Purity: 41 %) IPA salt ##	Valid, Category 4a	3669 mg/kg bw
CA 5.2.1/036	[redacted] 1983	*in vivo:* Kasauli mice, ♂ / ♀	Glyphosate Technical (Batch: R&D sample (9-7-83), Purity: 95 %)	Supportive, Category 3a	4000 mg/kg bw
CA 5.2.1/037	[redacted], 1983	*in vivo:* rats	Glyphosate (tech.)	Supportive, Category 4a	Not applicable
CA 5.2.1/038	[redacted] 1981	*in vivo:* Sprague-Dawley (Crl:CD® (SD)BR) rats, ♂ / ♀	Glyphosate (MON 0139) (Batch: SSRT-11012, Purity: 65 %) IPA salt ##	Supportive, Category 2a	>5000 mg/kg bw
CA 5.2.1/039	[redacted] *et al.,* 1979	*in vivo:* Wistar rats, ♂ / ♀	Glyphosate technical (Batch: XHI-180, Purity: 99 %)	Supportive, Category 2a	5600 mg/kg bw

#: This study was performed at the [redacted]
##: This study was performed with a glyphosate salt
§: The category describes the acceptability/reliability of the study within the AIR 5 submission. This category does not describe hazard classification. (for details please refer to the Doc ID: 110054-B-GRG_Jun_2020)

1. Information on the study

Data point:	CA 5.2.1/001
Report author	[redacted]
Report year	2014
Report title	Glyphosate: Acute Oral Toxicity in the Rat – Fixed Dose Method
Report No	41401853
Document No	Not reported
Guidelines followed in study	OECD 420 (2001) Method B1 bis (EC) No.440/2008
Deviations from current test guideline (OECD 420, 2001)	None
Previous evaluation	Accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The acute oral toxicity of glyphosate technical was investigated in female rats (5 animals) of the Wistar strain. Following a sighting test at a dose level of 2000 mg/kg bw, an additional four fasted female animals were given a single dose of test item, as a dispersion/suspension in dimethyl sulphoxide by oral gavage to each animal at a dose level of 2000 mg/kg bw and constant dose volume of 10 mL/kg bw. Mortality, body weight and clinical signs were recorded during the subsequent 14 days. All animals were subjected to a gross necropsy at the end of the study.

No clinical signs of sytemic toxicity were noted in the first treated animal during the observation period, whereas hunched posture was noted during the day of dosing in four additional treated animals. All animals showed expected body weight gains over the observation period. No mortality occurred. No abnormal necropsy findings were noted.
The acute oral LD_{50} was

LD_{50}, oral, female rat > 2000 mg/kg bw

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate

Identification:	Glyphosate technical
Description:	White crystalline solid
Lot/Batch #:	04062014
Purity:	85.79 %
Stability of test compound:	Not specified

2. **Vehicle and/or positive control:** Dimethyl sulphoxide
3. **Test animals:**

Species:	Rat
Strain:	RccHanTM:Wistar
Source:	[redacted]
Age:	8 – 12 weeks
Sex:	Female
Weight at dosing:	141 – 171 g
Acclimation period:	At least 5 days
Diet/Food:	2014C Teklad Global Rodent diet supplied by Harlan Laboratories UK Ltd., Oxon, UK, *ad libitum* (except overnight fast prior to dosing and 3-4 hours after dosing)
Water:	Tap water, *ad libitum*
Housing:	Maximum of 4 animals / cage in suspended solid-floor polypropylene cages furnished with woodflakes
Environmental conditions:	Temperature: 19 – 25 °C Humidity: 30 – 70 % Air changes: at least 15 per hour 12-hour light / dark cycle (light during 06:00 – 18:00)

B. STUDY DESIGN AND METHODS

In life dates: 2014-07-10 to 2014-07-30

Animal assignment and treatment:
Female Wistar (RccHanTM:Wist) strain rats were supplied by [redacted]
On receipt the animals were randomly allocated to cages. The females were nulliparous and non-pregnant. The body weight variation did not exceed ±20 % of the body weight of the initially dosed animal. With the exception of an overnight fast immediately before dosing and for approximately three to four hours after dosing, free access to mains drinking water and food was allowed throughout the study.

For the purpose of the study the test item was freshly prepared, as required, as a dispersion/suspension in dimethyl sulphoxide. Dimethyl sulphoxide was used because the test item did not dissolve/suspend in distilled water or arachis oil BP. The test item was formulated within two hours of being applied to the test system. It is assumed that the formulation was stable for this duration.

No analysis was conducted to determine the homogeneity, concentration or stability of the test item formulation. This is an exception with regard to GLP and has been reflected in the GLP compliance statement.

Using available information on the toxicity of the test item, 2000 mg/kg bw was chosen as the starting dose (one animal) and administered at a dosing volume of 10 mL/kg. In the absence of toxicity at a dose level of 2000 mg/kg bw, an additional group of four animals was treated. All animals were dosed once only by gavage, using a metal cannula attached to a graduated syringe.

Clinical observation was made 0.5, 1, 2, and 4 hours after dosing and then daily for fourteen days. Morbidity and mortality checks were made twice daily.

Individual body weights were recorded on day 0 (the day of dosing) and on days 7 and 14.

At the end of the observation period the animals were killed by cervical dislocation. All animals were subjected to gross necropsy. This consisted of an external examination and opening of the abdominal and thoracic cavities. The appearance of any macroscopic abnormalities was recorded. No tissues were retained.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortality occurred during the 14-day observation period after administration.

B. CLINICAL OBSERVATIONS

No signs of systemic toxicity were noted in the initial treated animal during the observation period. Hunched posture was noted during the day of dosing in four additional treated animals (see table below).

Table 5.2.1-1: Glyphosate: Acute Oral Toxicity in the Rat – Fixed Dose Method ([redacted] 2014): Summary of clinical observations

Dose group		**2000 mg/kg bw**				
Sex		**Females**				
Time after treatment		0.5 h	1 h	2 h	4 h	Day 1 - 14
Total animals examined	**n**	5	5	5	5	5
Clinical sign	**n**	4	2	1	1	0
Hunched posture	n	4	2	1	1	0
Single animals observations						
Animal No.						
1-0		*	*	*	*	*
2-0		A	*	*	*	*
2-1		A	A	A	A	*
2-2		A	A	*	*	*
2-2		A	*	*	*	*

* = No abnormalities detected, A = ruffled fur

C. BODY WEIGHT

All animals showed expected gains in body weight over the observation period. Individual body weights are provided in the table below.

Table 5.2.1-2: Glyphosate: Acute Oral Toxicity in the Rat – Fixed Dose Method ([redacted] 2014): Body weight and body weight gain

Dose level	**2000 mg/kg bw**				
Sex	**Females**				
Day	**0**	**7**	**14**	**0-7**	**7-14**
Animal No.	**Body weight [g]**			**Body weight gain [g]**	
1-0	171	194	203	23	9
2-0	167	192	211	25	19
2-1	160	180	191	20	11
2-2	141	155	165	14	10
2-3	142	160	178	18	18
Mean ± SD	**156.2 ± 14.0**	**176.2 ± 18.0**	**189.6 ± 18.6**	**20 ± 4.3**	**13.4 ± 4.7**

8 = animal No. of females

Note: Mean and standard deviation were not given in the study report. Values were calculated retrospectively.

D. NECROPSY

No macroscopic findings were recorded at the scheduled necropsy. No tissues were retained.

III. CONCLUSIONS

The acute oral LD_{50} of glyphosate technical in female rats, observed over a period of 14 days was greater than 2000 mg/kg bw in females.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD 420 (2001). The outcome can be therefore reported as valid. The acute oral LD_{50} is above 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.1/002
Report author	[redacted]
Report year	2011
Report title	Glyphosate technical: Acute oral toxicity study in the rat (up and down procedure)
Report No	10/218-001P
Document No	Not reported
Guidelines followed in study	OECD 425 (2008) OPPTS 870.1100 (2002)
Deviations from current test guideline (OECD 425, 2008)	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

Two limit tests with different dose levels were performed. As there were no clinical signs or macroscopic findings observed at the dose level of 2000 mg/kg bw a limit test at a higher dose level (5000 mg/kg bw) was requested by the Sponsor, data from the animals treated at 2000 mg/kg bw were archived in the raw data without any further reporting.

In an acute oral toxicity study (limit test), a group of three, fasted, 10-11 week old, RjHan:WI female rats was given a single oral dose of Glyphosate Technical (96.3 % w/w Glyphosate technical) in 0.5 % carboxymethylcellulose (CMC) at a concentration of 5000 mg/kg bw and administered at a dosing volume of 10 mL/kg bw.

All animals were examined for clinical signs once during the first 30 minutes and at approximately 1, 2, 3, 4 and 6 hours after treatment on day 1 and once daily for 14 days thereafter. Body weights were recorded on day -1 (prior to removal of food), day 0 (prior to administration) and on days 7 and 14. All animals were necropsied and examined macroscopically.

Single animals were dosed sequentially at no less than approximately 48 hour intervals. The time intervals between dosing were determined by the onset, duration and severity of clinical signs.
No deaths occurred during the study. No clinical signs were observed in the 3 animals treated at 5000 mg/kg bw. There was no treatment related changes in the body weights. The body weights of the animals were within the range commonly recorded for this strain and age. No test item-related macroscopic findings were observed.

LD_{50}, oral, female rat > 5000 mg/kg bw

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate technical
Description:	Technical, dry white powder
Lot/Batch #:	569753(BX20070911)
Purity:	96.3 %
Stability of test compound:	Stable under storage conditions (room temperature range < 30 °C), recertification date end August 2011

2. Vehicle and/or positive control: 0.5 % Carboxymethylcellulose (CMC)

3. Test animals:

Species:	Rat
Strain:	RjHan:WI
Source:	[redacted]
Age:	10 – 11 weeks
Sex:	Female
Weight at dosing:	228 – 231 g
Acclimation period:	At least 21 days
Diet/Food:	ssniff® SM R/M-Z+H "Autoclavable complete feed for rats and rats – breeding and maintenance" produced by ssniff Spezialdiäten GmbH, D-59494 Soest Germany, *ad libitum* (except for pre-dose fast and 3 hours after dosing)
Water:	Tap water, *ad libitum*
Housing:	Individually in Type II. polypropylene / polycarbonate cages with Lignocel Bedding for Laboratory Animals
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 30 – 70 % Air changes: 15 – 20 / hour 12-hour light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2011-01-20 to 2011-02-10

Animal assignment and treatment:

In an acute oral toxicity study, a group of three, fasted, 10 – 11 week old, RjHan:WI female rats was given a single oral dose of glyphosate technical (96.3 % w/w glyphosate technical) at a concentration of 5000 mg/kg bw by gavage. The test substance was diluted in vehicle (0.5 % carboxymethylcellulose) and administered at a dosing volume of 10 mL/kg bw.

Single animals were dosed sequentially at no less than approximately 48 hour intervals. The time intervals between dosing were determined by the onset, duration and severity of clinical signs. Treatment of an animal at the next dose was only performed when no significant clinical signs were noted in the previous animal.

All animals were examined for clinical signs once during the first 30 minutes and at approximately 1, 2, 3, 4 and 6 hours after treatment on day 1 and once daily for 14 days thereafter.

Body weights were recorded on day -1 (prior to removal of food), day 0 (prior to administration) and on days 7 and 14.

All animals were exsanguinated under pentobarbital anaesthesia at the end of the observation period, necropsied and examined macroscopically.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities.

B. CLINICAL OBSERVATIONS
No clinical signs were observed in the 3 animals treated at 5000 mg/kg bw.

C. BODY WEIGHT
The body weight of the animals was within the range commonly recorded for this strain and age. Individual body weights are listed below in the table below.

Table 5.2.1-3: Glyphosate technical: Acute oral toxicity study in the rat (up and down procedure) ([redacted] 2011): Body weight development and gain of rats administered glyphosate

Dose group	**5000 mg/kg bw**							
Sex	**Females**							
Day	**-1**	**0**	**7**	**15**	**-1 to 0**	**0 to 7**	**7 to 14**	**-1 to 14**
Animal No.	**Body weight (g)**				**Body weight gain (g)**			
3512	242	229	252	266	-13	23	14	24
3213	247	228	253	263	-19	25	10	16
3511	241	231	245	257	-10	14	12	16
Mean (± SD)	**243.3 ± 3.2**	**229.3 ± 1.5**	**250.0 ± 4.4**	**262.0 ± 4.6**	**-14.0 ± 4.6**	**20.7 ± 5.9**	**12.0 ± 2.0**	**18.7 ± 4.6**

D. NECROPSY
No macroscopic findings were recorded at the scheduled necropsy.

III. CONCLUSIONS

The acute oral LD_{50} of glyphosate technical in female rats, observed over a period of 14 days was greater than 5000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in accordance with the current OECD guideline 425 (2008). The outcome can be therefore reported as valid. The acute oral LD_{50} is above 5000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.1/003
Report author	[redacted]
Report year	2010
Report title	Acute Oral Toxicity Study of Glyphosate TC in Rats
Report No	24874
Document No	Not reported
Guidelines followed in study	EC method B.1 tris (2004/73/EC), OECD 423 (ATC method, 2001) and OPPTS 870.1100
Deviations from current test guideline (OECD 423, 2001)	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid#
Category study in AIR 5 dossier (L docs)	Category 2a

#: Following the feedback from the Assessment Group Glyphosate in March 2020, the Notifier is aware of concerns regarding work conducted at this specific contract laboratory ([redacted]). For the sake of completeness, this study is included in the dossier submission since it was part of the data package of previous EU evaluation processes.

2. Full summary

Executive Summary

The test substance, glyphosate TC, was evaluated for its acute oral toxicity potential in female albino rats when administered as a gavage dose at a level of 2000 mg/kg bw. The Acute Toxic Class Method (ATC method) was employed to establish the required information for hazard assessment and hazard classification. No mortality occurred during the study and no clinical signs were observed. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute oral LD_{50} was determined to be

LD_{50}, oral, female rat > 2000 mg/kg bw

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	Glyphosate TC
Identification:	Glyphosate technical grade
Description:	White powder
Lot/Batch #:	2009051501
Purity:	96.4 %
Stability of test compound:	Expiry date: 2011-05-15
2. Vehicle and/ or positive control:	0.8 % aqueous hydroxypropylmethylcellulose
3. Test animals:	
Species:	Rat albino
Strain / Stock:	CD / Crl:CD(SD)
Source:	[redacted]
Age:	Approx. 7 weeks
Sex:	Female
Weight at dosing:	171 – 192 g
Acclimation period:	5 days
Diet/Food:	ssniff® R/M-H V1534 (ssniff Spezialdiäten GmbH), *ad libitum* except for approx. 16 h before dosing
Water:	Tap water, *ad libitum*
Housing:	Groups of 3 animals were kept in MAKROLON cages (type III plus) with granulated textured wood as bedding material
Environmental conditions:	Temperature: 22 ± 3 °C Rel. humidity: 40 – 70 % 12-hour light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2010-10-15 to 2010-11-10

Animal assignment and treatment:

A group of three fasted females received the test material at a single dose level of 2000 mg/kg bw by oral gavage. The dosing volume was 10 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made before, immediately, 5, 15, 30 and 60 min, as well as 3, 6 and 24 hours after administration and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and thereafter in weekly intervals up to the end of the study.
On Day 14 after dosing, all animals were sacrificed, dissected and inspected macroscopically. All gross pathological changes were recorded. No microscopic examination was performed as no pathological findings were noted at necropsy.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

No clinical signs were observed during the study.

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance (see table below).

Table 5.2.1-4: Acute Oral Toxicity Study of Glyphosate TC in Rats ([redacted] 2010): Body weight development

Dose group		**2000 mg/kg bw**				
Sex		**Females**				
Step	**Animal No.**	**Body weight (g)**		**Body weight gain (%)**	**Body weight (g)**	**Body weight gain (%)**
		Day 1	**Day 8**		**Day 15**	
1	1	188	219	+16.5	230	+22.3
	2	180	217	+20.6	227	+26.1
	3	183	212	+15.8	226	+23.5
	Mean (± SD)	**183.7 ± 4.0**	**216.0 ± 3.6**	**+17.6**	**227.7 ± 2.1**	**+24**
2	4	171	198	+15.8	208	+21.6
	5	192	218	+13.5	226	+17.7
	6	187	214	+14.4	223	+19.3
	Mean (± SD)	**183.3 ± 11.0**	**210.0 ± 10.6**	**+14.6**	**219 ± 9.6**	**+19.5**

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute oral LD_{50} of glyphosate technical in female rats, observed over a period of 14 days was greater than2000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study is in concordance with the current OECD guideline 423 (2001). The outcome can be therefore reported as valid. The acute oral LD_{50} is above 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.1/004
Report author	[redacted]
Report year	2010
Report title	Acute Oral Toxicity Study of Glyphosate TC in Rats
Report No	24602
Document No	Not reported
Guidelines followed in study	EC method B.1 tris (2004/73/EC), OECD 423 (ATC method, 2001) and OPPTS 870.1100 (1998)
Deviations from current test guideline (OECD 423, 2001)	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid#
Category study in AIR 5 dossier (L docs)	Category 2a

#: Following the feedback from the Assessment Group Glyphosate in March 2020, the Notifier is aware of concerns regarding work conducted at this specific contract laboratory ([redacted]). For the sake of completeness, this study is included in the dossier submission since it was part of the data package of previous EU evaluation processes.

2. Full summary

Executive Summary

The test substance, glyphosate technical, was evaluated for its acute oral toxicity potential in female albino rats when administered as a gavage dose at a level of 2000 mg/kg bw. The Acute Toxic Class Method (ATC method) was employed to establish the required information for hazard assessment and hazard classification. No mortality occurred during the study and no clinical signs were observed. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute oral LD_{50} was determined to be

LD_{50}, oral, female rat > 2000 mg/kg bw

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate TC

Identification: Glyphosate technical grade
Description: White powder
Lot/Batch #: 20090506
Purity: 97.3 %
Stability of test compound: Expiry date: May 2011

2. Vehicle and/or positive control: 0.8 % aqueous hydroxypropylmethylcellulose

3. Test animals:

Species:	Rat albino
Strain / Stock:	CD / Crl:CD(SD)
Source:	[redacted]
Age:	Approx. 7 – 8 weeks
Sex:	Female
Weight at dosing:	154 – 196 g
Acclimation period:	5 days
Diet/Food:	ssniff® R/M-H V1534 (ssniff Spezialdiäten GmbH), *ad libitum* except for approx. 16 h before dosing
Water:	Tap water, *ad libitum*
Housing:	Groups of 3 animals were kept in MAKROLON cages (type III plus) with granulated textured wood as bedding material.
Environmental conditions:	Temperature: 22 ± 3 °C Rel. humidity: 40 – 70 % 12-hour light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2009-10-26 to 2009-11-24

Animal assignment and treatment:

A group of three fasted females received the test material at a single dose level of 2000 mg/kg bw by oral gavage. The dosing volume was 10 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made before, immediately, 5, 15, 30 and 60 min, as well as 3, 6, and 24-hours after administration and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and thereafter in weekly intervals up to the end of the study.

On Day 14 after dosing, all animals were sacrificed, dissected and inspected macroscopically. All gross pathological changes were recorded. No microscopic examination was performed as no pathological findings were noted at necropsy.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

No clinical signs were observed during the study.

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance (see table below).

Table 5.2.1-5: Acute Oral Toxicity Study of Glyphosate TC in Rats ([redacted] 2010): Body weight development

Dose group		2000 mg/kg bw				
Sex		Females				
Step	Animal No.	Body weight (g)		Body weight gain (%)	Body weight (g)	Body weight gain (%)
		Day 1	Day 8		Day 15	
1	1	160	207	+29.4	228	+42.5
	2	154	189	+22.7	212	+37.7
	3	159	188	+18.2	205	+28.9
	Mean (± SD)	**157.7 ± 3.2**	**194.7 ± 10.7**	**+23.5**	**215.0 ± 11.8**	**+36.3**
2	4	171	202	+18.1	217	+26.9
	5	185	209	+13.0	226	+22.2
	6	196	252	+28.6	284	+44.9
	Mean (± SD)	**184.0 ± 12.5**	**221.0 ± 27.1**	**+20.1**	**242.3 ± 36.4**	**+31.7**

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute oral LD_{50} of glyphosate technical in female rats, observed over a period of 14 days was greater than 2000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study is in concordance with the current OECD guideline 423 (2001). The outcome can be therefore reported as valid. The acute oral LD_{50} is above 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.1/005
Report author	[redacted]
Report year	2009
Report title	Acute Oral Toxicity Study of Glyphosate TC in Rats
Report No	23910
Document No	Not reported
Guidelines followed in study	EC method B.1 tris (2004/73/EC), OECD 423 (ATC method, 2001) and OPPTS 870.1100 (1998)
Deviations from current test guideline (OECD 423, 2001)	None
Previous evaluation	Yes, accepted in RAR (2015)

GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid#
Category study in AIR 5 dossier (L docs)	Category 2a

#: Following the feedback from the Assessment Group Glyphosate in March 2020, the Notifier is aware of concerns regarding work conducted at this specific contract laboratory ([redacted]). For the sake of completeness, this study is included in the dossier submission since it was part of the data package of previous EU evaluation processes.

2. Full summary

Executive Summary

The test substance, glyphosate technical, was evaluated for its acute oral toxicity potential in female albino rats when administered as a gavage dose at a level of 2000 mg/kg bw. The Acute Toxic Class Method (ATC method) was employed to establish the required information for hazard assessment and hazard classification. No mortality occurred during the study and no clinical signs were observed. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute oral LD_{50} was determined to be

LD_{50}, oral, female rat > 2000 mg/kg bw

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate TC

Identification:	Glyphosate technical grade
Description:	White, solid
Lot/Batch #:	20080801
Purity:	98.8 %
Stability of test compound:	Expiry date: 2010-08-01

2. Vehicle and/or positive control: 0.8 % aqueous hydroxypropylmethylcellulose gel

3. Test animals:

Species:	Rat albino
Strain / Stock:	CD / Crl:CD(SD)
Source:	[redacted]
Age:	50 – 51 days
Sex:	Female
Weight at dosing:	167 – 186 g
Acclimation period:	5 days
Diet/Food:	ssniff® R/M-H V1534 (ssniff Spezialdiäten GmbH), *ad libitum* except for approx. 16 h before dosing
Water:	Tap water, *ad libitum*
Housing:	Groups of 3 animals were kept in MAKROLON cages (type III plus) with granulated textured wood as bedding material

Environmental conditions: Temperature: 22 ± 3 °C
Rel. humidity: 40 – 70 %
12-hour light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2009-02-04 to 2009-03-04

Animal assignment and treatment:

A group of three fasted females received the test material at a single dose level of 2000 mg/kg bw by oral gavage. The dosing volume was 10 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made before, immediately, 5, 15, 30 and 60 min, as well as 3, 6 and 24-hours after administration and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and thereafter in weekly intervals up to the end of the study.

On Day 14 after dosing, all animals were sacrificed, dissected and inspected macroscopically. All gross pathological changes were recorded. No microscopic examination was performed as no pathological findings were noted at necropsy.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

No clinical signs were observed during the study.

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance (see table below).

Table 5.2.1-6: Acute Oral Toxicity Study of Glyphosate TC in Rats ([redacted] 2009): Body weight development

Dose group		**2000 mg/kg bw**				
Sex		**Females**				
Step	Animal No.	**Body weight (g)**		**Body weight gain (%)**	**Body weight (g)**	**Body weight gain (%)**
		Day 1	**Day 8**		**Day 15**	
1	1	167	205	+22.8	213	+27.5
	2	182	213	+17.0	229	+25.8
	3	171	200	+17.0	216	+26.3
	Mean (± SD)	**173.3 ± 7.8**	**206.0 ± 6.6**	**+18.9**	**219.3 ± 8.5**	**+26.5**
2	4	172	209	+21.5	221	+28.5
	5	186	221	+18.8	224	+20.4
	6	183	214	+16.9	225	+23.0
	Mean (± SD)	**180.3 ± 7.4**	**214.7 ± 6.0**	**+19.1**	**223.3 ±2.1**	**+23.8**

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute oral LD_{50} of glyphosate technical in female rats, observed over a period of 14 days was greater than 2000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD guideline 423 (2001). The outcome can be therefore reported as valid. The acute oral LD_{50} is above 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.1/006
Report author	[redacted]
Report year	2009
Report title	Glyphosate Technical: Acute Oral Toxicity Study in Rats
Report No	C22864
Document No	Not reported
Guidelines followed in study	OECD 423 (2001) Commission Regulation (EC) No 440/2008 (2008), method B.1 tris
Deviations from current test guideline (OECD 423, 2001)	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The test substance, glyphosate technical, was evaluated for its acute oral toxicity potential in female HanRcc:WIST (SPF) rats when administered as a gavage dose at a level of 2000 mg/kg bw. No mortality occurred during the study. No clinical signs were observed during the course of the study. There was no effect on body weight gain. No macroscopic findings were recorded at necropsy. The acute oral LD_{50} was determined to be

LD_{50}, oral, female rat > 2000 mg/kg bw

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate Technical
Description:	Solid
Lot/Batch #:	GI-1045
Purity:	96.66 %
Stability of test compound:	(Stable under storage conditions) Expiry date: July 2010

2. Vehicle and/ or positive control: Purified water

3. Test animals:

Species:	Rat
Strain:	HanRcc: WIST (SPF)
Source:	[redacted]
Age:	11 weeks
Sex:	Female
Weight at dosing:	181.0 – 198.7 g
Acclimation period:	7 days
Diet/Food:	Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet, batch no. 6108 (Provimi Kliba AG, 4303 Kaiseaugst / Switzerland) ad libitum (except for the overnight fasting period rior to intubation and approximately 3-4 hours post dose)
Water:	Tap water, *ad libitum*
Housing:	In groups of three in Makrolon type-4 cages with wire mesh tops and standard softwood bedding ('Lignocel' Shill AG, 4132 Muttenz / Switzerland)
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 30 – 70 % Air changes: 10 – 15 / hour 12-hour light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2009-02-13 to 2009-02-03 and 2009-02-05

Animal assignment and treatment:

Two groups of three fasted females each received the test material at a dose level of 2000 mg/kg bw by oral gavage. The dosing volume was 10 mL/kg bw. Observations for mortality and clinical signs of toxicity were made at least five times on the day of dosing (Day 1) and at twice daily thereafter during days 2 – 15. Individual body weights were recorded just prior to dosing and on Days 8 and 15.
On Day 15 after dosing, each animal was euthanised by CO_2 asphyxiation. All study animals were subjected to gross necropsy and all abnormalities were recorded.

II. RESULTS AND DISCUSSION

A. MORTALITY
No deaths occurred during the study.

B. CLINICAL OBSERVATIONS
No clinical signs were observed during the course of the study.

C. BODY WEIGHT
The body weight of the animals was within the range commonly recorded for this strain and age.

Table 5.2.1-7: Glyphosate Technical: Acute Oral Toxicity Study in Rats ([redacted] 2009): Body weight development

Dose group	2000 mg/kg bw		
Sex	Females		
Animal No.	Body weight (g)		
	Day 1	Day 8	Day 15
1	182.3	194.4	197.6
2	181.0	190.8	202.4
3	198.7	209.8	220.8
Mean (± SD)	**187.3 ± 9.9**	**198.3 ± 10.1**	**206.9 ± 12.2**
4	198.6	221.0	227.1
5	183.8	204.8	216.5
6	192.2	206.8	222.0
Mean (± SD)	**191.5 ± 7.4**	**210.8 ± 8.9**	**221.9 ± 5.3**

D. NECROPSY
No macroscopic findings were recorded at necropsy.

III. CONCLUSIONS
The acute oral LD_{50} of glyphosate technical in female rats, observed over a period of 14 days was greater than 2000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD guideline 423 (2001). The outcome can be therefore reported as valid. The acute oral LD_{50} is above 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.1/007
Report author	[redacted]
Report year	2009
Report title	Glyphosate: Acute Oral Toxicity Study (UDP) In Rats
Report No	12170-08
Document No	Not reported
Guidelines followed in study	US EPA OPPTS 870.1100 Equivalent to OECD 425 (2008)
Deviations from current test guideline (OECD 425, 2008)	Humidity was in the range of 33 – 89 % instead of 30 – 70 %.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The test substance, glyphosate technical, was evaluated for its acute oral toxicity potential in female albino rats when administered as a gavage dose at a level of 5000 mg/kg bw. No mortality occurred during the study. Clinical signs included activity decrease, diarrhoea, piloerection, polyuria and salivation, which were no longer evident by Day 8. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute oral LD_{50} was determined to be

LD_{50}, oral, female rat > 5000 mg/kg bw

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate

Identification: Glyphosate Tech Grade Mixed 5-Batch
Description: White powder
Lot/Batch #: 080704-1 thru 5
Purity: 96.40 %
Stability of test compound: No data given in the report.

2. Vehicle and/or positive control: Deionised water

3. Test animals:

Species: Rat albino
Strain: Sprague-Dawley

Source:	[redacted]
Age:	8 weeks
Sex:	Female
Weight at dosing:	160 – 187 g
Acclimation period:	5 days
Diet/Food:	Formulab #5008 (PMI Feeds Inc.), *ad libitum* except for approx. 16 h before dosing
Water:	Tap water, *ad libitum*
Housing:	Individual housing in suspended, wire bottom, stainless steel cages
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 30 – 89 % Air changes: 10 – 12 / hour 12-hour light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2008-11-11 to 2008-11-27

Animal assignment and treatment:
A group of three fasted females received the test material at a dose level of 5000 mg/kg bw by oral gavage in a sequential manner according to the up-and-down procedure (limit test). The dosing volume was 12.5 mL/kg bw. Observations for mortality and clinical / behavioural signs of toxicity were made at least three times on the day of dosing (Day 0) and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and on Days 7 and 14.
On Day 14 after dosing, animals were sacrificed, subjected to gross necropsy and all abnormalities were recorded.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. CLINICAL OBSERVATIONS
Clinical signs in one animal included activity decrease, diarrhoea, piloerection, polyuria and salivation, which were no longer evident at Day 8 (see table below).

Table 5.2.1-8: Glyphosate: Acute Oral Toxicity Study (UDP) In Rats ([redacted] 2009): Cinical observations

Dose level		5000 mg/kg bw (12.5 mL/kg bw)										
Sex		Female										
Time after dosing		Day 0*			Days							
Animal No.	Parameter	1st	2nd	3rd	1	2	3	4	5	6	7	8 - 14
291	Salivation	-	s	s	s	s	-	-	-	-	-	-
	Piloerection	-	-	-	m	m	m	s	s	s	s	-
	Diarrhea	-	-	-	s	m	m	m	m	-	-	-
	Polyuria	-	-	-	-	m	m	m	m	-	-	-
	Activity decrease	-	-	-	-	-	-	-	s	-	-	-
292	Appeared normal at each observation											
293	Appeared normal at each observation											

s = slight, m = moderate

Table 5.2.1-8: Glyphosate: Acute Oral Toxicity Study (UDP) In Rats ([redacted] 2009): Cinical observations

Dose level		5000 mg/kg bw (12.5 mL/kg bw)									
Sex		Female									
Time after dosing		Day 0*			Days						
Animal No.	Parameter	1st	2nd	3rd	1	2	3	4	5	6	7 - 8 - 14

* = Observations were performed at three time points on the day of dosing (Day 0)

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance. Individual and mean body weights see table below.

Table 5.2.1-9: Glyphosate: Acute Oral Toxicity Study (UDP) In Rats ([redacted] 2009): Body weights

Animal No.	Body Weights (g)		
	Day 0	Day 7	Day 14
291	187	203	231
292	168	199	204
293	160	183	194
Mean ± SD	**171.7 ± 13.9**	**195.0± 10.6**	**209.7 ± 19.1**

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute oral LD_{50} of glyphosate technical in female rats, observed over a period of 14 days was greater than 5000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance to the current OECD guideline 425 (2008). Therefore, the outcome can be reported as valid. The acute oral LD_{50} is above 5000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.1/008
Report author	[redacted]
Report year	2008
Report title	Acute Oral Toxicity Study in Wistar Hannover Rats for Glyphosate Technical
Report No	[redacted]-3996.305.475.07 (including amendment 1)
Document No	Not reported
Guidelines followed in study	OECD guideline 423 (2001)
Deviations from current test guideline (OECD 423 (2001)	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The test substance, Glyphosate technical, was tested for acute oral toxicity in female albino rats using a stepwise procedure. The test item was administered orally at single dose levels of 2000 mg/kg bw. No mortality occurred during the study and no clinical signs were observed. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute oral LD_{50} was determined to be

LD_{50} cut-off value, oral, female rat > 2000 mg/kg bw

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate Technical

Identification: Glyphosate Technical
Description: Solid
Lot/Batch #: 20070606
Purity: 98.05 %
Stability of test compound: No data given in the report

2. Vehicle and/or positive control: Deionised water

3. Test animals:

Species: Rat albino
Strain: Wistar Hannover
Source: [redacted]

Age:	8 - 9 weeks
Sex:	Female
Weight at dosing:	172 - 205 g
Acclimation period:	6 days
Diet/Food:	Autoclaved Nuvilab CR-1 pellet diet type for rodents (Nuvital Nutrients Ltda.), *ad libitum* except for fasting overnight before dosing
Water:	Filtered drinking water, *ad libitum*
Housing:	Groups of three rats per cage were held in polypropylene rodents cages with wire mesh tops and bedding material
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 30 - 70 % Air changes: min. 10/hour 12-hour light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2007-09-12 to 2008-06-11

Animal assignment and treatment:

A group of three fasted females received the test material at a dose level of 2000 mg/kg bw by oral gavage in a stepwise manner. As no mortality was observed, a second group of three fasted female rats was dosed in the same manner. Observations for mortality and clinical/behavioural signs of toxicity were made once within the first 30 minutes after dosing, three times more during the first 4 hours after dosing, and daily thereafter for a period of 14 days. Individual body weights were recorded just prior to dosing (Day 0) and on Days 7 and 14. On Day 14 after dosing, each animal was euthanised by an overdose of CO_2. All study animals were subjected to gross necropsy and all abnormalities were recorded.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

No clinical signs of toxicity were observed in females treated with 2000 mg/kg bw.

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance (see table below).

Table 5.2.1-10: Acute Oral Toxicity Study in Wistar Hannover Rats for Glyphosate Technical ([redacted] 2008): Body weight and body weight gain.

Dose group		2000 mg/kg bw					
Sex		Females					
Time after treatment		Day 0	Day 7	Day 14	Day 0 to 7	Day 7 to 14	Day 0 to 14
Step	Animal No.	Body weight (g)			Body weight gain g)		
1	25	180	203	213	23	10	33
	26	205	229	239	24	10	34
	27	203	230	243	27	13	40
	Mean (± SD)	**196.0 ± 13.9**	**220.7 ± 15.3**	**231.7 ± 16.3**	**24. 7 ± 2.1**	**11.0 ± 1.7**	**35.7 ± 3.8**

Table 5.2.1-10: Acute Oral Toxicity Study in Wistar Hannover Rats for Glyphosate Technical ([redacted] 2008): Body weight and body weight gain.

Dose group		2000 mg/kg bw					
Sex		Females					
Time after treatment		Day 0	Day 7	Day 14	Day 0 to 7	Day 7 to 14	Day 0 to 14
Step	Animal No.	Body weight (g)			Body weight gain g)		
2	28	183	206	208	23	2	25
	29	186	210	217	24	7	31
	30	172	198	206	26	8	34
	Mean (± SD)	**180.3 ± 7.4**	**204.67 ± 6.1**	**210.3 ± 5.9**	**24.33 ± 1.5**	**5.67 ± 3.2**	**30.00 ± 4.6**

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute oral LD_{50} of glyphosate technical in female rats, observed over a period of 14 days was greater than 2000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study is in concordance with the current OECD guideline 423 (2001). The outcome can be therefore reported as valid. The acute oral LD_{50} is above 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.1/009
Report author	[redacted]
Report year	2007
Report title	Glyphosate technical material: Acute oral toxicity study in the rat (up and down procedure)
Report No	B02755
Document No	Not reported
Guidelines followed in study	OECD 425 (2001) US EPA OPPTS 870.1100 (2002) Japanese MAFF 12 NohSan No. 8147 (2001)
Deviations from current test guideline (OECD 425, 2008)	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

In an acute oral toxicity study (limit test), a group of three, fasted, 11 week old, HanRcc:WIST (SPF), female rats was given a single oral dose of glyphosate technical material (96.1 % w/w glyphosate acid) in purified water at a concentration of 5000 mg/kg bw and administered at a dosing volume of 10 mL/kg bw.

The animals were examined daily during the acclimatisation period and mortality, viability and clinical signs were recorded. All animals were examined for clinical signs once during the first 30 minutes and at approximately 1, 2, 3 and 5 hours after treatment on day 1 and once daily during test days 2 – 15. Mortality/viability was recorded once during the first 30 minutes and at approximately 1, 2, 3 and 5 hours after administration on test day 1 (with the clinical signs) and twice daily during days 2-15. Body weights were recorded on day -1 (prior to removal of food), day 1 (prior to administration) and on days 8 and 15. All animals were necropsied and examined macroscopically.

Single animals were dosed sequentially at no less than approximately 48 hour intervals. The time intervals between dosing were determined by the onset, duration and severity of clinical signs.

All animals survived until the end of the study period. Slightly ruffled fur was noted in all animals from the 30-minute reading to the 5-hour reading and persisted in one animal until test day 3. Hunched posture was also noted in the animals from the 1- or 2-hour reading to the 5-hour reading. The body weight of the animals was within the range commonly recorded for this strain and age. No macroscopic findings were recorded at the scheduled necropsy.

LD_{50}, oral, female rat > 5000 mg/kg bw

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate technical material
Description:	Technical, white powder
Lot/Batch #:	0507
Purity:	96.1 %
Stability of test compound:	Re-certification date August 2008. Stable under storage conditions (room temperature range 20 ± 5 °C, protected from light and humidity)

2. Vehicle and/or positive control: Purified water (deionised water processed and treated by the PURELAB Option-R unit which links four purification technologies: reverse osmosis, adsorption, ion-exchange and photo oxidation)

3. Test animals:

Species:	Rat
Strain:	HanRcc:WIST (SPF)
Source:	[redacted]
Age:	11 weeks

Sex:	Female
Weight at dosing:	183.0 – 188.9 g
Acclimation period:	5 – 7 days
Diet/Food:	Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet (Provimi Kliba AG, CH-4303 Kaiseraugst, Switzerland), *ad libitum* (except for pre-dose fast and 3 hours after dosing)
Water:	Community tap water, *ad libitum*
Housing:	Individually in Makrolon type-3 cages with standard softwood bedding
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 30 – 70 % Air changes: 10 – 15 / hour 12-hour light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2006-12-06 to 2006-12-26

Animal assignment and treatment:
In an acute oral toxicity study, a group of three, fasted, 11 week old, HanRcc:WIST (SPF), female rats was given a single oral dose of glyphosate technical material (96.1 % w/w glyphosate acid) at a concentration of 5000 mg/kg bw by gavage. The test substance was diluted in vehicle (purified water) and dosed at a volume dosage of 10 mL/kg bw.
Single animals were dosed sequentially at no less than approximately 48 hour intervals. The time intervals between dosing were determined by the onset, duration and severity of clinical signs. The first animal was treated at a dose level of 5000 mg/kg bw. As no mortality or significant clinical signs were observed, two additional animals were sequentially dosed at 5000 mg/kg bw such that a total of 3 animals were tested. No mortalities were observed; therefore, the study was terminated.

The animals were examined daily during the acclimatization period and mortality, viability and clinical signs were recorded. All animals were examined for clinical signs once during the first 30 minutes and at approximately 1, 2, 3 and 5 hours after treatment on day 1 and once daily during test days 2 – 15. Mortality / viability was recorded once during the first 30 minutes and at approximately 1, 2, 3 and 5 hours after administration on test day 1 (with the clinical signs) and twice daily during days 2 – 15.

Body weights were recorded on day -1 (prior to removal of food), day 1 (prior to administration) and on days 8 and 15.

All animals were killed at the end of the observation period by carbon dioxide asphyxiation, necropsied and examined macroscopically.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities.

B. CLINICAL OBSERVATIONS
Slight ruffled fur was noted in all animals from the 30-minute reading to the 5-hour reading and persisted in one animal until test day 3. Hunched posture was also noted in the animals from the 1- or 2-hour reading to the 5-hour reading (see table below).

Table 5.2.1-11: Glyphosate technical material: Acute oral toxicity study in the rat (up and down procedure) ([redacted] 2007): Summary of observations during the study.

Dose group	**5000 mg/kg bw**							
Sex	**Females**							
Time after treatment	0.5 h	1 h	2 h	3 h	5 h	2 days	3 days	4-15 days
Total animals examined n	3	3	3	3	3	3	3	3
Clinical sign n	3	3	3	3	3	1	1	0
Ruffled fur n	3	3	3	3	3	1	1	0
Hunched posture n	0	1	3	3	3	0	0	0
Single animals observations								
Animal No.								
1	s	sh	sh	sh	sh	s	s	*
2	s	s	sh	sh	sh	*	*	*
3	s	s	sh	sh	sh	*	*	*

* = No abnormalities detected, s = slight ruffled fur, h = hunched posture

C. BODY WEIGHT

The body weight of the animals was within the range commonly recorded for this strain and age. The individual body weights are listed below in the table below.

Table 5.2.1-12: Glyphosate technical material: Acute oral toxicity study in the rat (up and down procedure) ([redacted] 2007): Individual body weights recorded during the study.

Dose group	**5000 mg/kg bw**			
Sex	**Females**			
Animal No.	**Day -1**	**Day 1**	**Day 8**	**Day 15**
	Body weight (g)			
1	185.4	183.0	190.9	195.9
2	198.8	184.1	205.9	215.8
3	193.7	188.9	204.8	211.6
Mean (± SD)	**192.6 ± 6.8**	**185.3 ± 3.1**	**200.5 ± 8.4**	**207.8 ± 10.5**

Note: Mean and standard deviation were not given in the study report. Values were calculated retrospectively

D. NECROPSY

No macroscopic findings were recorded at the scheduled necropsy.

III. CONCLUSIONS

The acute oral LD_{50} of glyphosate technical in female rats, observed over a period of 14 days was greater than 5000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in accordance with the current OECD guideline 425 (2008). The outcome can be therefore reported as valid. The acute oral LD_{50} is above 5000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.1/010
Report author	[redacted]
Report year	2007
Report title	Glyphosate Technical (NUP05068): Acute Oral Toxicity Study in Rats
Report No	B02272
Document No	Not reported
Guidelines followed in study	Japanese guideline Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF), Guidelines for Preparation of Study Results, Acute oral toxicity studies, Guideline 2"1-1 Notification 12 NohSan No. 8147, as partly revised in 16-Shouan-9260, on 16 March 2005. English translation by ACIS on 17 Oct 2005. Directive 2004/73/EC, 8.1 tris "Acute Oral Toxicity-Acute Toxic Class Method", April 29,2004. OECD Guidelines for the Testing of Chemicals, Number 423 "Acute Oral Toxicity – Acute Toxic Class Method", adopted 17 December 2001
Deviations from current test guideline (OECD 423, 2001)	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The test substance, glyphosate, was evaluated for its acute oral toxicity potential in female rats when administered as a 2000 mg/kg bw gavage dose. No mortality occurred during the study. The only clinical sign observed was slightly ruffled fur. There was no effect on body weight gain and no macroscopic findings were recorded at necropsy.
The median lethal dose of glyphosate technical (NUP 05068) after single oral administration to female rats, observed over a period of 14 days is:

LD_{50} (female rat) > 2000 mg/kg bw

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate Technical (NUP 05068)
Description:	White powder
Lot/Batch #:	200609062
Purity:	95.1 %
Stability of test compound:	Stable under storage conditions.

2. Vehicle and/or positive control: Polyethylene glycol 300 (PEG 300)

3. Test animals:

Species:	Rat
Strain:	HanRcc:WIST (SPF)
Source:	[redacted]
Age:	11 weeks
Sex:	Female
Weight at dosing:	160 – 187 g
Acclimation period:	5 days
Diet/Food:	Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet, batch no. 67/06 (Provimi Kliba AG, CH-4303 Kaiseraugst/Switzerland), *ad libitum* except for 18 – 19 hours before dosing and 3 hours after dosing.
Water:	Tap water, *ad libitum*
Housing:	In groups of three in Makrolon type-4 cages with wire mesh tops and standard softwood bedding ('Lignocel' Schill AG, CH-4132 Muttenz/Switzerland)
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 30 – 70 % Air changes: 10 – 15 / hour 12-hour light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2006-12-12 to 2007-01-04

Animal assignment and treatment:
The animals received a single dose of the test item by oral gavage administration at 2000 mg/kg bw after being fasted for approximately 18 – 19 hours (access to water was permitted). Food was provided again approximately 3 hours after dosing. The dosing volume was 10 mL/kg bw. Observations for mortality and viability: Daily during the acclimatization period, during the first 30 minutes and at approximately 1, 2, 3 and 5 hours after administration on test day 1 (with the clinical signs) and twice daily during days 2 – 15.

Body weights: On test days 1 (prior to administration), 8 and 15.

Clinical signs: Daily during the acclimatization period, during the first 30 minutes and at approximately 1, 2, 3 and 5 hours after administration on test day 1. Once daily during days 2 – 15. All abnormalities were recorded.

Necropsy: All animals were sacrificed at the end of the observation period and subjected to macroscopic examinations.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

A slightly ruffled fur was noted in all six animals treated at 2000 mg/kg bw after dosing. Otherwise no clinical signs were observed in any animal at any observation time (see table below).

Table 5.2.1-13: GLYPHOSATE TECHNICAL (NUP05068): Acute oral toxicity study ([redacted] 2007): Summary of clinical observations

Dose group		**2000 mg/kg bw**					
Sex		**Females**					
Time after treatment		0.5 h	1 h	2 h	3 h	5 h	Day 2 - 15
Total animals examined	**n**	6	6	6	6	6	6
Clinical sign	**n**	0	3	6	6	0	0
Ruffled fur	n	0	3	6	6	0	0
Single animals observations							
Animal No.							
1		*	*	A	A	*	*
2		*	*	A	A	*	*
3		*	*	A	A	*	*
4		*	A	A	A	*	*
5		*	A	A	A	*	*
6		*	A	A	A	*	*

* = No abnormalities detected, A = ruffled fur

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance (table below**Table 5.2.1-14**).

Table 5.2.1-14: GLYPHOSATE TECHNICAL (NUP05068) : Acute oral toxicity study ([redacted] 2007): Body weight development

Dose group		**2000 mg/kg bw**		
Sex		**Females**		
Time after treatment		**Day 1**	**Day 8**	**Day 15**
Step	**Animal No.**	**Body weight (g)**		
1	1	189.6	206.3	220.7
	2	189.4	209.8	213.6
	3	184.4	203.6	207.6
2	4	175.0	197.9	198.9
	5	186.2	208.8	219.7
	6	187.6	211.6	222.8
	Mean ± SD	**185.4 ± 5.4**	**206.3 ± 5.0**	**213.9 ± 9.2**

Note: Mean and standard deviation were not given in the study report. Values were calculated retrospectively

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute oral LD_{50} of glyphosate technical in female rats, observed over a period of 14 days was greater than 2000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant: The study is in concordance with the current OECD guideline 423 (2001). The outcome can be therefore reported as valid. The acute oral LD_{50} is above 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.1/011
Report author	[redacted]
Report year	2005
Report title	Glyphosate Acid Technical – Acute Oral Toxicity Up and Down Procedure in Rats
Report No	15274
Document No	Not reported
Guidelines followed in study	US EPA OPPTS 870.1100 (2002) OECD 425 (2001)
Deviations from current test guideline (OECD 425, 2008)	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The test substance, glyphosate acid technical, was evaluated for its acute oral toxicity potential in female albino rats when administered as a gavage dose at a level of 5000 mg/kg bw. No mortality occurred during the study. Clinical signs included diarrhea, ano-genital and facial staining, and/or reduced fecal volume. All animals recovered by Day 4 and appeared active and healthy for the remainder of the 14-day observation period. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute oral LD_{50} was determined to be

LD_{50}, oral, female rat > 5000 mg/kg bw

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	Glyphosate Acid Technical
Identification:	Glyphosate Acid Technical
Description:	White crystalline powder
Lot/Batch #:	040205
Purity:	97.23 %
Stability of test compound:	Test substance was expected to be stable for the duration of testing
2. Vehicle and/or positive control:	Distilled water
3. Test animals:	
Species:	Rat albino
Strain:	Sprague-Dawley derived
Source:	[redacted]
Age:	11 weeks
Sex:	Female
Weight at dosing:	222 – 235 g
Acclimation period:	21 or 23 days
Diet/Food:	Purina Rodent Chow #5012, *ad libitum* except for overnight fasting before dosing and 3 – 4 hours after dosing
Water:	Filtered tap water, *ad libitum*
Housing:	Individual housing in suspended stainless steel cages with mesh floors. Litter paper was placed beneath the cage and was changed at least three times per week
Environmental conditions:	Temperature: 19 – 23 °C 12-hour light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2004-05-04 to 2004-05-20

Animal assignment and treatment:
A group of three fasted females received the test material at a dose level of 5000 mg/kg bw by oral gavage in a sequential manner according to the up-and-down procedure (limit test). The test substance was administered as a 50 % w/w suspension in distilled water. Observations for mortality and clinical/behavioural signs of toxicity were made during the first several hours post-dosing and at least once daily thereafter for 14 days after dosing. Individual body weights were recorded just prior to dosing and on Days 7 and 14.
On Day 14 after dosing, each animal was euthanised by an overdose of CO_2. All study animals were subjected to gross necropsy and all abnormalities were recorded.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

Clinical signs noted for all animals included diarrhea, ano-genital and facial staining, and/or reduced fecal volume. All animals recovered by Day 4 and appeared active and healthy for the remainder of the 14-day observation period (see table below).

Table 5.2.1-15: Glyphosate Acid Technical – Acute Oral Toxicity Up and Down Procedure in Rats ([redacted] 2005): Clinical observations after treatment

Dose group	**5000 mg/kg bw**							
Sex	**Females**							
Time after treatment	**1 h**	**2 h**	**3 h**	**5 h**	**Day 1**	**Day 2**	**Day 3**	**Day 4-14**
Total animals n examined	3	3	3	3	3	3	3	3
Clinical sign n	0	0	1	2	3	2	1	0
Diarrhea n	0	0	0	1	0	0	0	0
Anogenital staining n	0	0	0	2	2	2	1	0
Facial staining n	0	0	1	0	0	0	0	0
Reduced fecal volume n	0	0	0	0	1	0	0	0
Single animals observations								
Animal No.								
4765	*	*	*	AD	A	*	*	*
4856	*	*	*	A	A	A	*	*
4857	*	*	C	*	B	A	A	*

* = No abnormalities detected, A = anogenital staining, B = reduced fecal volume, C = facial staining, D = diarrhea

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance (see table below).

Table 5.2.1-16: Glyphosate Acid Technical – Acute Oral Toxicity Up and Down Procedure in Rats ([redacted] 2005): Body weight development during the study

Dose group	**5000 mg/kg bw**		
Sex	**Females**		
Time after treatment	**Day 0**	**Day 7**	**Day 14**
Animal No.	**Body weight (g)**		
4765	234	247	269
4856	235	247	271
4857	222	239	263
Mean ± SD	**230 ± 7.2**	**244.3 ± 4.6**	**167.7 ±4.2**

Note: Mean and standard deviation were not given in the study report. Values were calculated retrospectively

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute oral LD_{50} of glyphosate acid technical in female rats, observed over a period of 14 days was greater than 5000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD guideline 425 (2008). The outcome can be therefore reported as valid. The acute oral LD_{50} is above 5000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.1/012
Report author	[redacted]
Report year	1999
Report title	NUP5a99 62 % glyphosate MUP: Acute oral toxicity study in rats – Limit test
Report No	7907
Document No	Not reported
Guidelines followed in study	US EPA Health Effects Test Guidelines, OPPTS 870.1100 (1998)
Deviations from current test guideline (OECD 420, 2001)	Animals of both sexes used, humidity and air changes not specified, purity of only 62 %.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

NUP5a99 62 % glyphosate MUP was administered to ten healthy rats by oral gavage at the dose of 5000 mg/kg bw. All animals survived and gained weight during the study. Following administration, most females exhibited anogenital staining and two females exhibited soft feces or diarrhea, but recovered by Day 2 and appeared active and healthy for the remainder of the study. Gross necropsy fmdings at terminal sacrifice were unremarkable.

Based on the results of this study, the single dose acute oral LD_{50} of the test substance is >5000 mg/kg of body weight.

LD_{50}, oral, male and female rat > 5000 mg/kg bw

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	NUP5a99 62 % glyphosate MUP
Description:	clear viscous amber liquid
Lot/Batch #:	Drum Sample E
Purity:	62 % (isopropylamine glyphosate)
Stability of test compound:	No data available

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley derived, albino
Source:	[redacted]
Age:	Not specified
Sex:	5 males and 5 females
Weight at dosing:	Young adult / males 227 – 254 grams and females 178 – 200 grams at experimental start
Acclimation period:	14 days
Diet/Food:	Purina Rodent Chow #5012 (Fasting approximately 17.25 h before and 3.5 h after dosing)
Water:	Tap water, *ad libitum*
Housing:	singly housed in suspended stainless steel caging with mesh floors. Litter paper was placed beneath the cage and was changed at least three times per week.
Environmental conditions:	Temperature: 22 – 24 °C Humidity: not specified Air changes: not specified 12-hour light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 1999-08-03 – 1999-08-17

Animal assignment and treatment:

Prior to dosing, a group of animals was fasted for approximately 17.25 hours by removing feed from their cages. After weighing and clinical examination, ten (five male and five female) healthy rats were selected for test. Individual doses were calculated based on the initial body weights, taking into account the specific gravity (determined by [redacted]) of the test substance. Each animal received 5000 mg/kg bw of the test substance via gavage. Feed was replaced approximately 3.5 hours after dosing. The day of administration was considered Day zero of the study. Animals were weighed prior to test substance administration (initial) and again on Days 7 and 14 (termination). Clinical signs were recorded at 1, 3 and 22 hours post-dosing and at least once daily thereafter for 14 days. Observations included gross evaluation of skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and product safety labs central nervous systems, somatomotor activity and behavior pattern. Particular attention was directed to observation of tremors, convulsions, salivation, diarrhea and coma. All rats were euthanised via CO_2 inhalation on Day 14. Gross necropsies were performed on all animals.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

Most females exhibited anogenital staining and two females exhibited soft feces or diarrhea, but recovered by Day 2. Details on clinical observations are provided in the table below.

Table 5.2.1-17: NUP5a99 62 % glyphosate MUP: Acute oral toxicity study in rats – Limit test ([redacted] 1999): Summary of clinical observations

Dose group		5000 mg/kg bw							
Sex		Males				Females			
Time after treatment		1 h	3 h	22 h	Day 1-14	1 h	3 h	22 h	Day 1-14
Total animals examined	n	5	5	5	5	5	5	5	5
Clinical sign	n	0	0	0	0	0	1	4	0
Anogenital staining	n	0	0	0	0	0	1	4	0
Soft feces	n	0	0	0	0	0	0	1	0
Diarrhea	n	0	0	0	0	0	0	1	0
Single animals observations									
Animal No.§									
7733 (7738)		*	*	*	*	*	*	A	*
7734 (7739)		*	*	*	*	*	A	A	*
7735 (7740)		*	*	*	*	*	*	*	*
7736 (7741)		*	*	*	*	*	*	AB	*
7737 (7742)		*	*	*	*	*	*	AC	*

§ = animal No. of males and (females), * = No abnormalities detected, A = anogenital staining, B = soft feces, C = diarrhea

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance. Individual and group mean body weights are listed below in the table below.

Table 5.2.1-18: NUP5a99 62 % glyphosate MUP: Acute oral toxicity study in rats – Limit test ([redacted] 1999): Body weight development after treatment with glyphosate

Dose level	5000 mg/kg bw					
Sex	Males			Females		
Day	0	7	14	0	7	14
Animal No.§	Body weight (g)					
7733 (7738)	240	313	364	178	203	244
7734 (7739)	254	324	355	184	203	251
7735 (7740)	242	321	371	200	224	247
7736 (7741)	244	310	329	180	206	239
7737 (7742)	227	300	347	183	220	250
Mean (± SD)	**241.4 ± 9.7**	**313.6 ± 9.5**	**353.2 ± 16.3**	**185.0 ± 8.7**	**211.2 ± 10.0**	**246.2 ± 4.9**

§ = animal No. of males and (females), note: Mean and standard deviation were not given in the study report. Values were calculated retrospectively

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The single dose acute oral LD_{50} of NUP5a99 62 % glyphosate MUP is greater than 5000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was performed according to a national guideline similar to the current OECD guideline 420 (2001). The study outcome was therefore considered valid. Nevertheless, the study is considered as supportive due to the low purity of the test substance. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.1/013
Report author	[redacted]
Report year	1996
Report title	Glyphosate Acid: Acute Oral Toxicity Study In Rats
Report No	[redacted]/P/4660
Document No	Not reported
Guidelines followed in study	OECD 401 (1987) US EPA OPPTS 870.1100 (2002)
Deviations from current test guideline (OECD 420, 2001).	Animals of both sexes used. Clinical observation was performed twice after treatment, once within 2 hours instead of the first 30 minutes.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary
In an acute oral toxicity study, a group of five male and five female, fasted, young adult Alpk:AP_fSD (Wistar-derived) rats were given a single oral dose of 5000 mg/kg bw of glyphosate acid in deionised water and observed for 15 days.

None of the animals died. There were no signs of systemic toxicity and no treatment-related findings at examination *post mortem*. All animals gained weight during the study.

LD_{50}, oral, male and female rat > 5000 mg/kg bw

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate Acid
Description:	Technical, white solid
Lot/Batch #:	P24
Purity:	95.6 %
Stability of test compound:	The test substance was used within the expiry date.

2. Vehicle and/or positive control: Deionised water

3. Test animals:

Species:	Rat
Strain:	Alpk:AP$_f$SD (Wistar-derived)
Source:	[redacted]
Age:	Young adult
Sex:	Male and female
Weight at dosing:	233 – 260 g (males), 197 – 225 g (females)
Acclimation period:	At least 6 days
Diet/Food:	Diet (PCD), supplied by Special Diet Services Limited, Witham, Essex, UK, *ad libitum* (except overnight immediately prior to dosing).
Water:	Mains water, *ad libitum*
Housing:	5/cage, sexes separately in multiple rat racks suitable for animals of this strain and the weight range expected during the course of the study
Environmental conditions:	Temperature: 21 ± 2 °C Humidity: 40 – 70 % Air changes: Approximately 25 – 30 / hour 12-hour light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 1995-03-16 to 1995-03-30

Animal assignment and treatment:
In an acute oral toxicity study, a group of five male and five female, fasted, young adult Alpk:AP$_f$SD (Wistar-derived) rats were given a single oral dose of 5000 mg/kg bw of glyphosate acid by gavage. The test substance was diluted in deionised water. The volume of the dose was calculated for each animal according to its weight at the time of dosing and a standard volume of 10 mL/kg bw of the dosing preparation was administered.

Prior to the start of the study, all rats were examined to ensure that they were physically normal and exhibited normal activity. The animals were observed for signs of systemic toxicity once within 2 hours of dosing and again between 4 and 7 hours after dosing. Subsequent observations were made daily, up to day 15.

The animals were weighed on the day before dosing (day -1), immediately before dosing (day 1) and on days 3, 5, 8 and 15.

All animals were subjected to an examination *post mortem*. This involved an external observation and a careful examination of all thoracic and abdominal viscera.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities.

B. CLINICAL OBSERVATIONS

There were no signs of systemic toxicity.

C. BODY WEIGHT

All animals lost weight initially due to the pre-dose fast, but all had exceeded their initial weight by day 3, and apart from a transient weight loss in one female, continued to gain weight throughout the remainder of the study (see table below).

Table 5.2.1-19: Glyphosate Acid: Acute Oral Toxicity Study In Rats ([redacted] 1996): Individual body weights

Dose	**5000 mg/kg bw**											
Sex	**Males**						**Females**					
Animal No.§	**Day -1**	**Day 1**	**Day 3**	**Day 5**	**Day 8**	**Day 15**	**Day -1**	**Day 1**	**Day 3**	**Day 5**	**Day 8**	**Day 15**
	Body weight (g)											
11 (67)	255	237	265	283	304	359	197	177	208	209	214	232
12 (68)	260	232	261	279	300	339	225	203	232	230	227	243
13 (69)	233	210	243	263	285	330	222	201	233	247	262	272
14 (70)	254	228	265	278	303	345	216	192	224	230	239	244
15 (71)	260	233	270	280	304	333	218	197	223	227	244	255
Mean (± SD)	**252.4 ± 11.2**	**228.0 ± 10.6**	**260.8 ± 10.4**	**276.6 ± 7.8**	**299.2 ± 8.1**	**341.2 ± 11.5**	**215.6 ± 11.0**	**194.0 ± 10.4**	**224.0 ± 10.0**	**228.6 ± 13.5**	**237.2 ± 18.1**	**249.2 ± 15.1**

§ = animal No. of males and (females)

D. NECROPSY

Red or mottled areas in the lung or red areas in the thymus were seen in three males and two females. These are common spontaneous findings in rats of this age and strain and are considered not to be treatment-related. Individual findings at necropsy are listed in the table below.

Table 5.2.1-20: Glyphosate Acid: Acute Oral Toxicity Study In Rats ([redacted] 1996): Necropsy findings

Animal No. and sex	**Organ**	**Observation**
11 M	Lung	Red area/s: (moderate) on all lobes and surfaces
12 M	Thymus	Red area/s: covering most of right lobe
13 M	-	-
14 M	Lung	Mottled: (moderate) red
15 M	-	-

Table 5.2.1-20: Glyphosate Acid: Acute Oral Toxicity Study In Rats ([redacted] 1996): Necropsy findings

67 F	Lung	Mottled: (slight) red
68 F	-	-
69 F	-	-
70 F	Lung	Red area/s: (slight) on all lobes and surfaces
71 F	-	-

- = No findings

III. CONCLUSIONS

The acute oral LD_{50} of glyphosate acid in male and female rats was greater than 5000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in accordance with the current OECD guideline 420 (2001). The outcome can be therefore reported as valid. The acute oral LD_{50} is above 5000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.1/014
Report author	[redacted]
Report year	1995
Report title	An Acute Toxicity Study of MON 0139 by Oral Administration in Mice
Report No	B-3101XX-95-205
Document No	Not reported
Guidelines followed in study	JMAFF 59 NohSan No. 4200 (January 28, 1985)
Deviations from current test guideline (OECD 420, 2001)	Animals of both sexes used, animals 7 weeks old instead of 8 and 12 weeks, 9 instead of 5 animals per sex were used in the sighting study, no fasting after dosing.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The test substance, MON 0139, was evaluated for its acute oral toxicity potential in male and female Crj:CD-1(ICR) strain SPF mice when administered as a gavage dose at 5000 mg/kg bw. No mortality

occurred during the study. No clinical signs of toxicity were observed. There was a slight tendency toward retardation of body weight gain as compared with the control group for males from 7 days after administration. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute oral LD_{50} was calculated to be

LD_{50}, oral, mouse > 5000 mg/kg body weight

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	MON 0139
Description:	Light yellow viscous solution
Lot/Batch #:	LBRV-11092
Purity:	62.34 % (isopropyl amine salt of glyphosate)
Stability of test compound:	Stable under room temperature, expiry July, 1996

2. Vehicle and/or positive control: Water for injection

3. Test animals:

Species:	Mouse
Strain:	Crj:CD-1(ICR)
Source:	[redacted]
Age:	6 weeks
Sex:	Male and female
Weight at dosing:	♂ 31.1 – 34.5 g; ♀ 22.2 – 26.2 g
Acclimation period:	Approximately 1 week
Diet/Food:	CRF-1 pelleted diet, sterilised by radiation (Oriental Yeast Co., Ltd.), *ad libitum* except during fasting prior to dosing
Water:	Tap water; *ad libitum*
Housing:	Plastic cages with wood chip bedding in groups of 5 (groups of 5 or 6 during quarantine/acclimation)
Environmental conditions:	Temperature: 23 ± 3 ºC Humidity: 50 ± 20 % Air changes: 11 – 13 per hour Light cycle: 12 hour illumination per day

B. STUDY DESIGN AND METHODS

In life dates: 1995-08-16 – 1995-09-06

Animal assignment and treatment:

Preliminary study:
During the quarantine/acclimatization period, a preliminary study was conducted using 9 males and 9 females. The animals were fasted for approximately 4 hours prior to administration and the test article was administered once orally, by gavage, adjusting the dose volume according to each dose level. Three male and female animals were dosed with 1000, 2000, or 5000 mg/kg bw. The animals were fed again after

administration, and had free access to water throughout the experimental period.

Main Study:
In the preliminary study, no deaths were observed in either sex in any of the dose groups. Based on these results, the dose level of 5000 mg/kg bw was selected for the main test. The animals were ranked by individual body weights and randomly assigned to groups so as to ensure the homogeneity of group means as far as possible. The animals were fasted for approximately 4 hours prior to administration and the test article was administered once orally, by gavage. The animals in the 5000 mg/kg bw group and control group were given 4.1 mL/kg body weight of test article and 'water for injection', respectively. Each group consisted of 5 animals per sex. Animals were fed again after administration, and had free access to water throughout the experimental period.

The animals were observed frequently for the first 6 hours after administration, and then once daily for 14 days for mortality, signs of toxicity and abnormalities. Body weights were recorded prior to fasting, immediately before dosing, and on days 1, 2, 3, 7, 10, and 14 after dosing. A gross necropsy was performed on all animals at the time of terminal sacrifice at the end of the 14-day observation period.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. CLINICAL OBSERVATIONS
No clinical signs of toxicity were observed.

C. BODY WEIGHT
In males, a slight tendency toward retardation of body weight gain as compared with the control group was observed in the 5000 mg/kg bw group from 7 days after administration (see table below). In females, no compound-related changes were observed in the 5000 mg/kg bw group.

Table 5.2.1-21: Acute Toxicity Study of MON 0139 by Oral Administration in Mice ([redacted] 1995): Body weight development in mice after treatment with glyphosate

Day		0[a]	0	1	2	3	7	10	14	0-14
Sex		Males								
Dose level (mg/kg bw)	Animal No.[§]	Body weight [g]								Bw gain [g]
0	1001	32.6	31.3	33.7	33.8	34.1	37.0	35.2	37.3	6.0
	1002	33.3	31.1	33.8	34.4	34.6	35.9	36.5	37.6	6.5
	1003	33.3	32.2	33.5	34.2	34.3	36.4	40.1	37.4	5.2
	1004	36.0	34.5	35.1	36.1	36.3	38.2	39.2	41.1	6.6
	1005	36.6	34.1	36.5	37.3	37.7	39.2	39.7	40.4	6.3
	Mean	**34.4**	**32.6**	**34.5**	**35.2**	**35.4**	**37.3**	**38.1**	**38.8**	**6.1**
	± SD	**± 1.8**	**± 1.6**	**± 1.3**	**± 1.5**	**± 1.6**	**± 1.3**	**± 2.2**	**± 1.8**	**± 0.6**
5000	2001	34.4	32.0	31.2	30.8	31.2	33.5	33.5	35.3	3.3
	2002	34.6	31.7	34.1	34.1	34.5	34.7	35.2	35.8	4.1
	2003	34.9	33.3	35.3	36.2	36.8	37.3	38.1	39.4	6.1
	2004	35.0	33.4	34.9	33.8	34.0	34.2	35.4	37.7	4.3
	2005	35.3	33.7	35.3	35.7	35.7	36.0	37.1	38.2	4.5
	Mean	**34.8**	**32.8**	**34.2**	**34.1**	**34.4**	**35.1**	**35.9**	**37.3**	**4.5**
	± SD	**0.4**	**± 0.9**	**± 1.7**	**± 2.1**	**± 2.1**	**± 1.5**	**± 1.8**	**± 1.7**	**± 1.0**

Table 5.2.1-21: Acute Toxicity Study of MON 0139 by Oral Administration in Mice (1995): Body weight development in mice after treatment with glyphosate

Day		0[a]	0	1	2	3	7	10	14	0-14
Sex		Males								
Dose level (mg/kg bw)	Animal No.§	Body weight [g]								Bw gain [g]
			Females							
0	1101	25.2	24.3	25.3	24.9	24.4	25.2	25.5	26.4	2.1
	1102	25.3	23.9	25.5	26.0	24.5	26.3	26.9	26.6	2.7.
	1103	26.7	25.2	27.3	27.2	25.2	25.8	26.4	26.9	1.7
	1104	27.2	26.2	26.7	26.8	26.7	28.2	29.2	29.2	3.0
	1105	25.9	24.4	26.8	27.1	25.4	27.5	27.4	29.8	5.4
Mean		**26.1**	**24.8**	**26.3**	**26.4**	**25.2**	**26.6**	**27.1**	**27.8**	**3.0**
± SD		**± 0.9**	**± 0.9**	**± 0.9**	**± 1.0**	**± 0.9**	**± 1.2**	**± 1.4**	**± 1.6**	**± 1.4**
5000	2101	22.9	22.2	23.3	24.0	23.7	24.7	24.5	25.8	3.6
	2102	24.6	23.7	23.5	23.8	23.1	24.8	24.2	23.4	-0.3
	2103	25.6	25.1	26.7	26.7	26.2	28.1	29.3	31.4	6.3
	2104	26.0	24.9	26.3	26.5	25.9	25.6	27.1	28.7	3.8
	2105	27.0	25.1	28.1	28.2	26.0	27.5	27.4	27.5	2.4
Mean		**25.2**	**24.2**	**25.6**	**25.8**	**25.0**	**26.1**	**26.5**	**27.4**	**3.2**
± SD		**± 1.6**	**± 1.3**	**± 2.1**	**± 1.9**	**± 1.5**	**± 1.6**	**± 2.1**	**± 3.0**	**± 2.4**

§ = animal No. of males and (females), a = prior to fasting

D. NECROPSY

No abnormalities were observed.

III. CONCLUSIONS

The acute oral LD_{50} of the test material (MON 0139; 62.34 % (isopropyl amine salt of glyphosate)) in male and female mice was greater than 5000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study was performed according to a national guideline similar to the current OECD guideline 420 (2001). The outcome can be therefore reported as valid. The acute oral LD_{50} is above 5000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:

RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.1/015
Report author	[redacted]
Report year	1995
Report title	HR-001: Acute Oral Toxicity Study In Rats
Report No	[redacted] 94-0134
Document No	Not reported
Guidelines followed in study	OECD 401 (1987), JMAFF 59 NohSan 4200 (1985), US EPA (1984)
Deviations from current test guideline (OECD 420, 2001):	At dosing, animals were 5 weeks old instead of 8 to 12 weeks. After administration the first observation was after 1 h instead of during the first 30 minutes. Temperature 23 ºC (± 3 ºC) instead of 22 ºC (± 3 ºC).
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The test substance, glyphosate technical (HR-001), was evaluated for its acute oral toxicity potential in Sprague Dawley rats when administered as a gavage dose at a level of 5000 mg/kg bw. No mortality occurred during the study. Clinical signs included decreased spontaneous motor activity and salivation 1 and 3 hours after administration. No body weight losses were recorded on Day 7 and 14 after administration when compared with the body weights on the day of administration. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute oral LD_{50} was determined to be

LD_{50}, oral, rats > 5000 mg/kg bw

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate technical, Code: HR-001
Description:	White crystal
Lot/Batch #:	940908-1
Purity:	95.68 %
Stability of test compound:	No data given in the report

2. Vehicle and/or positive control: 0.5 % carboxymethyl-cellulose (CMC)

3. Test animals:

Species: Rat

Strain:	Sprague-Dawley (Crj:CD), SPF
Source:	[redacted]
Age:	5 weeks
Sex:	Males and females
Weight at dosing:	♂ 168 – 179 g; ♀ 125 – 142 g
Acclimation period:	7 days
Diet/Food:	Pellet Diet MF (Oriental Yeast Co., Japan), *ad libitum* except for an overnight fast before dosing and about 3 h after dosing
Water:	Tap water, *ad libitum*
Housing:	Wire-mesh stainless steel cages in groups of 5 animals/sex/cage
Environmental conditions:	Temperature: 23 ± 3 °C Humidity: 55 ± 15 % Air changes: 12 / hour 12-hour light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 1995-01-24 to 1995-02-07

Animal assignment and treatment
A group of five fasted rats per sex received the test material at a dose level of 5000 mg/kg bw by oral gavage (limit test). The dosing volume was 20 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made three times on the day of dosing (Day 0) and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and on Days 7 and 14. On Day 14 after dosing, each animal was euthanised under ether anesthesia and subjected to gross necropsy.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. CLINICAL OBSERVATIONS
Decreased spontaneous motor activity was observed in five males and three females as well as salivation in one male. These signs were observed at 1 and 3 hours after the administration (see table below).

Table 5.2.1-22: HR-001: Acute Oral Toxicity Study In Rats ([redacted] 1995): Summary of clinical observations

Dose group		**5000 mg/kg bw**							
Sex		**Males**				**Females**			
Time after treatment		1 h	3 h	6 h	Day 1-14	1 h	3 h	6 h	Day 1-14
Total animals examined	**n**	5	5	5	5	5	5	5	5
Clinical sign	**n**	4	3	0	0	2	3	0	0
Decreased spontaneous motor activity	n	4	3	0	0	2	3	0	0
Salivation	n	1	0	0	0	0	0	0	0
Single animals observations									
Animal No.§									
1 (101)		A	A	*	*	A	A	*	*
2 (102)		A	*	*	*	A	A	*	*
3 (103)		A	*	*	*	*	*	*	*

Table 5.2.1-22: HR-001: Acute Oral Toxicity Study In Rats ([redacted] 1995): Summary of clinical observations

Dose group	5000 mg/kg bw							
Sex	Males				Females			
Time after treatment	1 h	3 h	6 h	Day 1-14	1 h	3 h	6 h	Day 1-14
4 (104)	AB	A	*	*	*	*	*	*
5 (105)	*	A	*	*	*	A	*	*

§ = animal No. of males and (females), * = No abnormalities detected, A = decreased spontaneous motor activity, B = salivation

C. BODY WEIGHT

No body weight losses were recorded on Day 7 and 14 after administration when compared with the body weights on the day of administration (Day 0). Individual and group mean body weights are provided in the table below.

Table 5.2.1-23: HR-001: Acute Oral Toxicity Study In Rats ([redacted] 1995): Body weight development

Dose group	5000 mg/kg bw					
Sex	Males			Females		
Day	0	7	14	0	7	14
Animal No.§	Body weight [g]					
1 (101)	178	266	320	136	187	216
2 (102)	172	251	310	129	169	191
3 (103)	171	246	297	125	165	181
4 (104)	168	239	296	129	180	214
5 (105)	179	265	322	142	187	211
Mean (± SD)	**173.6 ± 4.7**	**253.4 ± 11.8**	**309 ± 12.3**	**132.2 ± 6.8**	**177.6 ± 10.2**	**202.6 ± 15.7**

§ = animal No. of males and (females)
Note: Standard deviation was not given in the study report. Values were calculated retrospectively.

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute oral LD_{50} of glyphosate technical (HR-001) in male and female rats was greater than 5000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is performed according to OECD guideline 401, which is obsolete nowadays. The study is in concordance with the current OECD guideline 420 (2001). The outcome can be therefore reported as valid. The acute oral LD_{50} is above 5000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.1/016
Report author	[redacted]
Report year	1995
Report title	HR-001: Acute Oral Toxicity Study In Mice
Report No	[redacted] 94-0133
Document No	Not reported
Guidelines followed in study	OECD 401 (1987), JMAFF 59 NohSan 4200 (1985), US EPA (1984)
Deviations from current test guideline (OECD 420, 2001)	2 hours fasting instead of overnight fasting, animals were 6 weeks old at start of administration instead of 8 to 12 weeks. These deviations did not affect the study outcome.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The test substance, glyphosate technical (HR-001), was evaluated for its acute oral toxicity potential in ICR mice when administered as a gavage dose at a level of 5000 mg/kg bw. No mortality occurred during the study. Clinical signs included decreased spontaneous motor activity, sedation and crouching position 1 and 3 hours after administration. There were no body weight losses at 14 days after administration when compared with the body weights on the day of administration. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute oral LD_{50} was determined to be

LD_{50}, oral, mice > 5000 mg/kg bw

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification: Glyphosate technical, Code: HR-001
Description: White crystal
Lot/Batch #: 940908-1
Purity: 95.68 %
Stability of test compound: No data given in the report.

2. Vehicle and/or positive control: 0.5 % carboxymethyl-cellulose (CMC)

3. Test animals:

Species: Mice
Strain: ICR (Crj:CD-1), SPF
Source: [redacted]

Age:	6 weeks
Sex:	Males and females
Weight at dosing:	♂ 29.4 – 32.7 g; ♀ 22.8 – 25.8 g
Acclimation period:	7 days
Diet/Food:	Pellet Diet MF (Oriental Yeast Co., Japan), *ad libitum* except for approx. 2 h before dosing, and 3 h after dosing
Water:	Tap water, *ad libitum*
Housing:	Aluminium cages with wire-mesh floors in groups of 5 animals/sex/cage
Environmental conditions:	Temperature: 23 ± 3 °C Humidity: 55 ± 15 % Air changes: 12 / hour 12-hour light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 1995-01-24 to 1995-02-07

Animal assignment and treatment:
A group of five fasted mice per sex received the test material at a dose level of 5000 mg/kg bw by oral gavage (limit test). The dosing volume was 20 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made three times on the day of dosing (Day 0) and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and on Days 7 and 14. On Day 14 after dosing, animals were sacrificed and subjected to gross necropsy.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. CLINICAL OBSERVATIONS
Decreased spontaneous motor activity was observed in one male and one female as well as sedation and crouching position in another male. These signs were observed at 1 and 3 hours after the administration. A summary of all recorded clinical signs is provided in the table below.

Table 5.2.1-24: HR-001: Acute Oral Toxicity Study In Mice ([redacted] 1995): Clininal signs after dosing

Dose group		**5000 mg/kg bw**							
Sex		**Males**				**Females**			
Time after treatment		1 h	3 h	6 h	Day 1-14	1 h	3 h	6 h	Day 1-14
Total animals examined	**n**	5	5	5	5	5	5	5	5
Clinical sign	**n**	2	1	0	0	1	0	0	0
Decreased spontaneous motor activity	n	1	0	0	0	1	0	0	0
Sedation	n	1	1	0	0	0	0	0	0
Crouching	n	1	1	0	0	0	0	0	0
Single animals observations									
Animal No.§									
1 (101)		*	*	*	*	*	*	*	*
2 (102)		*	*	*	*	*	*	*	*
3 (103)		A	*	*	*	A	*	*	*
4 (104)		*	*	*	*	*	*	*	*

Table 5.2.1-24: HR-001: Acute Oral Toxicity Study In Mice ([redacted] 1995): Clinical signs after dosing

Dose group	5000 mg/kg bw							
Sex	Males				Females			
Time after treatment	1 h	3 h	6 h	Day 1-14	1 h	3 h	6 h	Day 1-14
5 (105)	BC	BC	*	*	*	*	*	*

§ = animal No. of males and (females), * = No abnormalities detected, A = decreased spontaneous motor activity, B = sedation, C = crouching

C. BODY WEIGHT

7 days after administration, a slight body weight loss (0.5 g) was observed in one male when compared with the body weight on the day of administration. No body weight losses were recorded in any animal 14 days after the administration. A summary of the recorded bodyweights is provided in the table below.

Table 5.2.1-25: HR-001: Acute Oral Toxicity Study In Mice ([redacted] 1995): Body weight development

Dose group	5000 mg/kg bw					
Sex	Males			Females		
Day	0	7	14	0	7	14
Animal No.§	Body weight [g]					
1 (101)	29.4	30.7	32.3	22.8	24.7	26.8
2 (102)	30.3	32.0	32.5	24.1	26.1	27.9
3 (103)	30.1	31.5	33.4	23.8	25.5	25.9
4 (104)	31.8	33.1	36.3	23.2	24.8	25.9
5 (105)	32.7	32.2$	33.6	25.8	26.5	28.9
Mean (± SD)	**30.9 ± 1.4**	**31.9 ± 0.9**	**33.6 ± 1.6**	**23.9 ± 1.2**	**25.5 ± 0.8**	**27.1 ± 1.3**

§ = animal No. of males and (females)
$ = Body weight loss when compared with the pre-treatment value.
Note: Standard deviation was not given in the study report. Values were calculated retrospectively

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute oral LD_{50} of glyphosate technical (HR-001) in male and female mice was greater than 5000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is performed according to OECD guideline 401, which is obsolete nowadays. The study is in concordance with the current OECD guideline 420 (2001). The outcome can be therefore reported as valid. The acute oral LD_{50} is above 5000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.1/017
Report author	[redacted]
Report year	1995
Report title	Final report for "Oral and dermal LD_{50} tests with Sanachem Glyphosate acid technical in rats, limit test"
Report No	00917
Document No	Not reported
Guidelines followed in study	OECD 401 (1987), EPA Pesticide Assessment Guidelines, Subdivison F, Series 81-1 (1984)
Deviations from current test guideline (OECD 420, 2001)	5 animals of both sexes used. Air changes not specified. Body weights only recorded once prior to start of study and not once a week during the study. Individual body weights were not reported. Individual clinical signs were not reported, however it was mentioned that no clinical findings were observed.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The test substance, glyphosate acid technical, was evaluated for its acute oral toxicity potential using 5 female and 5 male rats. The test substance was administered by oral gave in a single dose at 2000 mg/kg bw. No clinical signs or mortality were recorded during the study period of 14 days. The gross necropsy conducted at termination of the study revealed slightly congested lungs, splenomegaly and centrilobular hepatic congestion in male and female animals. The acute oral LD_{50} was determined to be

LD_{50}, oral, rat > 2000 mg/kg bw

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** Glyphosate acid
   - Identification: Glyphosate acid technical
   - Description: Fine white granular powder
   - Lot/Batch #: 1073
   - Purity: 97.6 % (certificate of analysis)
   - Stability of test compound: Expiry date: Aug 1996
2. **Vehicle and/or positive control:** Cotton seed oil
3. **Test animals:**
   - Species: Rat

Strain:	Sprague-Dawley
Source:	[redacted]
Age:	Not specified
Sex:	Male and female
Weight at receipt:	150.2 – 237.2 g (males) and 205.6 – 260.9 g (females)
Acclimation period:	At least 5 days
Diet/Food:	Pelleted feed, *ad libitum,* except for a fasting period the night before dosing (approximately 14 – 18 h) and 3 - 4 hours after dosing
Water:	Not specified, *ad libitum*
Housing:	Groups of 5/sex in standard rodent polycarbonate cages
Environmental conditions:	Temperature: 19 – 21 °C Humidity: 62 – 73 % Air changes: Not specified 12-hour light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 23 Feb 1995 – 13 Mar 1995
Finalisation date: 08/08/1995

Animal assignment and treatment:
Glyphosate acid was tested for acute oral toxicity using 5 males and 5 female rats. Prior to dosing, all animals were fasted overnight (approximately 14 – 18 h). Glyphosate acid was administered as a single oral dose by gavage at a concentration of 2000 mg/kg bw. A dose volume of 20 mL per kg body weight was not exceeded. After dosing, the animals were fasted 3 – 4 hours. Individual body weights were measured prior to dosing. The animals were observed for clinical signs and mortality 1 – 2 hours after treatment and daily thereafter for the rest of the study period of 14 days. All animals were sacrificed at the end of the study period and subjected to a gross pathological examination.

II. RESULTS AND DISCUSSION

A. MORTALITY
No mortality occurred during the study.

B. CLINICAL OBSERVATIONS
No clinical signs were observed in any of the animals.

C. BODY WEIGHT
Body weights were not reported.

D. NECROPSY
The gross necropsy conducted at termination of the study revealed slightly congested lungs, splenomegaly and centrilobular hepatic congestion in male and female animals.

III. CONCLUSIONS
The acute oral LD_{50} of glyphosate acid technical in male and female rats was greater than 2000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is performed according to OECD guideline 401, which is obsolete nowadays. Nevertheless, except for deviations provided above, the study is in concordance to the current OECD guideline 420 (2001). Therefore, the outcome can be reported as valid. The acute oral LD_{50} in rat is above 2000 mg/kg bw.
According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.1/018
Report author	[redacted]
Report year	1995
Report title	Final report for "Oral and dermal LD50 tests with Sanachem Glyphosate 62 % IPA in rats, limit test"
Report No	00926
Document No	Not reported
Guidelines followed in study	OECD 401 (1987), EPA Pesticide Assessment Guidelines, Subdivison F, Series 81-1 (1984)
Deviations from current test guideline (OECD 420, 2001)	5 animals of both sexes were used. Air changes not specified. Body weights only recorded once prior to start of study and not once a week during the study. Individual body weights were not reported. Individual clinical signs were not reported, however it was mentioned that no clinical findings were observed.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier	Category 2a

2. Full summary

Executive Summary

The test substance, glyphosate salt, was evaluated for its acute oral toxicity potential using 5 female and 5 male rats. The test substance was administered by oral gave in a single dose at 2000 mg/kg bw. No clinical signs or mortality were recorded during the study period of 14 days. The gross necropsy conducted at termination of the study revealed severe lung congestion, splenomegaly, hepatomegaly with centrilobular congestion and subcapsular renal petechiae in all male and female animals. The acute oral LD_{50} was calculated to be

LD_{50}, oral, rat > 2000 mg/kg bw

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate

Identification:	Sanachem Glyphosate 62 % IPA
Description:	Light greenish viscous liquid
Lot/Batch #:	940950
Purity:	62 % (certificate of analysis)
Stability of test compound:	Aug 1996 (expiry date)

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	Not specified (young adult)
Sex:	Male and female
Weight at receipt:	153 – 212.2 g (males) and 197.2 – 248.7 g (females)
Acclimation period:	At least 5 days
Diet/Food:	Pelleted feed, *ad libitum,* except for a fasting period overnight (approximately 14 to 18 hours) before dosing and 3 - 4 hours after dosing
Water:	Not specified, *ad libitum*
Housing:	Groups of 5/sex in standard rodent polycarbonate cages
Environmental conditions:	Temperature: 19 – 21 °C Humidity: 62 – 73 % Air changes: Not specified 12-hour light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 23 Feb 1995 – 13 Mar 1995
Finalisation date: 08/08/1995

Animal assignment and treatment:
Glyphosate salt was tested for oral toxicity using 5 male and 5 female rats. Prior to dosing, all animals were fasted overnight (approximately 14 – 18 hours). Glyphosate salt was administered as a single oral dose by gavage at a concentration of 2000 mg/kg bw and a dose volume of 20 mL per kg body weight was not exceeded. After dosing, the animals were fasted 3 – 4 hours. Individual body weights were measured prior to dosing. The animals were observed for clinical signs and mortality 1 – 2 hours after treatment and daily thereafter for the rest of the study period of 14 days. All animals were sacrificed at the end of the study period and subjected to a gross pathological examination.

II. RESULTS AND DISCUSSION

A. MORTALITY
No mortality occurred during the study.

B. CLINICAL OBSERVATIONS
No clinical signs were observed in any of the animals.

C. BODY WEIGHT
Body weights were not reported.

D. NECROPSY
The gross necropsy conducted at termination of the study revealed severe lung congestion, splenomegaly, hepatomegaly with centrilobular congestion and subcapsular renal petechiae in all male and female animals (see table below).

Table 5.2.1-26: Final report for "Oral and dermal LD50 tests with Sanachem Glyphosate 62 % IPA in rats, limit test" ([redacted] 1995): Findings at gross necropsy

Dose level	2000 mg/kg bw	
Sex	Males	Females
Findings		
Severe lung congestion	5/5	5/5
Splenomegaly	5/5	5/5
Hepatomegaly with centrilobular congestion	5/5	5/5
Subcapsular renal petechiae	5/5	5/5

III. CONCLUSIONS
The acute oral LD_{50} of the glyphosate salt in male and female rats was greater than 2000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance to the former OECD guideline 401 (1987). Therefore, the outcome can be reported as valid. Minor deviations such as number of air changes or the use of more animals than requested, did not affect the study outcome. The study is however only considered supportive. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.1/019
Report author	[redacted]
Report year	1995
Report title	Acute oral toxicity (LD_{50}) test in rats
Report No	10670
Document No	Not reported
Guidelines followed in study	No final conclusion possible.
GLP	No final conclusion possible.
Previous evaluation	Yes, accepted in RAR (2015)
Short description of study design and observations:	Not available
Short description of results:	Piloerection, subdued behaviour, hunched appearance
Reasons for why the study is not considered relevant/reliable or not considered as key study:	Full study report is not available to the applicant. Only a short summary is provided in the Monograph, B5, 2000
Reasons why the study report is not available for submission	The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance" (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL
Category study in AIR 5 dossier (L docs)	Category 4a

1. Information on the study

Data point:	CA 5.2.1/020
Report author	[redacted]
Report year	1994
Report title	Glyphosate Premix: Acute Oral Toxicity (Limit Test) in the Rat
Report No	545/37
Document No	Not reported
Guidelines followed in study	EPA OTS 798.1175 (Acute Oral Toxicity) EPA OPP 81-1 (Acute Oral Toxicity)
Deviations from current test guideline (OECD 420, 2001)	Both sexes, each with 5 animals per dose group instead of one sex (usually females) with 5 animals per dose group. At dosing, animals were 5 to 8 weeks instead of 8 to 12 weeks old. Animals in range-finding study were observed for 5 days instead for at least 14 days.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The acute oral toxicity of glyphosate premix was investigated in male and female rats. Therefore, a dose-range finding study with 1 animal per sex was performed. The animals were dosed once by oral gavage at a concentration of 5000 mg/kg bw. As no signs of toxicity were observed during the 5-day observation time following dosing, the main study was conducted. Therefore, 5 further animals of each sex were administered the test substance by oral gavage once at a concentration of 5000 mg/kg bw and a dose volume of 4.1 mL/kg. The animals were regularly observed for clinical signs after 0.5, 1, 2, and 4 hours after dosing and once daily thereafter for 14 days. Body weights were determined immediately prior to dosing, at day 7 and day 14. At the end of the observation period, all animals were sacrificed and subjected to gross necropsy.

No adverse clinical signs were noticed. Changes of body weight were in the normal range. No mortality occurred. No abnormal necropsy findings were noted.
The acute oral LD_{50} was determined to be

LD_{50}, oral, rats > 5000 mg/kg bw

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	Glyphosate
Identification:	Glyphosate Premix
Description:	Pale yellow liquid, aqueous solution containing the isopropylamine salt of glyphosate as the active ingredient
Lot/Batch #:	290-JaK-146-4
Purity:	46.1 % (Glyphosate), 62.2 % (Glyphosate isopropylamine salt)
Stability of test compound:	Not specified
2. Vehicle and/ or positive control:	None
3. Test animals:	
Species:	Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	5 – 8 weeks
Sex:	Males and females
Weight at dosing:	128 – 155 g (males), 125 – 137 g (females)
Acclimation period:	At least 5 days
Diet/Food:	Rat and mouse expanded diet no. 1, *ad libitum* (Except for a fasting period the night prior to dosing and 2 hours after dosing)
Water:	Tap water, *ad libitum*
Housing:	5 animals/sex in solid-floor polypropylene cages with woodflakes
Environmental conditions:	Temperature: 19 – 22 °C Humidity: 42 – 55 % Air changes: 15 times / hour 12-hour light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 28/03/1994 to 26/04/1994 (Duration of the study- not further specified)

Animal assignment and treatment:

Rats were housed by sex (5 males and 5 females) and starved the night prior to dosing. The undiluted test material was administered by a single oral gavage at dose level of 5000 mg/kg bw with an application volume of 4.1 mL/kg bw. Animals were starved for a further 2 hours following dosing. All animals were observed for clinical signs of toxicity at several time points on the day of administration (0.5, 1, 2 and 4 hours) and at least once a day, thereafter. Body weights were recorded prior to administration, on day 7 and prior to sacrifice on day 14.
At study termination all animals were sacrificed followed by gross necropsy examination.

II. RESULTS AND DISCUSSION

A. MORTALITY
No mortality occurred during the range-finding or main study.

B. CLINICAL OBSERVATIONS
No adverse observations were made in the range-finding or main study.

C. BODY WEIGHT
Body weight gain of all animals was within the normal range (see table below).

Table 5.2.1-27: Glyphosate Premix: Acute Oral Toxicity (Limit Test) in the Rat ([redacted] 1994): Body weight data of the main study

Dose level	5000 mg/kg bw									
Sex	Males			Females			Males		Females	
Day	0	7	14	0	7	14	0-7	0-14	0-7	0-14
Animal No.§	Body weight [g]						Body weight gain [g]			
3-0 (4-0)	155	205	253	129	165	185	50	48	36	20
3-1 (4-1)	154	210	250	125	158	174	56	40	33	16
3-2 (4-2)	128	158	184	137	174	202	30	26	37	28
3-3 (4-3)	140	196	243	127	160	175	56	47	33	15
3-4 (4-4)	137	198	255	132	168	186	61	57	36	18
Mean ± SD	**142.8 ± 11.6**	**193.4 ± 20.6**	**237.0 ± 30.0**	**130.0 ± 4.7**	**165.0 ± 6.4**	**184.4 ± 11.3**	**50.6 ± 12.2**	**43.6 ± 11.5**	**35.0 ± 1.9**	**19.4 ± 5.2**

§ = animal No. of males and (females)
Note: Mean and standard deviation were not given in the study report. Values were calculated retrospectively.

D. NECROPSY
No abnormal necropsy findings were noticed.

III. CONCLUSIONS

The acute oral LD_{50} of the test material (glyphosate premix) in male and female rats was greater than 5000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was performed according to a national guidelines similar to the current OECD guideline 420 (2001). Nevertheless, due to deviations and the composition of the test material the study is considered as supplementary information. The acute oral LD_{50} is above 5000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.1/021
Report author	[redacted]
Report year	1994
Report title	Glyphosate: Acute oral toxicity (limit test) in the rat
Report No	710/14
Document No	Not reported
Guidelines followed in study	No final conclusion possible.
GLP	No final conclusion possible.
Previous evaluation	Yes, accepted in RAR (2015)
Short description of study design and observations:	Not available
Short description of results:	LD_{50} > 2000 mg/kg bw No findings
Reasons for why the study is not considered relevant/reliable or not considered as key study:	Full study report is not available to the applicant. Only a short summary is provided in the Monograph, B5, 2000, *Category 4a*
Reasons why the study report is not available for submission	The notifier has not access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance" (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL.
Category study in AIR 5 dossier (L docs)	Category 4a

1. Information on the study

Data point:	CA 5.2.1/022
Report author	[redacted]
Report year	1994
Report title	Acute oral toxicity in rats
Report No	GHA-94-401/R
Document No	Not reported
Guidelines followed in study	OECD 401 (1987)
Deviations from current test guideline (OECD 420, 2001)	5 animals of both sexes per group used. Control group included. Organ weights measured.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing	Yes

facilities	
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The test substance, glyphosate technical was evaluated for its acute oral toxicity potential. 5 female and 5 male rats were administered glyphosate technical (in 3 % carboxymethyl-cellulose in water) by oral gavage in a single dose at 5000 mg/kg bw. A control group was included with 5 male and 5 female animals. No clinical signs or mortality were recorded during the study period of 14 days. Body weights of treated male animals were statistically significantly lower at the end of the study period. The gross necropsy conducted at termination of the study did not reveal any abnormalities. Organ weights were comparable to the control, except for male heart weight, which was statistically significantly decreased but not considered to be of toxicological relevance. The acute oral LD_{50} was determined to be

LD_{50}, oral, rat > 5000 mg/kg bw

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate

Identification: Glyphosate Technical

Description: White or almost white crystalline powder

Lot/Batch #: 36300892

Purity: 99.6 % (certificate of analysis)

Stability of test compound: Expiry date: Sep 1994

2. Vehicle and/ or positive control: 3 % carboxymethyl-cellulose in water

3. Test animals:

Species: Rat

Strain: Wistar

Source: [redacted]

Age: Not specified

Sex: Male and female

Weight at receipt: Not specified

Acclimation period: Approximately 3 weeks

Diet/Food: Altromin rodent chow, *ad libitum,* except for a fasting period of 16 hours before dosing and 4 hours after dosing

Water: Tap water, *ad libitum*

Housing: Groups of 5/sex in marolon III. box

Environmental conditions: Temperature: 20 ± 2 °C
Humidity: 45 – 70 %
Air changes: 10 times/h
12-hour light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 17 Jan 1994 – 31 Jan 1994
Finalisation date: 06 Apr 1994

Animal assignment and treatment:
Glyphosate technical was tested for acute oral toxicity using 5 males and 5 female rats per dose group. 16 hours prior to dosing, all animals were fasted. Glyphosate technical was administered as a single oral dose by gavage at a concentration of 5000 mg/kg bw. A control group was included as well. After dosing, the animals were fasted 4 hours. Individual body weights were measured prior to dosing and weekly thereafter. The animals were observed for clinical signs and mortality on the day of dosing and twice a day thereafter for the rest of the study period of 14 days. All animals were sacrificed at the end of the study period and subjected to a gross pathological examination. Furthermore, organ weights of the brain, heart, thymus, stomach, spleen, lung, liver, kidneys and adrenals were measured of both sexes. In addition, organ weights of testes and epididymides were measured of male animals. The ratio of organ to brain weight was calculated.

Statistical analysis:
The data were analysed for statistical significance using the t-test. A p-value of <0.05 indicated statistical significance.

II. RESULTS AND DISCUSSION

A. MORTALITY
No mortality occurred during the study.

B. CLINICAL OBSERVATIONS
No clinical signs were observed in any animal of the control or treated group.

C. BODY WEIGHT
No differences in body weight or body weight gain were observed in female rats when compared to the control group. In males however, a statistically significant decrease in body weight gain (-14.9 %) was observed in the treated group during the second week (see table below).

Table 5.2.1-28: Acute oral toxicity in rats ([redacted] 1994): Summary of body weights recorded during the study in control and treated animals.

Day		0	7	14	0-14	0	7	14	0-14
Sex		Males				Females			
Dose level (mg/kg bw)	Animal No.§	Body weight [g]			Bw gain [g]	Body weight [g]			Bw gain [g]
0	6 (16)	205	250	285	80	135	160	175	40
	7 (17)	210	270	315	105	150	175	195	45
	8 (18)	200	260	300	100	160	190	205	45
	9 (19)	190	255	280	90	145	170	175	30
	10 (20)	195	250	290	95	145	165	175	30
	Mean	**200.0**	**257.0**	**294.0**	**94.0**	**147.0**	**172.0**	**185.0**	**38.0**
	± SD	**± 7.9**	**± 8.4**	**± 13.9**	**± 9.6**	**± 9.1**	**± 11.5**	**± 14.1**	**± 7.6**
5000	1 (11)	195	240	270	75	150	170	180	30
	2 (12)	205	250	275	70	160	185	195	35

Table 5.2.1-28: Acute oral toxicity in rats ([redacted] 1994): Summary of body weights recorded during the study in control and treated animals.

Day		0	7	14	0-14	0	7	14	0-14
Sex		Males				Females			
Dose level (mg/kg bw)	Animal No.§	Body weight [g]			Bw gain [g]	Body weight [g]			Bw gain [g]
	3 (13)	190	245	275	85	145	165	180	35
	4 (14)	200	250	280	80	145	170	185	40
	5 (15)	205	260	295	90	145	165	185	40
	Mean	**199.0**	**249.0**	**279.0**	**80.0***	**149.0**	**171.0**	**185.0**	**36.0**
	± SD	**± 6.5**	**± 7.4**	**± 9.6**	**± 7.9**	**± 6.5**	**± 8.2**	**± 6.1**	**± 4.2**

§ = animal No. of males and (females), * $p<0.05$

D. ORGAN WEIGHTS

The absolute organ weights were similar to the controls. The heart weight was significantly lower in male rats at 5000 mg/kg bw. However, this finding is not considered to be of toxicological relevance (see table below).

Table 5.2.1-29: Acute oral toxicity in rats ([redacted] 1994): Summary of absolute organ weights and organ to brain weight ratios.

Dose group (mg/kg bw)		0	5000	0	5000	0	5000	0	5000
Sex		Males		Females		Males		Females	
		Absolute organ weight [g]				Relative organ weight [g]			
Brain	Mean	1.89	1.88	1.66	1.68	1.89	1.88	1.66	1.68
	± SD	± 0.07	± 0.10	± 0.07	± 0.09	± 0.07	± 0.10	± 0.07	± 0.09
Heart	Mean	0.92	0.84	0.64	0.65	0.49	0.45	0.39	0.39
	± SD	± 0.07	± 0.04*	± 0.10	± 0.06	± 0.04	± 0.04	± 0.07	± 0.05
Thymus	Mean	0.66	0.58	0.46	0.44	0.35	0.31	0.28	0.27
	± SD	± 0.11	± 0.04	± 0.12	± 0.07	± 0.06	± 0.03	± 0.07	± 0.04
Stomach	Mean	1.53	1.54	1.19	1.19	0.81	0.82	0.72	0.71
	± SD	± 0.13	± 0.14	± 0.12	± 0.09	± 0.05	± 0.10	± 0.09	± 0.07
Spleen	Mean	0.70	0.65	0.51	0.49	0.37	0.35	0.31	0.29
	± SD	± 0.11	± 0.05	± 0.04	± 0.07	± 0.04	± 0.03	± 0.03	± 0.05
Lung	Mean	1.69	1.63	1.29	1.35	0.90	0.87	0.78	0.81
	± SD	± 0.19	± 0.14	± 0.11	± 0.06	± 0.10	± 0.09	± 0.09	± 0.08
Liver	Mean	13.33	13.00	8.27	8.34	7.05	6.93	5.00	4.99
	± SD	± 0.86	± 1.00	± 1.25	± 0.87	± 0.27	± 0.69	± 0.83	± 0.69
Kidneys	Mean	2.12	2.06	1.29	1.29	1.12	1.10	0.78	0.77
	± SD	± 0.23	± 0.18	± 0.20	± 0.09	± 0.09	± 0.11	± 0.13	± 0.05
Adrenals	Mean	0.05	0.05	0.06	0.07	0.03	0.03	0.04	0.04
	± SD	± 0.01	± 0.00	± 0.02	± 0.01	± 0.00	± 0.00	± 0.01	± 0.01
Testes	Mean	3.17	2.98			1.68	1.58		
	± SD	± 0.17	± 0.20			± 0.10	± 0.10		
Epididymis	Mean	0.75	0.66			0.40	0.35		
	± SD	± 0.02	± 0.08			± 0.02	± 0.03		

*$p \leq 0.05$

E. NECROPSY

No abnormal findings were recorded during necropsy.

III. CONCLUSIONS

Based on the results the test substance glyphosate technical is considered as non-toxic concerning oral toxicity.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is performed according to OECD guideline 401, which is obsolete nowadays. Nevertheless, except for deviations provided above, the study is in concordance to the current OECD guideline 420 (2001). Therefore, the outcome can be reported as valid. The acute oral LD_{50} in rat is above 5000 mg/kg bw as no mortality occurred during the study. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.1/023
Report author	[redacted]
Report year	1994
Report title	Glyphosate technical: Acute oral toxicity study in mice
Report No	940020
Document No	Not reported
Guidelines followed in study	No final conclusion possible.
GLP	No final conclusion possible.
Previous evaluation	Yes, accepted in RAR (2015)
Short description of study design and observations:	Not available
Short description of results:	LD_{50} > 2000 mg/kg bw Piloerection, hunched posture, hypoactivity (all mice)
Reasons for why the study is not considered relevant/reliable or not considered as key study:	Full study report is not available to the applicant. Only a short summary is provided in the Monograph, B5, 2000
Reasons why the study report is not available for submission	The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance" (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL
Category study in AIR 5 dossier (L docs)	Category 4a

1. Information on the study

Data point:	CA 5.2.1/024
Report author	[redacted]
Report year	1992
Report title	Glyphosate technical Acute oral toxicity (Limit test) in the rat
Report No	134/37

Document No	Not reported
Guidelines followed in study	OECD 401 (1987)
Deviations from current test guideline (OECD 420, 2001)	6 animals per sex used instead of 5 in total. Animals were 5 – 8 weeks old instead of 8 – 12 weeks.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The acute oral toxicity of glyphosate was investigated in 5 male and 5 female rats of the Sprague-Dawley strain. The test substance was administered undiluted by oral gavage to each animal at a dosage of 2000 mg/kg bw. Mortality, body weight and clinical signs were recorded during the subsequent 14 days. All animals were subjected to a gross necropsy at the end of the study.

No mortality occurred. No clinical signs were noticed. Changes of body weight were in the normal range. No abnormalities at necropsy were observed.
The acute oral LD_{50} was determined to be

LD_{50}, oral, rat > 2000 mg/kg bw

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate

Identification: Glyphosate technical
Description: White powder
Lot/Batch #: L3258
Purity: Not specified
Stability of test compound: Not specified

2. Vehicle and/ or positive control: Distilled water

3. Test animals:

Species: Rat
Strain: Sprague-Dawley
Source: [redacted]
Age: 5 – 8 weeks
Sex: Male and female
Weight at dosing: 142 – 150 g (males), 129 – 142 g (females)
Acclimation period: At least 5 days

Diet/Food:	Rat and mouse extended Diet No. 1 (SDS Limited), *ad libitum* (except for fasting period overnight before dosing)
Water:	Not specified, *ad libitum*
Housing:	Five per sex in solid-floor polypropylene cages with sawdust bedding.
Environmental conditions:	Temperature: 19 – 22 °C Humidity: 52 – 63 % Air changes: 15 per hour light/dark cycle: 12 h / 12 h

B. STUDY DESIGN AND METHODS

In life dates: 11/12/1991 to 20/01/1992

Animal assignment and treatment:
A group of 5 male and 5 female rats was fasted overnight and dosed with the test substance at a concentration of 2000 mg/kg bw. After treatment, animals were observed for mortality and clinical signs four times within the first 4 hours after dosing and once daily thereafter. Surviving animals were sacrificed after 14 days and were subjected to necropsy. Body weights were recorded immediately before dosing and on day 7 and 14 during the study.

II. RESULTS AND DISCUSSION

A. MORTALITY
No mortality occurred.

B. CLINICAL OBSERVATIONS
No clinical signs of toxicity were observed during the 14 days observation period.

C. BODY WEIGHT
All animals gained weight during the study (see table below).

D. NECROPSY
No abnormalities were observed during necropsy.

III. CONCLUSIONS

The acute oral LD_{50} of the test material in male and female rats was greater than 2000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance to the former OECD guideline 401 (1987). Therefore, the outcome can be reported as valid. The acute oral LD_{50} is above 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

Table 5.2.1-30: Acute oral toxicity of Glyphosate ([redacted], 1992): Mean body weight

Dose group	2000 mg/kg bw	
Sex	Males	Females

Day	0	7	14	0	7	14
Animal No.§	**Body weight [g]**					
281 (286)	146	209	272	141	177	206
282 (287)	142	213	275	138	182	219
283 (288)	150	220	294	129	170	197
284 (289)	150	210	279	133	173	209
285 (290)	149	210	277	142	174	209
Mean (± SD)	**147.4 ± 3.4**	**212.4 ± 4.5**	**279.4 ± 8.6**	**136.6 ± 5.5**	**165.2 ± 23.5**	**208.0 ± 7.9**

§ = animal No. of males and (females)
Note: Mean and standard deviation were not given in the study report. Values were calculated retrospectively

1. Information on the study

Data point:	CA 5.2.1/025
Report author	[redacted]
Report year	1991
Report title	Assessment of acute oral toxicity of "Glyphosate technical" to mice
Report No	12321
Document No	Not reported
Guidelines followed in study	OECD 401 (1987)
Deviations from current test guideline (OECD 420, 2001)	Both sexes used instead of one sex (usually females). Fasting periods in mice prior to dosing 18 hours instead of 3-4 hours and 4 hours instead of 1-2 hours after dosing. No observation 30 minutes after dosage. Individual weights are not reported.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary
The acute oral toxicity of glyphosate technical was investigated in male and female mice (5 animals/sex/group) of the Bom:NMRI strain. The test substance, was administered once by oral gavage to the starved animals at a dosage of 2000 mg/kg bw and constant dose volume of 10 mL/kg bw. The animals were observed 1, 3 and 6 hours after dosing. Furthermore, mortality, body weight and clinical signs were recorded during the subsequent 14 days. All animals were subjected to a gross necropsy at the end of the study.

No adverse clinical signs were noticed. Changes of body weight were in the normal range. No mortality occurred. No abnormal necropsy findings were noted.
The acute oral LD_{50} was calculated to be

LD_{50}, oral, mice > 2000 mg/kg bw

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate

Identification:	Glyphosate Technical (PMG)
Description:	White powder
Lot/Batch #:	206-JaK-25-1
Purity:	98.6 %
Stability of test compound:	Not specified

2. Vehicle and/ or positive control: Distilled water

3. Test animals:

Species:	Mice
Strain:	Bom:NMRI
Source:	[redacted]
Age:	4 – 5 weeks
Sex:	Male and female
Weight at dosing:	21 – 24 g
Acclimation period:	5 days
Diet/Food:	Complete rodent diet "Altromin 1314", *ad libitum* (Except for a fasting period of 18 hours prior to dosing and 4 hours after dosing)
Water:	Tap water, *ad libitum*
Housing:	5 animals/sex in Macrolone cages Type II (26 × 20 × 14 cm) Pinewood sawdust "Spanvall Special White" bedding
Environmental conditions:	Temperature: 21 ± 3 °C Humidity: 55 ± 15 % Air changes: 6 times / hour 12-hour light / dark cycle (light during 06:00 – 18:00)

B. STUDY DESIGN AND METHODS

In life dates: No information

Animal assignment and treatment:

Mice were housed by sex (5 males and 5 females) and starved for approximately 18 hours prior to dosing. Test material was suspended in distilled water and administered by a single oral gavage at dose level of 2000 mg/kg bw with an application volume of 10 mL/kg bw. Animals were starved for a further 3-4 hours following dosing.
All animals were observed for clinical signs of toxicity at several time points on the day of administration and at least once a day, thereafter. Body weights were recorded prior to administration, on day 7 and prior to sacrifice on day 14.

At study termination all animals were sacrificed with carbon dioxide followed by gross necropsy examination.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortality occurred during the 14-days observation period.

B. CLINICAL OBSERVATIONS

No adverse observations were made. 1 and 3 hours after treatment piloerection and sedation were observed. Piloerection was observed up to 6 hours after treatment.

Table 5.2.1-31: Assessment of acute oral toxicity of "Glyphosate technical" to mice ([redacted], [redacted] 1991): Summary of clinical observations

Dose group	**2000 mg/kg bw**							
Sex	**Males**				**Females**			
Time after treatment	1 h	3 h	6 h	Day 1 - 14	1 h	3 h	6 h	Day 1 - 14
Total animals n examined	5	5	5	5	5	5	5	5
Clinical sign n	5	5	5	0	5	5	5	0
Piloerection n	5	5	5	0	5	5	5	0
Sedation n	5	5	0	0	5	5	0	0
Single animals observations								
Animal No.§								
6 (1)	AB	AB	A	*	AB	AB	A	*
7 (2)	AB	AB	A	*	AB	AB	A	*
8 (3)	AB	AB	A	*	AB	AB	A	*
9 (4)	AB	AB	A	*	AB	AB	A	*
10 (5)	AB	AB	A	*	AB	AB	A	*

§ = animal No. of males and (females), * = No abnormalities detected, A = piloerection, B = sedation

C. BODY WEIGHT

Body weight gain of all animals was within the normal range. A summary of the body weights is provided in the table below.

Table 5.2.1-32: Assessment of acute oral toxicity of "Glyphosate technical" to mice ([redacted], [redacted] 1991): Body weight data

Dose level	**2000 mg/kg bw**					
Sex	**Males**			**Females**		
Day	**0**	**7**	**14**	**0**	**7**	**14**
Body weight [g]						
Mean ± SD	21.6 ± 0.5	32.0 ± 1.7	36.8 ± 2.3	23.8 ± 0.4	25.6 ± 0.5	28.0 ± 1.2

D. NECROPSY

No abnormal necropsy findings were noticed.

III. CONCLUSIONS

The acute oral LD_{50} of glyphosate) in mice was estimated to be greater than 2000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is performed according to OECD guideline 401, which is obsolete nowadays. Nevertheless, except for deviations provided above, the study is in concordance to the current OECD guideline 420 (2001). Therefore, the outcome can be reported as valid. The acute oral LD_{50} in mice is above 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.1/026
Report author	[redacted]
Report year	1991
Report title	Acute oral toxicity study with glyphosate technical (FSG 0309 H/05 March 90) in Wistar rats
Report No	ES.874.AOR
Document No	Not reported
Guidelines followed in study	OECD 401 (1987)
Deviations from current test guideline (OECD 420, 2001)	Both sexes, each with 5 animals per dose group instead of one sex (usually females) with 5 animals per dose group, no stepwise procedure; highest dose group above exceptionally considered 5000 mg/kg bw; maximum dose volume was exceeded at the highest dose level (25 mL/kg bw instead of 20 mL/kg bw).
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The test substance, glyphosate technical, was evaluated for its acute oral toxicity potential. 5 female and 5 male rats were used per dose group. The test substance was administered by oral gavage at levels of 2500, 5000 and 7500 mg/kg bw. No mortality and no clinical signs occurred at the two low dose groups. At the high dose level of 7500 mg/kg bw, 4/10 animals died and clinical signs included lethargy, ataxia and dyspnoea. Body weight decreased in animals that died during the study. Some surviving rats lost weight at day 7 and however gained weight until end of the study, except for 2 rats which lost weight up to the end of the observation period. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute oral LD_{50} was determined to be

LD_{50}, oral, rat > 7500 mg/kg bw

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	Glyphosate
Identification:	Glyphosate Technical
Description:	Solid white crystals
Lot/Batch #:	60
Purity:	96.80 %
Stability of test compound:	Expiry date July 1992
2. Vehicle and/or positive control:	Refined groundnut (peanut) oil
3. Test animals:	
Species:	Rat
Strain:	Wistar
Source:	Bred at the toxicology department [redacted]
Age:	11 weeks
Sex:	Male and female
Weight at dosing:	140 – 196 g (males), 122 – 210 g (females)
Acclimation period:	At least one week
Diet/Food:	Standard 'Gold Mohur' brand pelleted rat feed, *ad libitum* except for approx. 14 – 16 h before and 3 h after dosing
Water:	Deep borewell water passed through activated charcoal filter and exposed to UV rays, *ad libitum*
Housing:	Groups of 5/sex in standard polypropylene cages with stainless steel top grill. Bedding: steam sterilised clean paddy husk.
Environmental conditions:	Temperature: 23 ± 2 °C Humidity: 68 ± 6 % Air changes: 10 – 15 / hour 12-hour light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: No information given

Animal assignment and treatment:
Groups of 5 fasted females and 5 males were assigned to three different dose groups. The animals received the test substance at dose levels of 2500, 5000 and 7500 mg/kg bw by oral gavage. The dosing volume was 8.3, 16.6 and 25.0 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made four times on the day of dosing (Day 0) and once daily thereafter for 14 days (Days 7 – 14). Individual body weights were recorded just prior to dosing and on Days 7 and 14.
On Day 14 after dosing, surviving animals were sacrificed. All study animals (dead and surviving) were subjected to gross necropsy and all abnormalities were recorded.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities at 2500 and 5000 mg/kg bw. However, 40 % of the animals died at 7500 mg/kg bw (see tbale below).

Table 5.2.1-33 Acute oral toxicity study with glyphosate technical (FSG 03090 h/05 March 90) in Wistar rats ([redacted] 1991): Mortality for all dose groups

Dose level (mg/kg bw)	Animal No.		No. of deaths/No. of animals examined (Day of death)	
	Males	Females	Males	Females
2500	0441 – 0445	446 – 450	-	-
5000	0451 – 0455	456 – 460	-	-
7500	0461	0466	1/5 (day 3)	-
	0462	0467	-	1/5 (day 2)
	0463	0468	-	-
	0464	0469	1/5 (day 1)	1/5 (day 3)
	0465	0410	-	-

- = No death

B. CLINICAL OBSERVATIONS

Clinical signs were noted in animals of the high dose group (7500 mg/kg bw) and included lethargy, ataxia and dyspnoea. The table below shows individual clinical observations.

Table 5.2.1-34: Acute oral toxicity study with glyphosate technical (FSG 03090 h/05 March 90) in Wistar rats ([redacted] 1991): Individual clinical observations

Dose level (mg/kg bw)	Animal No.		Clinical observations	
	Males	Females	Males	Females
2500	0441 – 0445	446 – 450	-	-
5000	0451 – 0455	456 – 460	-	-
7500	0461	0466	-	-
	0462	0467	-	Lethargy, ataxia and dyspnoea
	0463	0468	-	-
	0464	0469	-	Lethargy
	0465	0470	-	Lethargy

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance at 2500 and 5000 mg/kg bw, except for a few animals that lost weight on day 7 but exceeded their initial weight on day 14. Two animals in the mid dose group lost weight. Dead animals from the high dose group had lost weight. Individual body weights are provided in the table below.

Table 5.2.1-35: Acute oral toxicity study with glyphosate technical (FSG 03090 h/05 March 90) in Wistar rats ([redacted] 1991): Body weight development

Day		**0**	**7**	**14**	**0**	**7**	**14**
Sex		**Males**			**Females**		
Dose level (mg/kg bw)	**Animal No.**	**Body weight [g]**					
2500	0441 (0446)	172	172	174	126	152	152
	0442 (0447)	140	156	156	134	136	162
	0443 (0448)	186	202	204	180	174	220
	0444 (0449)	186	204	218	122	112	150
	0445 (0450)	148	166	166	180	194	196
	Mean	**166.4**	**180**	**183.6**	**148.4**	**153.6**	**176**
	± SD	**±21.4**	**± 21.8**	**± 26.3**	**± 29.2**	**± 32.0**	**± 30.8**

Table 5.2.1-35: Acute oral toxicity study with glyphosate technical (FSG 03090 h/05 March 90) in Wistar rats ([redacted] 1991): Body weight development

Day		0	7	14	0	7	14
Sex		Males			Females		
Dose level (mg/kg bw)	Animal No.	Body weight [g]					
5000	0451 (0456)	170	162	210	154	172	190
	0452 (0457)	176	192	208	168	140	150
	0453 (0458)	170	166	202	156	118	154
	0454 (0459)	194	204	234	148	154	166
	0455 (0460)	142	144	144	172	188	196
	Mean	**170.4**	**173.6**	**199.6**	**159.6**	**154.4**	**171.2**
	± SD	**± 18.7**	**± 24.1**	**± 33.4**	**± 10.0**	**± 27.3**	**± 20.9**
7500	0461 (0466)	196	-	-(176)*	210	230	258
	0462 (0467)	186	202	222	184	-	-(164)*
	0463 (0468)	174	184	184	184	152	194
	0464 (0469)	190	-	-(174)*	150	-	-(126)*
	0465 (0470)	176	182	206	152	174	174
	Mean	**184.4**	**189.3**	**204.0**	**176**	**191.0**	**208.7**
	± SD	**± 9.3**	**± 11.0**	**± 19.1**	**± 25.2**	**± 40.2**	**± 43.9**

Note: Mean and standard deviation were not given in the study report. Values were calculated retrospectively. *Body weight of decedents, not used for calculation of the mean and the SD.

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities in dead and surviving animals.

III. CONCLUSIONS

The oral LD_{50} of glyphosate technical in male and female rats was determined to be greater than 7500 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is performed according to OECD guideline 401, which is obsolete nowadays.
Nevertheless, except for deviations provided above, the study is in concordance to the current OECD guideline 420 (2001). Therefore, the outcome can be reported as valid. The acute oral LD_{50} is above 7500 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.1/027
Report author	[redacted]
Report year	1991
Report title	Acute oral toxicity study with glyphosate technical in swiss albino mice
Report No	ES.875.AOM
Document No	Not reported
Guidelines followed in study	OECD 401 (1987)
Deviations from current test guideline (OECD 420, 2001)	Both sexes instead of one sex (usually females) with 5 animals per dose group. No stepwise procedure. Highest dose group above exceptionally considered 5000 mg/kg bw. Maximum dose volume was exceeded at the mid and high dose level as the test substance was administered in groundnut (peanut) oil. Animals 14 weeks old instead of 8 to 12 weeks. Day of clinical observations not provided.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The test substance, glyphosate, was evaluated for its acute oral toxicity potential. 5 female and 5 male mice were used per dose group. The test substance was administered by oral gavage at levels of 2500, 5000 and 7500 mg/kg bw. Mortality occurred in the respective dose groups as follows: 10 %, 20 %, and 40 %. Clinical signs included lethargy in all dose groups, ataxia and dyspnoea at 7500 mg/kg bw. Body weight decreased in animals that died during the study. A few surviving mice lost weight at day 7 however gained weight until end of the study, except for 2 mice which lost weight. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute oral LD_{50} was determined to be

LD_{50}, oral, mice > 7500 mg/kg bw

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate

Identification: Glyphosate Technical

Description: Solid white crystals

Lot/Batch #: 60

Purity: 96.80 %

Stability of test compound: Expiry date July 1992

2. Vehicle and/or positive control: Refined groundnut (peanut) oil

3. Test animals:

Species:	Mice
Strain:	Swiss albino
Source:	Bred at the toxicology department. [redacted]
Age:	14 weeks
Sex:	Male and female
Weight at dosing:	28 – 40 g (males), 28 – 40 g (females)
Acclimation period:	At least one week
Diet/Food:	Standard 'Gold Mohur' brand pelleted mice feed, *ad libitum* except for approx. 14 – 16 h before and 1 h after dosing
Water:	Deep borewell water passed through activated charcoal filter and exposed to UV rays. Kept in glass bottles, *ad libitum*
Housing:	Groups of 5/sex in standard polypropylene cages with stainless steel top grill. Individual housing after dosing. Bedding: steam sterilised clean paddy husk.
Environmental conditions:	Temperature: 23 ± 2 °C Humidity: 68 ± 6 % Air changes: 10 – 15 / hour 12-hour light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: No information given

Animal assignment and treatment:
Groups of 5 fasted females and 5 males were assigned to three different dose groups. The animals received the test substance at dose levels of 2500 (G1), 5000 (G2) and 7500 (G3) mg/kg bw by oral gavage. The dosing volume was 6.25, 12.5 and 18.75 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made four times on the day of dosing (Day 0) and once daily thereafter for 14 days (Days 7-14). Individual body weights were recorded just prior to dosing and on Days 7 and 14.

14 days after dosing, each animal was euthanised using ether anaesthesia. All study animals (dead and surviving) were subjected to gross necropsy and all abnormalities were recorded.

II. RESULTS AND DISCUSSION

A. MORTALITY

Mortality was observed between 1 and 8 days following treatment at dose levels of 2500 mg/kg bw (1/5 males, day 2), 5000 mg/kg bw (1/5 males, day 5 and 1/5 females, day 8), and 7500 mg/kg bw (3/5 males, days 1, 2) and 1/5 females, days 2). Details are provided in the table below.

Table 5.2.1-36: Acute oral toxicity study with glyphosate technical in swiss albino mice ([redacted] 1991): Mortality for all dose groups

Dose level (mg/kg bw)	Animal No.		Mortality*	
	Males	Females	Males	Females
2500	001 – 005	006 – 010	1 (004)§ /5 (day 2)	0
5000	011 – 015	016 – 020	1 (013) /5 (day 5)	1 (020) /5 (day 8)
7500	021	026	1/5 (day 1)	-
	022	027	1/5 (day 2)	-
	023	028	-	-

Table 5.2.1-36: Acute oral toxicity study with glyphosate technical in swiss albino mice ([redacted] 1991): Mortality for all dose groups

Dose level (mg/kg bw)	Animal No.		Mortality*	
	Males	Females	Males	Females
	024	029	-	-
	025	030	1/5 (day 1)	1/5 (day 2)

* = No. of deaths/No. of animals examined (Day of death), - = No death, § = animal number

B. CLINICAL OBSERVATIONS

Clinical signs of toxicity in decedents and survivors were observed in all treatment groups and included and included lethargy, urine incontinence, ataxia and dyspnoea. For detail see table below.

Table 5.2.1-37: Acute oral toxicity study with glyphosate technical in swiss albino mice [redacted], 1991): Individual clinical observations

Dose level (mg/kg bw)	Animal No.		Clinical observations	
	Males	Females	Males	Females
2500	001 – 005	006 – 010	Lethargy (002 – 004)§	-
5000	011 – 015	016 – 020	Lethargy, urine incontinence (013)	Lethargy (020)
7500	021	026	Lethargy	-
	022	027	Lethargy	-
	023	028	-	Lethargy
	024	029	Lethargy	-
	025	030	Lethargy, ataxia and dyspnoea	Lethargy

- = No observations made, §= animal number from decedents

C. BODY WEIGHT

Surviving animals with body weight loss or maintenance of body weight from days 1 to 7 gained weight during the 14-day observation period. Individual body weights are provided in the table below.

Table 5.2.1-38: Acute oral toxicity study with glyphosate technical in swiss albino mice ([redacted] 1991): Body weight data

Day		0	7	14	0	7	14
Sex		**Males**			**Females**		
Dose level (mg/kg bw)	**Animal No.§**	**Body weight [g]**					
2500	001 (006)	24	24	26	34	34	34
	002 (007)	28	28	30	30	30	30
	003 (008)	32	34	32	32	32	- (32)*
	004 (009)	30	-	- (24)*	30	30	30
	005 (010)	34	32	34	30	32	32
	Mean	**29.6**	**29.5**	**30.5**	**31.2**	**31.6**	**31.5**
	± SD	**± 3.8**	**± 4.4**	**± 3.4**	**± 1.8**	**± 1.7**	**± 1.9**
5000	011 (016)	36	38	36	26	30	28
	012 (017)	40	40	40	30	26	30
	013 (018)	36	-	- (26)*	26	30	28

Table 5.2.1-38: Acute oral toxicity study with glyphosate technical in swiss albino mice ([redacted] 1991): Body weight data

Day		0	7	14	0	7	14
Sex		Males			Females		
Dose level (mg/kg bw)	Animal No.§	Body weight [g]					
	014 (019)	34	36	36	24	32	26
	015 (020)	34	34	34	28	18	20
	Mean	**36**	**37.0**	**36.5**	**26.8**	**27.2**	**26.4**
	± SD	**± 2.4**	**± 2.6**	**± 2.5**	**± 2.3**	**± 5.6**	**± 3.8**
7500	021 (026)	36	-	- (32)*	28	34	34
	022 (027)	36	-	- (30)*	28	26	30
	023 (028)	36	30	34	24	22	24
	024 (029)	32	30	32	30	30	30
	025 (030)	34	-	- (30)*	26	-	- (22)*
	Mean	**34.8**	**30.0**	**33.0**	**27.2**	**28.0**	**29.5**
	± SD	**± 1.8**	**± 0.0**	**± 1.4**	**± 2.3**	**± 5.2**	**± 4.1**

§ = animal No. of males and (females), - = no determination of body weight, * = body weight of decedents, not used for calculation of the mean and the SD.
Note: Mean and standard deviation were not given in the study report. Values were calculated retrospectively.

D. NECROPSY

No necropsy findings in decedents and surviving animals.

III. CONCLUSIONS

The oral LD_{50} of glyphosate technical in male and female mice was greater than 7500 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is performed according to OECD guideline 401, which is cancelled. Nevertheless, except for deviations provided above, the study is in concordance to the current OECD guideline 420 (2001). Therefore, the outcome can be reported as valid. The acute oral LD_{50} in mice is above 7500 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.1/028
Report author	[redacted]
Report year	1990
Report title	Acute oral toxicity study in the rat: Glyphosate technical
Report No	AGC-900823B
Document No	Not reported
Guidelines followed in study	OECD 401 (1987)
Deviations from current test guideline (OECD 420, 2001)	Both sexes, each with 5 animals per dose group instead of one sex (usually females) with 5 animals per dose group. Young animals were used, age not specified in detail. No fasting of further 3-4 hours after treatment. Clinical signs were not reported for individual animals.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The acute oral toxicity of glyphosate technical was investigated in male and female rats (5 animals/sex/group) of the CD strain. The test substance, suspended in 1 % methylcellulose, was administered by oral gavage to each animal at a dosage of 0, 3000, 5000 and 8000 mg/kg bw at a constant dose volume of 20 mL/kg bw. Clinical signs were recorded at 0.5, 1 and 3 hours following dosing and daily thereafter for 14 days. All animals were subjected to a gross necropsy at the end of the study.

Clinical signs noticed were decreased activity, abnormal posture, abnormal limb position and abnormal gait in animals of the 5000 and 8000 mg/kg bw dose groups. Changes of body weight were in the normal range. No mortality occurred. No abnormal necropsy findings were noted.
The acute oral LD_{50} was determined to be

LD_{50}, oral, rat > 8000 mg/kg bw

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate

Identification:	Glyphosate Technical
Description:	Yellowish (transparent)
Lot/Batch #:	0190 A
Purity:	98.1 %
Stability of test compound:	Not specified

2. Vehicle and/or positive control: 1 % methylcellulose

3. Test animals:

Species:	Rat
Strain:	CD
Source:	[redacted]
Age:	Young adults (not further specified)
Sex:	Male and female
Weight at dosing:	184 – 229 g (males) 163 – 198 g (females)
Acclimation period:	5 days
Diet/Food:	Standard rat diet pellets (Redmills, Goresbridge, Co. Kilkenny Ireland), *ad libitum* (except the night prior to dosing)
Water:	Tap water, *ad libitum*
Housing:	5 animals/sex/cage in flat bottomed polypropylene cages with stainless steel
Environmental conditions:	Temperature: 13 – 25 °C Humidity: 43 – 61 % (Average) Air changes: Not specified 12-hour light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 14/03/1990 to 28/03/1990 (experimental phase – without acclimation period)
Finalisation date: 23 August 1990

Animal assignment and treatment:
Rats were housed by sex (5 males and 5 females) and fasted overnight prior to dosing. The test material was suspended in 1 % methylcellulose and administered by a single oral gavage at dose levels of 3000, 5000 and 8000 mg/kg bw with an application volume of 20 mL/kg bw. A control group with 5 male and 5 female rats was included which received 1 % methylcellulose as a single oral gavage.

All animals were observed for clinical signs of toxicity at 0.5, 1 and 3 hours post treatment and once a day, thereafter for 14 days. Body weights were recorded prior to administration, on day seven and prior to sacrifice on Day 14.

At study termination all animals were sacrificed followed by a gross necropsy examination.

II. RESULTS AND DISCUSSION

A. MORTALITY
No mortality occurred during the 14-days observation period after administration.

B. CLINICAL OBSERVATIONS
Animals of the control and low dose group did not show any signs of toxicity. Decreased activity was observed in 5/5 males at 5000 mg/kg bw and 8000 mg/kg bw 20 min after dosing. However, activity seems normal after 1 hour, except for 1 animal at 5000 mg/kg bw. A further 2/5 females at 8000 mg/kg bw showed decreased activity 1 hour after dosing. At the next observation time point animals had recovered. 2/5 males at 5000 mg/kg bw showed abnormal gait 1 hour after treatment and one male had recovered at hour 3. 1/5 males showed abnormal posture between 1 and 3 hours after test substance administration. 1/5 females showed decreased activity but recovered by hour 3. Abnormal body posture and limb position was observed in 2/5 females at 8000 mg/kg bw after 1 hour and one of them showed abnormal gait at 1 and 3 hours of the observation period. Clinical signs are listed in the table below.

Table 5.2.1-39: Acute oral toxicity study in the rat: Glyphosate technical ([redacted] 1989): Clinical observations after treatment with glyphosate technical

Dose group [mg/kg bw]	Control		3000		5000		8000	
Sex	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Total animals examined n	5	5	5	5	5	5	5	5
Clinical signs n	-	-	-	-	5	1	5	2
Decreased activity	-	-	-	-	5	1	5	2
Abnormal body posture	-	-	-	-	1	-	-	2
Abnormal gait	-	-	-	-	2	-	-	1
Abnormal limb position	-	-	-	-	-	-	-	1

C. BODY WEIGHT

Weight gain of all animals was within the normal range. For details on individual and mean body weights see table below.

Table 5.2.1-40: Acute oral toxicity study in the rat: Glyphosate technical ([redacted] 1989): Body weight development after treatment with glyphosate technical

Day		0	7	14	0	7	14
Sex		**Males**			**Females**		
Dose level (mg/kg bw)	**Animal No.§**	**Body weight [g]**					
0	31 (36)	212	275	304	198	243	258
	32 (37)	211	302	332	189	233	240
	33 (38)	222	306	333	163	183	201
	34 (39)	184	253	282	177	218	216
	35 (40)	213	288	310	173	204	205
	Mean	**208.4**	**284.8**	**312.2**	**180.0**	**216.2**	**224.5**
	± SD	**± 14.3**	**± 21.6**	**± 21.3**	**± 13.7**	**± 23.7**	**± 24.3**
3000	21 (26)	197	259	300	170	200	210
	22 (27)	218	285	319	166	200	215
	23 (28)	229	300	336	172	209	213
	24 (29)	213	284	325	171	197	215
	25 (30)	217	302	360	198	249	261
	Mean	**214.8**	**286**	**328**	**175.4**	**211**	**222.8**
	± SD	**± 11.6**	**± 17.2**	**± 22.1**	**± 12.8**	**± 21.7**	**± 21.5**
5000	11 (16)	196	272	313	184	222	238
	12 (17)	212	291	329	189	226	234
	13 (18)	221	303	334	174	206	216
	14 (19)	219	286	323	164	200	210
	15 (20)	218	294	322	180	216	229
	Mean	**213.2**	**289.2**	**324.2**	**178.2**	**214**	**225.4**
	± SD	**± 10.2**	**± 11.4**	**± 7.9**	**± 9.7**	**± 10.9**	**± 11.9**
8000	1 (6)	212	297	345	195	231	247
	2 (7)	201	272	314	179	204	234
	3 (8)	218	284	326	174	207	233
	4 (9)	191	257	305	191	221	234
	5 (10)	**209**	279	317	181	223	233
	Mean	**206.2**	**277.8**	**321.4**	**184.0**	**217.2**	**236.2**

Table 5.2.1-40: Acute oral toxicity study in the rat: Glyphosate technical ([redacted] 1989): Body weight development after treatment with glyphosate technical

Day		0	7	14	0	7	14
Sex		Males			Females		
Dose level (mg/kg bw)	Animal No.§	Body weight [g]					
	± SD	± 10.5	± 14.8	± 15.2	± 8.7	± 11.4	± 6.1

§ = animal No. of males and (females)
Note: Standard deviation were not given in the study report. Values were calculated retrospectively.

D. NECROPSY

No abnormal necropsy findings were noticed.

III. CONCLUSIONS

The oral LD_{50} of glyphosate technical in male and female rats was greater than 8000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is performed according to OECD guideline 401, which is cancelled. Nevertheless, except for deviations provided above, the study is in concordance to the current OECD guideline 420 (2001). Therefore, the outcome can be reported as valid. The acute oral LD_{50} is above 8000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.1/029
Report author	[redacted]
Report year	1989
Report title	Glyphosate Technical: Acute oral toxicity (limit) test in rats
Report No	5883
Document No	Not reported
Guidelines followed in study	OECD, EEC, EPA guidelines. (Guideline numbers are not specified in the report)
Deviations from current test guideline (OECD 420, 2001)	Both sexes, each with 5 animals per dose group instead of one sex (usually females) with 5 animals per dose group. Purity of the test substance not provided. From the batch number a purity of 98.6 % was concluded. Number of air changes during housing of the animals lacking. Only average data on the maximum and minimum temperature.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The acute oral toxicity of glyphosate technical was investigated in male and female rats (5 animals/sex/group) of the Sprague-Dawley strain. Test substance, suspended in 0.5 % Carboxymethylcellulose, was administered by oral gavage to each animal at a dosage of 5000 mg/kg bw and constant dose volume of 10 mL/kg bw. Mortality, body weight and clinical signs were recorded during the subsequent 14 days. All animals were subjected to a gross necropsy at the end of the study.

Clinical signs noticed were piloerection, reduced activity and ataxia. Changes of body weight were in the normal range. No mortality occurred. No abnormal necropsy findings were noted.
The acute oral LD_{50} was determined to be

LD_{50}, oral, rat > 5000 mg/kg bw

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate

Identification:	Glyphosate Technical (PMG)
Description:	White powder
Lot/Batch #:	206-JaK-25-1
Purity:	Not specified in the study report Batch 206-JaK-25-1 reported with 98.6 %, see also [redacted] 1991.
Stability of test compound:	Not specified

2. Vehicle and/or positive control: 0.5 % Carboxymethylcellulose (CMC)

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	6 – 8 weeks
Sex:	Male and female
Weight at dosing:	136 – 176 g
Acclimation period:	7 days
Diet/Food:	Expanded Rat and Mouse Maintenance Diet, *ad libitum* (except 18 hours prior to dosing and 3-4 hours after dosing)
Water:	Tap water, *ad libitum*
Housing:	Maximum of 5 animals/sex/cage in Polypropylene cages with mesh floors
Environmental conditions:	Temperature: 19 – 22 °C Humidity: 48 % (Average) Air changes: Not specified 12-hour light / dark cycle (light during 07:00 – 19:00)

B. STUDY DESIGN AND METHODS

In life dates: 25/05/1989 to 22/06/1989 (Arrival of the animals to termination of the study)
07/06/1989 to 22/06/1989 (experimental phase – without acclimation period)

Animal assignment and treatment:
Rats were housed by sex (5 males and 5 females) and starved for approximately 18 hours prior to dosing. Test material was suspended in 0.5 % CMC at concentration of 500 mg/mL and administered by a single oral gavage at dose level of 5000 mg/kg bw with an application volume of 10 mL/kg bw. Animals were starved for a further 3 – 4 hours.

All animals were observed for clinical signs of toxicity at several time points on the day of administration and at least once a day, thereafter. Body weights were recorded prior to administration, on day 7 and prior to sacrifice on day 14.

At study termination all animals were sacrificed followed by gross necropsy examination.

II. RESULTS AND DISCUSSION

A. MORTALITY
No mortality occurred during the 14-day observation period after administration.

B. CLINICAL OBSERVATIONS
The observed clinical signs were piloerection, reduced activity and ataxia. Females recovered after day 7, while piloerection in males lasted until the end of the study. All clinical signs are listed in the table below.

Table 5.2.1-41: Glyphosate Technical: Acute oral toxicity (limit) test in rats ([redacted] and [redacted] 1989): Clinical observations

Dose group	5000 mg/kg bw												
Sex	Males							Females					
Time after treatment	1 min	30 min	1 h	2 h	1-8 d	9 d	10–14 d	1 min	30 min	1 h	2 h	1-7 d	8 - 14 d
Total animals n examined	5	5	5	5	5	5	5	5	5	5	5	5	5
Clinical sign n	0	5	5	5	5	5	0	0	5	5	5	5	0
Piloerection n	0	5	5	5	5	0	0	0	5	5	5	5	0
Reduced activity n	0	0	5	5	5	5	0	0	0	5	5	5	0
Ataxia n	0	0	0	0	0	5	0	0	0	0	0	0	0

C. BODY WEIGHT
Weight gain of all animals was within the normal range. Individual body weights are depicted in the table below.

Table 5.2.1-42: Glyphosate Technical: Acute oral toxicity (limit) test in rats ([redacted] and [redacted] 1989): Body weight and body weight gain

Dose level	5000 mg/kg bw							
Sex	Males			Females			Males	Females
Day	0	7	14	0	7	14	0-14	0-14
Animal No.§	Body weight [g]						Body weight gain [g]	
1 M (6 F)	176	239	284	136	168	182	108	46

Table 5.2.1-42: Glyphosate Technical: Acute oral toxicity (limit) test in rats ([redacted] and [redacted] 1989): Body weight and body weight gain

2 M (7 F)	166	226	272	138	167	182	106	44
3 M (8 F)	169	255	316	151	192	202	147	51
4 M (9 F)	166	238	282	154	186	208	116	54
5 M (10 F)	162	232	269	137	165	179	107	42
Mean ± SD	**168 ± 5**	**238 ± 11**	**285 ± 19**	**143 ± 9**	**176 ± 12**	**191 ± 13**	**117 ± 17**	**47 ± 5**

§ = animal No. of males and (females)

D. NECROPSY

No abnormal necropsy findings were noticed.

III. CONCLUSIONS

The oral LD_{50} of glyphosate technical in male and female rats was greater than 5000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was performed according to guidelines similar to the current OECD guideline 420 (2001). Due to the deviations and the limited reporting, the study is only considered supplementary. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.1/030
Report author	[redacted]
Report year	1989
Report title	Acute oral toxicity study with glyphosate technical (isopropylamine salt 62 % in water equivalent of 46 % of N-phosphonomethylglycine acid) in rats
Report No	[redacted]439 / 238050
Document No	Not reported
Guidelines followed in study	No final conclusion possible.
GLP	No final conclusion possible.
Previous evaluation	Yes, accepted in RAR (2015)
Short description of study design and observations:	Not available
Short description of results:	LD_{50} > 2000 mg/kg bw No findings
Reasons for why the study is not considered relevant/reliable or not considered as key study:	Full study report is not available to the applicant. Only a short summary is provided in the Monograph, B5, 2000
Reasons why the study report is not available for submission	The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance" (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL

Category study in AIR 5 dossier (L docs)	Category 4a

1. Information on the study

Data point:	CA 5.2.1/031
Report author	[redacted]
Report year	1988
Report title	Acute Oral Toxicity Study of Glyphosate Batch/Lot/NBR No. XLI-55 in Sprague-Dawley Rats
Report No	88.2053.007
Document No	Not reported
Guidelines followed in study	US EPA 81-1, equivalent to OECD 420 (2001)
Deviations from current test guideline (OECD 420, 2001)	Yes, both sexes used instead of one sex (usually females), observation three times at the first day at unknown points in time and twice a day thereafter instead of once. Animals not fasted after dosing. The clinical signs per individual animal are not reported in the report.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The test substance, glyphosate, was evaluated for its acute oral toxicity potential in male and female Sprague-Dawley rats when administered as a gavage dose at level of 5000 mg/kg bw. No mortalities occurred. Clinical signs included diarrhoea, apparent urinary incontinence, and hair loss on the abdomen. Body weight gain was noted for all animals. No internal abnormalities were noted during gross necropsy examination of the animals. The acute oral LD_{50} was determined to be

LD_{50}, oral, rat > 5000 mg/kg bw

I. MATERIALS AND METHODS

A MATERIALS

1. Test material:

Identification:	Glyphosate
Description:	White powder
Lot/Batch #:	XLI-55
Purity:	97.76 %
Stability of test compound:	No data given in the report

2. Vehicle and/or positive control: Distilled water

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	Not specified
Sex:	Male and female
Weight at dosing:	♂ 300 – 332 g; ♀ 217 – 222 g
Acclimation period:	At least 5 days
Diet/Food:	NIH Open Formula 07 Rat and Mouse Diet, certified feed (Zeigler Brothers, Inc., Gardners, PA, US), *ad libitum* (except when fasted overnight prior to dosing)
Water:	Tap water, *ad libitum*
Housing:	Wire mesh cages
Environmental conditions:	Temperature: 20 – 23.9 °C Humidity: 40 – 70 % Air changes: Not specified Light cycle: 12 hour light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 1988-04-05 to 1988-04-19

Animal assignment and treatment:
Groups of five male and five female rats received the test material at a dose level of 5000 mg/kg bw by oral gavage as a 50 % w/v aqueous suspension. Observations for mortality and signs of toxicity were made three times on the day of dose administration and twice daily thereafter. Body weights were recorded prior dose administration on study day 1, and on days 8 and 15 (terminal sacrifice). A gross necropsy was performed on all animals at the time terminal sacrifice (day 15) and all abnormalities were recorded.

II. RESULTS AND DISCUSSION

A. MORTALITY
No mortalities occurred.

B. CLINICAL OBSERVATIONS
Clinical signs included diarrhea, apparent urinary incontinence, and hair loss on the abdomen (see table below).

Table 5.2.1-43: Acute Oral Toxicity Study of Glyphosate Batch/Lot/NBR No. XLI-55 in Sprague-Dawley Rats ([redacted] and [redacted] 1988): Clinical observations during the study.

Dose group		5000 mg/kg bw											
Sex		Males								Females			
Days after treatment		1	2	3	4	5	6	7	8-15	1	2	3	4-15
Total animals examined	n	5	5	5	5	5	5	5	5	5	5	5	5
Clinical sign	n	0	5	1-2[#]	1	1	1	0	1	0	5	0	1
Hair loss	n	0	0	0	1	1	1	0	1	0	0	0	1
Diarrhea	n	0	5	1	0	0	0	0	0	0	5	0	0

Table 5.2.1-43: Acute Oral Toxicity Study of Glyphosate Batch/Lot/NBR No. XLI-55 in Sprague-Dawley Rats ([redacted] and [redacted] 1988): Clinical observations during the study.

Wet abdomen	n	0	3	1	0	0	0	0	0	0	2	0	0

= From the reported data it cannot be concluded if 1 or 2 animals were affected in total

C. BODY WEIGHT

Body weight gain was noted for all animals (see table below).

Table 5.2.1-44: Acute Oral Toxicity Study of Glyphosate Batch/Lot/NBR No. XLI-55 in Sprague-Dawley Rats ([redacted] and [redacted] 1988): Body weight development

Dose group	**5000 mg/kg bw**					
Sex	**Males**			**Females**		
Time after treatment	**Day 1[a]**	**Day 8**	**Day 15**	**Day 1[a]**	**Day 8**	**Day 15**
Animal No.[§]	**Body weight (g)**					
70011 (70016)	310	357	394	220	232	243
70012 (70017)	332	371	411	222	245	255
70013 (70018)	300	349	382	221	241	250
70014 (70019)	309	342	380	217	248	258
70015 (70020)	318	363	389	217	243	253
Mean (± SD)	**313.8 ± 12.0**	**356.4 ± 11.4**	**391.2 ± 12.4**	**219.4 ± 2.3**	**241.8 ± 6.1**	**251.8 ± 5.7**

Note: Mean and standard deviation were not given in the study report. Values were calculated retrospectively.
§ = animal No. of males and (females), a= fasted body weight

D. NECROPSY

No internal abnormalities were noted during gross necropsy examination of the animals.

III. CONCLUSIONS

The oral LD_{50} of glyphosate in male and female rats was greater 5000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in accordance to the current OECD guideline 420 (2001). The outcome can be therefore reported as valid. The acute oral LD_{50} is above 5000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.1/032
Report author	[redacted]
Report year	1987
Report title	Acute oral LD_{50} study of MON-8750 in Sprague-Dawley rats
Report No	9308
Document No	Not reported
Guidelines followed in study	EPA OPP 81-1 (Acute Oral Toxicity)

Deviations from current test guideline (OECD 420, 2001)	Both sexes, each with 5 animals per dose group instead of one sex (usually females) with 5 animals per dose group. Number of air changes during housing of the animals lacking. Stability of test compound not reported. Age of the rats not provided. Individual clinical and necropsy findings were not reported.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The acute oral toxicity of glyphosate (MON 8750) was investigated in 1 female and 1 male rat in a limit test using a dose level of 5000 mg/kg bw. Subsequently, a dose-range study was conducted to determine suitable dose levels for the main study. On basis of the dose-range study and the limit test, 5 male and 5 female rats were orally dosed with the test substance at dose levels of 2222 and 7500 mg/kg bw. Mortality, body weight and clinical signs were recorded during the subsequent 14 days. All animals were subjected to a gross necropsy at the end of the study.

Mortality did not occur in the low dose group. However, 6/10 animals died in the middle dose group and 9/10 animals died in the high dose group. Clinical signs included but were not limited to ataxia, labored breathing and reduced activity. The findings were especially prominent in the middle and high dose group. Changes of body weight were in the normal range. Findings at necropsy concerned but were not limited to the lungs, the cecum and the intestines in the middle and high dose group. No findings were reported for the low dose group.

On basis of the limit test and the main study, the acute oral LD_{50} was calculated to be

LD_{50}, oral, female and male rat = 4613 mg/kg bw (95 % confidence interval (CI) 3511–5716 mg/kg bw)
LD_{50}, oral, female rat = 2222 < 5000 mg/kg bw
LD_{50}, oral, male rat = 5904 mg/kg bw (95 % CI 3420 – 8388 mg/kg bw)

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate

Identification: MON8750
Description: White powder
Lot/Batch #: XLG-255
Purity: 90.8 %
Stability of test compound: Not specified

2. Vehicle and/or positive control: Distilled/deionised water

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	Not specified
Sex:	Male and female
Weight at dosing:	222 – 311 g (limit test), 217 – 297 g (main study)
Acclimation period:	At least 5 days
Diet/Food:	Open formula, certified diet, Zeigler Brothers, *ad libitum* (except for fasting period overnight before dosing)
Water:	Tab water, *ad libitum*
Housing:	Wire mesh cages
Environmental conditions:	Temperature: 20 – 24 °C Humidity: 40 – 70 % Air changes: Not specified

B. STUDY DESIGN AND METHODS

In life dates: Not reported

Animal assignment and treatment:

A limit test was conducted by oral gavage administration. The test substance was administered after overnight fasting to five female and five male rats at a dose level of 5000 mg/kg bw. 6/10 animals died. Furthermore, a dose-range finding study was performed after overnight fasting on one female and one male rat per dose group (500, 1000, 3000, 5000 and 7000 mg/kg bw) to determine appropriate dose levels for the main study. One animal died in the highest dose group. The limit test should be used for determination of an LD_{50} together with the main study. Based on this and the dose-range finding study, dose levels of 2222 and 7500 mg/kg bw were chosen for the main study.

During the main study, 5 male and 5 female rats per dose group were fasted overnight and dosed by oral gavage with the test substance. Clinical observations and mortality were recorded three times on the day of dosing and twice daily thereafter for the duration of the study. Body weights were measured on day 1 prior to dosing, day 8 and day 15 or at death. All animals were sacrificed at the end of the study and subjected to gross necropsy.

II. RESULTS AND DISCUSSION

A. MORTALITY

Mortality occurred in one male rat and all 5 female rats during the limit test.
One animal of the highest dose group (7000 mg/kg bw) died during the dose-range finding study. During the main study, mortality occurred with 4 males and all 5 females in the highest dose group (7500 mg/kg bw).

Table 5.2.1-45: Acute oral LD50 study of MON-8750 in Sprague-Dawley rats ([redacted] 1987): Mortality during limit test and main study

Dose level (mg/kg bw)	Cumulative mortality at Day[1]				
Males	**1**	**2**	**3**	**4**	**5-15**
2222	0/5	0/5	0/5	0/5	0/5
5000[2]	0/5	0/5	1/5	1/5	1/5
7500	1/5	3/5	4/5	4/5	4/5
Females					
2222	0/5	0/5	0/5	0/5	0/5
5000[2]	1/5	4/5	4/5	5/5	5/5
7500	0/5	4/5	5/5	5/5	5/5

1 = Number dead animals / number of exposed animals per group, 2 = Dose level administered in limit test

Based on the mortality, the acute oral LD_{50} in males was calculated to be 5904 mg/kg bw with a 95 % CI of 3420 – 8388 mg/kg bw. The acute oral LD_{50} for females was calculated to be between 2222 and 5000 mg/kg bw. The combined LD_{50} was calculated to be 4613 mg/kg bw with a 95 % CI of 3511 – 5716 mg/kg bw.

B. CLINICAL OBSERVATIONS

Clinical signs of toxicity were observed during the limit test (5000 mg/kg bw) and in the high dose group of the main study (7500 mg/kg bw) and included but were not limited to ataxia, decreased activity, diarrhea and labored breathing (see table below).

Table 5.2.1-46: Acute oral LD50 study of MON-8750 in Sprague-Dawley rats ([redacted] 1987): Clinical observations recorded during the study in males and females

Dose level (mg/kg)	2222	5000[1]	7500	2222	5000[1]	7500
Sex	**Males**			**Females**		
Clinical observation	**Incidence[2] (study day)**					
Ataxia		2/5a (1)	2/5 (1) 1/2 (2) 1/1 (3-5)		1/5 (1) 1/1 (3)	3/5 (1) 1/1 (2)
Decreased activity		5/5 (1)	3/5 (1) 2/2 (2) 1/1 (3-6)		4/5 (1) 1/2 (2) 1/1 (3)	5/5 (1) 1/1 (2)
Diarrhea	2/5b (1)	3/5 (1) 2/5 (2) 1/4 (3,4)	2/5 (1) 2/2 (2) 1/1 (3,4)	4/5 (1)	2/5 (1) 2/2 (2) 1/1 (3)	1/1 (2)
Hair loss – base of tail		1/4 (4-11)				
Hair loss – abdomen		1/4 (5-15)				
Gasping						1/1 (2)
Hypothermia						1/1 (2)
Labored breathing			2/2 (2) 1/1 (3-5)		1/5 (1) 1/1 (3)	4/5 (1) 1/1 (2)
Lacrimation						1/1 (2)
Pale						1/1 (2)
Rales					1/1 (3)	
Sores- base of tail		1/4 (5-7)				
Wet abdomen		1/4 (3,4)				1/1 (2)

1 Dose level administered in limit test, 2 = number of animals with observed clinical signs / number of animals examined with study day(s) in parenthesis at which the clinical signs were observed.

C. BODY WEIGHT

Treatment with the test substance did not have an effect on body weights in surviving animals (see table

below).

Table 5.2.1-47: Acute oral LD50 study of MON-8750 in Sprague-Dawley rats ([redacted] 1987): Summary of body weights recorded during limit test and main study in males and females

Day		1[2]	8	15	1	8	15
Sex		Males			Females		
Dose level (mg/kg bw)	Animal No.§	Body weight [g]					
2222	93080051 (93080056)	297	361	385	234	264	267
	93080052 (93080057)	255	298	316	236	276	283
	93080053 (93080058)	251	297	328	217	246	258
	93080054 (93080059)	249	340	378	219	241	250
	93080055 (93080060)	258	301	316	217	239	245
Mean		**262.0**	**319.4**	**344.6**	**224.6**	**253.2**	**260.6**
± SD		**± 19.9**	**± 29.4**	**± 34.1**	**± 9.5**	**± 16.1**	**± 15.0**
5000[1]	93080021 (93080026)	310	372	397	226	-	-
	93080022 (93080027)	303	-	-	237	-	-
	93080023 (93080028)	311	326	363	222	-	-
	93080024 (93080029)	310	348	376	228	-	-
	93080025 (93080030)	299	334	360	230	-	-
Mean		**306.6**	**345.0**	**374.0**	**228.6**	-[4]	-[4]
± SD		**± 5.3**	**± 20.2**	**± 16.8**	**± 5.5**	-[4]	-[4]
7500	93080061 (93080066)	291	-	-	223	-	-
	93080062 (93080067)	291	-	-	219	-	-
	93080063 (93080068)	281	311	355	222	-	-
	93080064 (93080069)	269	-	-	217	-	-
	93080065 (93080070)	249	-	-	218	-	-
Mean		**276.2**	-[3]	-[3]	**219.8**	-[4]	-[4]
± SD		**± 17.7**	-[3]	-[3]	**± 2.6**	-[4]	-[4]

1 = Dose level administered in limit test, 2 = Fasted body weights,
3 = Group mean ± Standard deviation not calculated, insufficient number of animals.
4 = Group mean ± Standard deviation not calculated, 100 % mortality.
§ = animal No. of males and (females)
- = animal died
Note: Mean and standard deviation were not given in the study report. Values were calculated retrospectively.

D. NECROPSY

During necropsy, no abnormal changes on organs were noticed in the low dose group. Findings during necropsy in the middle and high dose group concerned the lungs, stomach and intestines. Observed findings in the stomach, most pronounced at mid and high dose levels are most likely due to the irritating properties of glyphosate. In addition, in short-term studies with rats and mice irritation with diarrhoea was observed in the gastro-intestinal tract, one of the main target organs of glyphosate (See MCA 5.3). All findings are listed in the table below.

Table 5.2.1-48: Acute oral LD50 study of MON-8750 in Sprague-Dawley rats ([redacted] 1987): Findings during gross necropsy

Dose level (mg/kg bw)	2222	5000[1]	7500	2222	5000[1]	7500
Sex	Males			Females		
Tissue/ Finding	Incidence[2]					
Cecum						

Table 5.2.1-48: Acute oral LD50 study of MON-8750 in Sprague-Dawley rats ([redacted] 1987): Findings during gross necropsy

- contains black substance - contains red fluid			1/5			1/5
Intestines – contain red fluid			1/5			1/5
Lungs- dark/red areas		2/5			2/5	
Stomach - contains dark fluid - red areas glandular mucosa - contains blood-like substance		1/5	4/5 2/5		1/5 1/5 1/5	5/5

1 = Dose level administered in limit test, 2 = number of animals with observed findings / number of animals examined.

III. CONCLUSIONS

The combined oral LD_{50} (males and females) of glyphosate (MON8750) in rats was calculated to be 4613 mg/kg bw with a 95 % CI of 3511 – 5716 mg/kg bw.

The acute oral LD_{50} in males was calculated to be 5904 mg/kg bw with a 95 % CI of 3420 – 8388 mg/kg bw. The acute oral LD_{50} in females was calculated to be between 2222 and 5000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was performed according to a national guideline similar to the current OECD guideline 420 (2001). Therefore, the outcome can be reported as valid. The combined acute oral LD_{50} is 4613 mg/kg bw (95 % CI 3511 – 5716 mg/kg bw). The acute oral LD_{50} in males was calculated to be 5904 mg/kg bw (95 % CI 3420 – 8388 mg/kg bw). The acute oral LD_{50} in females was calculated to be between 2222 and 5000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.1/033
Report author	[redacted]
Report year	1987
Report title	Acute oral toxicity study of MON 8722 in Sprague-Dawley rats
Report No	9308A
Document No	Not reported
Guidelines followed in study	No final conclusion possible.
GLP	No final conclusion possible.
Previous evaluation	Yes, accepted in RAR (2015)*
Short description of study design and observations:	Not available
Short description of results:	Ataxia, decreased activity, diarrhea, rectal sores
Reasons for why the study is not considered relevant/reliable or not considered as key study:	Full study report is not available to the applicant. Only a short summary is provided in the Monograph, B5, 2000

Reasons why the study report is not available for submission	The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance" (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL
Category study in AIR 5 dossier (L docs)	Category 4a

* Study not reported in RAR (2015), however short summary provided in Monograph (2000).

1. Information on the study

Data point:	CA 5.2.1/034
Report author	[redacted]
Report year	1987
Report title	Acute oral toxicity of 64 % SN750721 technical liquid in mice
Report No	TX58AO1
Document No	Not reported
Guidelines followed in study	No final conclusion possible.
GLP	No final conclusion possible.
Previous evaluation	Yes, accepted in RAR (2015)
Short description of study design and observations:	Not Available
Short description of results:	LD_{50} = 4373 mg/kg bw (confidence interval: 4144 – 4644 mg/kg bw) Dose related mortality in all IPA-treated groups; immobility, tremor, hyperemia of the ears
Reasons for why the study is not considered relevant/reliable or not considered as key study:	Full study report is not available to the applicant. Only a short summary is provided in the Monograph, B5, 2000
Reasons why the study report is not available for submission	The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance" (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL
Category study in AIR 5 dossier (L docs)	Category 4a

1. Information on the study

Data point:	CA 5.2.1/035
Report author	[redacted]
Report year	1987
Report title	Acute oral toxicity of 41 % SN750721 solution in mice
Report No	TX58O2
Document No	Not reported
Guidelines followed in study	No final conclusion possible.
GLP	No final conclusion possible.
Previous evaluation	Yes, accepted in RAR (2015)
Short description of study design and observations:	Not available

Short description of results:	LD_{50} = 3669 mg/kg bw (confidence interval: 3489 – 3858 mg/kg bw) Dose related mortality in all IPA-treated groups; immobility, tremor, hyperemia of the ears
Reasons for why the study is not considered relevant/reliable or not considered as key study:	Full study report is not available to the applicant. Only a short summary is provided in the Monograph, B5, 2000
Reasons why the study report is not available for submission	The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance" (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL
Category study in AIR 5 dossier (L docs)	Category 4a

1. Information on the study

Data point:	CA 5.2.1/036
Report author	[redacted]
Report year	1983
Report title	The acute oral toxicity (LD_{50}) to albino mice with glyphosate (tech) of Excel industries Ltd.
Report No	TOX95-51812
Document No	Not reported
Guidelines followed in study	None
Deviations from current test guideline (OECD 420 (2001))	5 animals of both sexes, high doses used, age not specified, housing conditions not specified, no gender specific differention of mortality data.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities (GLP was not compulsory at the time the study was performed)
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

Executive Summary

The test substance, glyphosate technical, was evaluated for its acute oral toxicity potential. 5 female and 5 male mice were used per dose group. The test substance was administered as a single oral gave at levels of 0, 2000, 3000, 4000, and 5000 mg/kg bw. Clinical signs included ataxia and loss of muscle tone at 3000, 4000 and 5000 mg/kg bw, which were no longer observed after 24-hours. Mortality occurred in the same dose groups. 20 %, 50 % and 70 % of the animals died at 3000, 4000 and 5000 mg/kg bw, respectively. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute oral LD_{50} was calculated to be

LD_{50}, oral, female and male mice = 4000 mg/kg bw (CI: 3330 mg/kg bw – 4800 mg/kg bw)

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate

Identification:	Glyphosate Technical
Description:	White amorphous powder
Lot/Batch #:	R&D sample (9-7-83)
Purity:	95 %
Stability of test compound:	Not specified

2. Vehicle and/ or positive control: No information provided

3. Test animals:

Species:	Mice
Strain:	Kasauli
Source:	[redacted]
Age:	Not specified
Sex:	Male and female
Weight at dosing:	20 – 25 g
Acclimation period:	Not specified
Diet/Food:	Pelleted feed (supplied by Hindustan Lever Ltd., Bombay), *ad libitum,* except for a fasting period the night before dosing
Water:	Not specified, *ad libitum*
Housing:	Groups of 5 / sex
Environmental conditions:	Temperature: Not specified Humidity: Not specified Air changes: Not specified 12-hour light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: No information provided
Finalisation date: 12/10/1983

Animal assignment and treatment:
Glyphosate technical was tested in a dose-range finding study with 5 mice/sex/group. Based on the mortality in the preliminary study, following dose groups were chosen for the main study: 0, 20000, 3000, 4000, and 5000 mg/kg bw. Prior to dosing, all animals were fasted overnight. The test substance was administered to 5 mice/sex/group by a single oral gavage. The animals were observed over the study period of 15 days for signs of toxicity and mortality.

II. RESULTS AND DISCUSSION

A. MORTALITY

2/10 animals died at 3000 mg/kg bw 24-hours after treatment. A total of 5/10 animals and 7/10 animals died between 8 hours and 2 days after treatment at 4000 and 5000 mg/kg bw, respectively. For details see table below.

Table 5.2.1-49: The acute oral toxicity (LD_{50}) to albino mice with glyphosate (tech) of excel industries Ltd. ([redacted] 1983): Mortality for all dose groups

Dose level (mg/kg bw)	Mortality*	Mortality rate (%)
0	0 / 10	0
2000	0 / 10	0
3000	2 /10 (24 h)	20
4000	1 / 10 (8 h); 2 / 10 (24 h); 2 / 10 (2 d)	50
5000	2 / 10 (8 h); 3 / 10 (24 h); 2 / 10 (2 d)	70

* = No. of deaths/No. of animals examined (Hours or Day of death)

B. CLINICAL OBSERVATIONS

No clinical signs were observed in the control group and at 2000 mg/kg bw. Sign observed in all mice at 3000, 4000 and 5000 mg/kg bw included ataxia and loss of muscle tone between 4 and 24-hours after treatment. No clinical signs were observed thereafter (see table below).

Table 5.2.1-50: The acute oral toxicity (LD_{50}) to albino mice with glyphosate (tech) of excel industries Ltd. ([redacted] 1983): Individual clinical observations

Clinical observation	Incidence[1]				
Dose level (mg/kg bw)	**0**	**2000**	**3000**	**4000**	**5000**
Sex	**Males**				
Ataxia	0	0	5/5 (4, 8, 24 h)	5/5 (4, 8, 24 h)	5/5 (4, 8, 24 h)
Loss of muscle tone	0	0	5/5 (4, 8, 24 h)	5/5 (4, 8, 24 h)	5/5 (4, 8, 24 h)
Sex	**Females**				
Ataxia	0	0	5/5 (4, 8, 24 h)	5/5 (4, 8, 24 h)	5/5 (4, 8, 24 h)
Loss of muscle tone	0	0	5/5 (4, 8, 24 h)	5/5 (4, 8, 24 h)	5/5 (4, 8, 24 h)

1 = number of animals with observed clinical signs / number of animals examined within the dose group and hour(s) in parenthesis at which the clinical signs were observed.

C. BODY WEIGHT

Body weights were not reported.

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities in deceased and surviving animals.

III. CONCLUSIONS

The oral LD_{50} of glyphosate technical in mice was estimated to be 4000 mg/kg bw (CI: 3330 mg/kg bw – 4800 mg/kg bw).

3. Assessment and conclusion

Assessment and conclusion by applicant:
Due to the deviations of the study and the fact that it was not performed according to any guideline and not according to GLP, the study provides supplementary information on acute oral toxicity of glyphosate, only.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.1/037
Report author	[redacted]
Report year	1983
Report title	The acute oral toxicity (LD50) to rats with glyphosate (tech.).
Report No	Not given
Document No	Not reported
Guidelines followed in study	No final conclusion possible.
GLP	No final conclusion possible.
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	Not available
Short description of results:	Slight ataxia (at 5000 mg/kg bw)
Reasons for why the study is not considered relevant/reliable or not considered as key study:	Full study report is not available to the applicant. Only a short summary is provided in the Monograph, B5, 2000
Reasons why the study report is not available for submission	The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance" (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL
Category study in AIR 5 dossier (I. docs)	Category 4a

1. Information on the study

Data point:	CA 5.2.1/038
Report author	[redacted]
Report year	1981
Report title	Acute oral toxicity of MON 0139 ro rats
Report No	800257
Document No	Not reported
Guidelines followed in study	None
Deviations from current test guideline (OECD 420, 2001)	Both sexes used instead of one sex (usually females). Purity and stability of test compound not reported. Number of air changes during housing of the animals lacking. Environmental conditions not reported. Type of diet not reported. Age of the rats and acclimation period not reported. Individual body weights and necropsy findings not reported.
Previous evaluation	Yes, accepted in RAR (2015)

GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities (GLP was not compulsory at the time the study was performed)
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary
The acute oral toxicity of glyphosate (MON 0139) was investigated in 5 male and 5 female rats of the Sprague-Dawley strain. The test substance was administered undiluted by oral gavage to each animal at a dosage of 5000 mg/kg bw. Mortality, body weight and clinical signs were recorded during the subsequent 14 days. All animals were subjected to a gross necropsy at the end of the study.

One animal died on the day of dosing. This was not attributed to the substance. No clinical signs were noticed. Changes of body weight were in the normal range. Pale kidneys (2 animals) and bilateral hydronephrosis (1 animal) were observed during necropsy.
The acute oral LD_{50} was determined to be

LD_{50}, oral, rat > 5000 mg/kg bw

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate

Identification:	MON 0139
Description:	Amber liquid
Lot/Batch #:	SSRT-11012
Purity:	Not specified
Stability of test compound:	Not specified

2. Vehicle and/or positive control: Undiluted

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley (Crl:CD® (SD)BR)
Source:	[redacted]
Age:	Not specified
Sex:	Male and female
Weight at dosing:	256 – 276 g (males), 178 – 196 g (females)
Acclimation period:	Not specified
Diet/Food:	Not specified, *ad libitum* (except for fasting period overnight before dosing)
Water:	Not specified, *ad libitum*
Housing:	Individually housed

Environmental conditions:	Temperature:	Not specified
	Humidity:	Not specified
	Air changes:	Not specified
	light/dark cycle:	Not specified

B. STUDY DESIGN AND METHODS

In life dates: 18/09/1980 to 02/10/1980 (Experimental phase)

Animal assignment and treatment:
A group of 5 male and 5 female rats was fasted overnight and dosed with the test substance at a concentration of 5000 mg/kg bw. After treatment, animals were housed individually and observed for mortality and clinical signs three times within the first 8 hours after dosing and twice daily (morning and afternoon) thereafter. Surviving animals were sacrificed after 15 days and were subjected to necropsy. Body weights were recorded immediately before dosing and on day 7 and 14 during the study. One animal died due to flawed administration.

II. RESULTS AND DISCUSSION

A. MORTALITY
One male animal died on the day of dosing probably as a result of an esophageal rupture as test material was found in the thoracic cavity.

B. CLINICAL OBSERVATIONS
No clinical signs of toxicity were observed during the 14 days observation period.

C. BODY WEIGHT
All animals gained weight during the study (see table below).

Table 5.2.1-51: Acute oral toxicity of MON 0139 [redacted] 1981): Mean body weight

Dose level	5000 mg/kg bw					
Sex	Males			Females		
Day	0	7	15	0	7	15
	Body weight [g]*					
Mean	267	339	378	188	235	250

* No body weight data of single animals reported.

D. NECROPSY
During necropsy, pale coloured kidneys were noticed in two females and bilateral hydronephrosis was noticed in one female. However, these findings were not considered as relevant to the test substance.

III. CONCLUSIONS
The oral LD_{50} of glyphosate (MON 0139) in male and female rats was greater than 5000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
Due to the deviations of the study and the fact that it was not performed according to GLP, the study provides supplementary information on acute oral toxicity of glyphosate, only.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.1/039
Report author	[redacted]
Report year	1979
Report title	Acute Oral Toxicity Study In Rats
Report No	[redacted]-77-428
Document No	Not reported
Guidelines followed in study	None (pre-guideline), similar to OECD 420 (2001)
Deviations from current test guideline (OECD 420 (2001)	5 animals per sex used, high doses, animals not fasted after dosing, for males and females clinical signs were provided not separately, environmental conditions not reported.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities (pre-GLP)
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The test substance, glyphosate, was evaluated for its acute oral toxicity potential in male and female Wistar strain albino rats when administered as a gavage dose at levels of 2500, 3500, 5000, 7000, and 9900 mg/kg bw (n=5 per sex per dose level). Mortalities at dose levels of 2500, 3500, 5000, 7000, and 9900 mg/kg bw were 1/10, 1/10, 3/10, 8/10, and 10/10, respectively. Clinical signs included ataxia, convulsions, muscle tremors, red nasal discharge, clear oral discharge, urinary staining of the abdomen, soft stool, piloerection, lethargy, and fecal staining of the abdomen. For the 2500, 3500, 5000 and 7000 mg/kg bw dose levels, although some animals lost weight between 7 and 14 days, all surviving animals gained weight throughout the study. The gross necropsy conducted at termination demonstrated discoloured lungs, liver and/or kidneys for the 2500 mg/kg bw group, discoloured lungs, liver and/or kidneys or air filled intestines for the 3500 mg/kg bw group, no findings for the 5000 mg/kg bw group, and discoloured lungs, liver and/or kidneys, and air filled intestines for the 7000 mg/kg bw group. No 9900 mg/kg bw animals survived to necropsy. The acute oral LD_{50} was calculated to be

LD_{50}, oral, rat 5600 mg/kg bw, with 95 % confidence limits of 4900 to 6300 mg/kg bw

I.MATERIALS AND METHODS

A. MATERIALS

1. Test material:	Glyphosate
Identification:	Glyphosate Technical
Description:	Fine white powder
Lot/Batch #:	XHI-180
Purity:	99 %
Stability of test compound:	No data given in the report
2. Vehicle and/or positive control:	Distilled water
3. Test animals:	
Species:	Rat
Strain:	Wistar
Source:	[redacted]
Age:	Not specified
Sex:	Male and female
Weight at dosing:	225 – 294 g
Acclimation period:	Not specified
Diet/Food:	*ad libitum* (except when fasted for approximately 18 hours prior to dosing)
Water:	*ad libitum*
Housing:	Individually
Environmental conditions:	Temperature: Not specified Humidity: Not specified Air changes: Not specified Light cycle: Not specified

B. STUDY DESIGN AND METHODS

In life dates: Not specified

Animal assignment and treatment:

Groups of five male and five female rats received the test material at a dose levels of 2500, 3500, 5000, 7000, and 9900 mg/kg body weight by oral gavage. The test material was administered by oral intubation as a 25 % w/v solution in distilled water. Observations for mortality and overt signs of effect were made at 0-2 and 4-6 hours following dosing and twice daily thereafter (early morning and late afternoon) for fourteen days. Body weights were recorded prior to fasting, on Day 7, and on Day 14 of the study. A gross necropsy was performed on all animals at the time of death or terminal sacrifice (Day 14). All abnormalities were recorded.

II. RESULTS AND DISCUSSION

A. MORTALITY

Mortalities in the study are as indicated in the table below.

Table 5.2.1-52: Acute Oral Toxicity Study In Rats ([redacted] 1979): Mortality observed after treatment

Dose Level (mg/kg body weight)	Mortality/Total Number of Animals Dosed
2500	1/10
3500	1/10
5000	3/10
7000	8/10
9900	10/10

Note: males and females were not reported separately.

B. CLINICAL OBSERVATIONS

Clinical signs included ataxia, convulsions, muscle tremors, red nasal discharge, clear oral discharge, urinary staining of the abdomen, soft stool, piloerection, lethargy, and fecal staining of the abdomen (see table below).

Table 5.2.1-53: Acute Oral Toxicity Study In Rats ([redacted] 1979): Clinical observations recorded after treatment during the study

Dose level [mg/kg bw]	2500	3500	5000	7000	9900
Clinical observation	**Incidence***				
Ataxia	1/10 (d1-3,4)	2/10 (h0-2) 1/9 (d7)	1/9 (d1) 1/8 (d7) 0/7 (d14)	1/3 (h0-6) 1/2 (d2) 0/2 (d3-4) 0/2 (d7,14)	1/2 (h0-2) 1/1 (h4-6)
Convulsion	1/10 (d3-4)	-	-	-	-
Muscle tremor	1/10 (d2)	1/10 (d2)	1/9 (d1) 4-5 (h0-6)	-	1/2 (h0-2)
Red nasal discharge	1/10 (d2)	-	1/9 (d1) 1/8 (d7) 0/7 (d14)	-	-
Rales	-	-	1/10 (h4-6)	-	-
Clear oral discharge	-	-	3/10 (h0-2)	1/3 (d4-6)	1/2 (h0-2)
Lacrimation	-	-	-	1-2/3 (h0-6)	-
Urinary staining	1/10 (d3-4)	1/10 (d2) 3/10 (d3)	1/10 (h4-6) 5/10 (d1)	1-2/2 (d2)	-
General unhealthy appearance	1/10 (d3)	-	1/8 (d7)	-	1/2 (h0-2) 1/1 (h4-6)
Soft stool	-	1/10 (h0-4, d2)	2-3/10 (h0-6) 3-6/10 (d1) 1/8 (d7) 0/7 (d14)	1-2/2 (d2)	-
Piloerection	5/10 (h0-2) 7/10 (h4-6) 4-8/10 (d1-3) 1/9 (d7) 0/9 (d14)	5/10 (h0-6) 4-8/10 (d1-3) 2/9 (d7) 0/9 (d14)	5-6/10 (h0-6) 6/9 (d1) 0/8 (d2-4,7) 0/7 (d14)	3/3 (h0-6) 2/2 (d1-3) 0/2 (d4,7) 0/2 (d14)	1/1 (h4-6)
Prostration	1/10 (d3) 0/9 (d4,7,14)	-	-	-	1/2 (h0-2)
Lethargy	1/10 (h0-2) 2/10 (h4-6) 5-7/10 (d1) 2-4/10 (d2-3) 1-2/10 (d4) 0/9 (d7,14)	6/10 (h0-2) 4/10 (h4-6) 2-4/10 (d1-2) 1-2/10 (d3) 1/9 (d7) 0/9 (d14)	2/10 (h0-2) 4/10 (h4-6) 1-3/9-10 (d1) 0/8 (d2-4) 1/8 (d7) 0/7 (d14)	2/3 (h0-6) 2/2 (d1-2) 1/2 (d3) 0/2 (d4,7) 0/2 (d14)	1/2 (h0-2) 1/1 (h4-6)

Table 5.2.1-53: Acute Oral Toxicity Study In Rats ([redacted] 1979): Clinical observations recorded after treatment during the study

Dose level [mg/kg bw]	2500	3500	5000	7000	9900
Clinical observation	**Incidence***				
Hyperactivity	-	1/10 (h0-2)	4/10 (d1) 2-3/10 (h0-6)	-	-
Fecal staining	1/10 (d1); 1/10 (d3-4) 0/9 (d7,14)	1/10 (h4-6); 1/10 (d2-3) 1/9 (d7) 0/9 (d14)	7/9 (d1) 0/8 (d2-3) 1/8 (d4,7) 0/7 (d14)	1-2/2 (d2-3) 0/2 (d4,7) 0/2 (d14)	-
Hyperpnea	-	-	1/8 (d7)	-	-
Squinting eyes	-	-	-	2-3/3 (h0-6)	-
Dyspnea	-	-	-	1/2 (d1)	-
No observed abnormality	3-5/10 (h0-6) 1-2/10 (d1) 4-5/10 (d2-3) 8/10 (d4) 8/9 (d7) 9/9 (d14)	2-3/10 (h0-6) 3-5/10 (d1-3) 10/10 (d4) 8/9 (d7) 9/9 (d14)	1/10 (h0-6) 0-1/9-10 (d1) 7-8/8 (d2-4) 7-8/8 (d7) 7/7 (d14)	2/2 (d4,7)	0/2 (h0-2) 0/1 (h4-6)

* = number of clinical signs/number of surviving animals, point in time (h = hours, d = day) in parenthesis

C. BODY WEIGHT

For the 2500, 3500, 5000, and 7000 mg/kg body weight dose levels, although some animals lost weight between 7 and 14 days, all surviving animals gained weight throughout the study (see table below).

Table 5.2.1-54: Acute Oral Toxicity Study In Rats ([redacted] 1979): Body weight development

Dose level [mg/kg bw]	2500	3500	5000	7000	9900
	Body weight (g)				
Sex	**Males**				
Day 0					
	268	279	250	290**	291**
	274	262	245	292**	265**
	269	290	252	289**	271**
	281	294	248	294**	294**
	284	262	240	287**	284**
Mean ± SD	**275.2 ± 7.1**	**277.4 ± 15.1**	**247.0 ± 4.7**	**290 ± 2.7**	**281.0 ± 12.6**
Day 7					
	320	326	305	-	-
	307	323	310	-	-
	324	345	321	-	-
	-	369	276	-	-
	339	186	256	-	NR
Mean ± SD	**322.5 ± 13.2**	**309.8 ± 71.6**	**293.6 ± 26.8**		
Termination*					
	365	356	346	+	+
	340	360	352	+	+
	349	379	370	+	+
	201 (Day 5)**	396	331	+	+
	363	177 (Day 8)**	305	+	NR
Mean ± SD	**323.6 ± 69.3**	**333.6 ± 89.0**	**340.8 ± 24.4**		
Sex	**Females**				

Table 5.2.1-54: Acute Oral Toxicity Study In Rats ([redacted] 1979): Body weight development

Dose level [mg/kg bw]	2500	3500	5000	7000	9900
Body weight (g)					
Day 0					
	229	240	241	241**	239**
	266	236	246	291**	270**
	279	234	250	236	232**
	284	239	235	243**	236**
	288	249	225	225	260**
Mean ± SD	**269.2 ± 24.0**	**239.6 ± 5.8**	**239.4 ± 9.8**	**247.2 ± 25.5**	**247.4 ± 16.6**
Day 7					
	241	254	-	-	-
	291	253	274	-	-
	310	246	274	259	-
	312	250	-	-	-
	325	259	268	241	-
Mean ± SD	**295.8 ± 32.9**	**252.4 ± 4.8**	**272.0 ± 3.5**	**250.0 ± 12.7**	
Termination*					
	237	256	212 (Day 1)**	+	+
	296	247	285	+	+
	304	242	219 (Day 9)**	258	+
	298	257	200 (Day 2)**	+	+
	323	263	269	236	+
Mean ± SD	**291.6 ± 32.3**	**253.0 ± 8.4**	**237.0 ± 37.6**	**247.0 ± 15.6**	

* = Terminal body weight recorded on Day 14 unless otherwise noted in parenthesis, ** = Animal died spontaneously, + = No terminal body weights were recorded for animals dying spontaneously on Day 1 of the study.
Note: Mean and standard deviation were not given in the study report. Values were calculated retrospectively.

D. NECROPSY

A summary of the gross necropsy findings for the decedents and the animals necropsied at the conclusion of the 14-day observation period is presented in the table below.

Table 5.2.1-55: Acute Oral Toxicity Study In Rats ([redacted] 1979): Summary of Necropsy Findings

Dose Level (mg/kg bw)	Animals Necropsied at 14 Days	Decedents
2500	Discoloured lungs (in 6/9), liver (in 9/9), and/or kidneys (in 9/9)	Urinary and faecal staining of the abdomen (1/1) Discoloured lungs (1/1) Fluid filled stomach (1/1) Fluid filled and/or distended intestines (1/1)
3500	Discoloured lungs (9/9), liver (9/9), and/or kidneys (9/9) Air filled intestines (1/9)	Discoloured lungs (1/1)
5000	No observations (7/7)	Oral and/or nasal discharge (2/3) Urinary and/or faecal staining of the abdomen (3/3) Discoloured lungs (2/3) and/or liver (3/3) Fluid filled and/or discoloured stomach and/or intestines (3/3)

Table 5.2.1-55: Acute Oral Toxicity Study In Rats ([redacted] 1979): Summary of Necropsy Findings

Dose Level (mg/kg bw)	Animals Necropsied at 14 Days	Decedents
7000	Discoloured lungs (1/2), liver (2/2), and/or kidneys (1/2) Air filled intestines (1/2)	Oral discharge (3/8) Fluid filled intestines and/or stomach (7/8) Discoloured liver (1/8), and/or kidneys (8/8) Urinary and/or faecal staining of the abdomen (2/8)
9900	Not applicable	Discoloured lungs (3/10), liver (2/10), and/or kidneys (7/10) Fluid filled intestines and/or stomach (9/9) Oral and/or nasal discharge (7/9) Urinary staining of the abdomen (1/9)

III. CONCLUSIONS

The oral LD_{50} of the test material (glyphosate) in rats was calculated to be 5600 mg/kg body weight with 95 % confidence limits of 4900 to 6300 mg/kg body weight.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is considered supplementary only as the study was conducted prior to GLP and not according to any test Guideline and additionally some reporting deficiencies are apparent.
Nevertheless, the outcome of the study can be reported as valid.
The acute oral LD_{50} is >5000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

CA 5.2.2 Dermal

In total, 27 acute dermal toxicity studies in rats and rabbits were provided of which 22 were performed with glyphosate acid and 5 with glyphosate salts.

The LD_{50} in rabbits always exceeded the applied limit dose of 2000 and 5000 mg/kg bw. In two studies applying the upper limit dose of 5000 mg/kg bw one of five female rabbits was found dead. In both studies a total of 10 animals was treated with 5000 mg/kg bw (CA 5.2.2/023 and CA 5.2.2/025); neither death was considered test item relelated. Slight erythema formation was observed in one rabbit study (CA 5.2.2/026). The LD_{50} in rats always exceeded the applied limit dose of 2000 and 5000 mg/kg bw. No mortality was observed up to the highest dose applied (8000 mg/kg bw). Occasionally, slight erythema or scab formation was observed in rats and rabbits. Body weight loss, reduced activity, piloerection were observed in few treated animals.

The low acute dermal toxicity of glyphosate and its salts demonstrated in the 2001 EU evaluation and the re-evaluation in 2015 is confirmed during the current AIR 5 process. The dermal LD_{50} values for rats and rabbits conclusively exceeded 5000 mg/kg bw for glyphosate acid and its salts. Thus, glyphosate does not need to be classified for acute dermal toxicity according to the CLP Regulation (EU) No. 1272/2008 nor according to the criteria of GHS UN category 5.

Table 5.2.2-1: Studies on acute dermal toxicity with glyphosate

Annex Point	Study	Study type	Substance(s)	Reference list-related category§	Result [LD_{50}]
CA 5.2.2/001	[redacted] 2011	*in vivo:* RjHan:(WI) Wistar rats, ♂ / ♀	Glyphosate technical (Batch: 569753, Purity: 96.3 %)	valid, Category 2a	>5000 mg/kg bw
CA 5.2.2/002	[redacted], 2010	*in vivo:* CD / Crl:CD(SD) rats, ♂ / ♀	Glyphosate technical grade (Batch: 2009051501, Purity: 96.4 %)	valid#, Category 2a	>2000 mg/kg bw
CA 5.2.2/003	[redacted], 2010	*in vivo:* CD / Crl:CD(SD) rats, ♂ / ♀	Glyphosate technical grade (Batch: 20090506, Purity: 97.3 %)	valid#, Category 2a	>2000 mg/kg bw
CA 5.2.2/004	[redacted] 2009	*in vivo:* Sprague-Dawley rats, ♂ / ♀	Glyphosate Tech Grade Mixed 5-Batch (Batch: 080704-1 thru 5, Purity: 96.71 % / 96.40 %)	valid, Category 2a	>5050 mg/kg bw
CA 5.2.2/005	[redacted], 2009	*in vivo:* HanRcc: WIST (SPF) rats, ♂ / ♀	Glyphosate technical (Batch: GI-1045, Purity: 96.66 %)	valid, Category 2a	>2000 mg/kg bw
CA 5.2.2/006	[redacted] 2009	*in vivo:* CD / Crl:CD(SD) rats, ♂ / ♀	Glyphosate technical grade (Batch: 20080801, Purity: 98.8 %)	valid#, Category 2a	>2000 mg/kg bw
CA 5.2.2/007	[redacted], 2008	*in vivo:* Wistar Hannover rats, ♂ / ♀	Glyphosate technical (Batch: 20070606, Purity: 98.05 %)	valid, Category 2a	>2000 mg/kg bw
CA 5.2.2/008	[redacted] 2007	*in vivo:* HanRcc:WIST (SPF) rats, ♂ / ♀	Glyphosate technical (NUP 05068) (Batch: 200609062, Purity: 95.1 %)	valid, Category 2a	>2000 mg/kg bw
CA 5.2.2/009	[redacted], 2007	*in vivo:* HanRcc:WIST (SPF) rats, ♂ / ♀	Glyphosate technical material (Batch: 0507, Purity: 96.1 % w/w)	valid, Category 2a	>5000 mg/kg bw
CA 5.2.2/010	[redacted], 2005	*in vivo:* Sprague-Dawley derived, albino rats, ♂ / ♀	Glyphosate acid technical (Batch: 040205, Purity: 97.23 %)	valid, Category 2a	>5000 mg/kg bw
CA 5.2.2/011	[redacted] 1996	*in vivo:* Alpk:AP_fSD (Wistar-derived) rats, ♂ / ♀	Glyphosate acid (Batch: P24, Purity: 95.6 %)	valid, Category 2a	>2000 mg/kg bw
CA 5.2.2/012	[redacted], 1995	*in vivo:* Specific pathogen free SD rats (Crj:CD), ♂ / ♀	Glyphosate technical (Code HR-001) (Batch: 940908-1, Purity: 95.68 %)	valid, Category 2a	>2000 mg/kg bw
CA 5.2.2/013	[redacted], 1995	*in vivo:* Sprague-Dawley rats, ♂ / ♀	Glyphosate acid technical (Batch: 1073; Purity: 97.6 %)	valid, Category 2a	>2000 mg/kg bw
CA 5.2.2/014	[redacted], 1995	*in vivo:* Sprague-Dawley rats, ♂ / ♀	Glyphosate salt (Batch: 940950; Purity: 61.8 %)##	valid, Category 2a	>2000 mg/kg bw
CA 5.2.2/015	[redacted], 1994	*in vivo:* Sprague-Dawley rats, ♂ / ♀	Glyphosate technical (Purity: 95 %)	invalid, Category 4b	>2000 mg/kg bw
CA 5.2.2/016	[redacted] 1994	*in vivo:* Sprague-Dawley rats, ♂ / ♀	Glyphosate (Purity: 95 %)	invalid, Category 4b	>2000 mg/kg bw

Table 5.2.2-1: Studies on acute dermal toxicity with glyphosate

CA 5.2.2/017	[redacted] 1994	*in vivo:* LATI/Wistar rat, ♂ / ♀	Glyphosate technical (Batch: 36300892; Purity: 99.6 %)	valid, Category 2a	>2000 mg/kg bw
CA 5.2.2/018	[redacted], 1992	*in vivo:* Sprague-Dawley rat, ♂ / ♀	Glyphosate technical (Batch: L3258)	valid, Category 2a	>2000 mg/kg bw
CA 5.2.2/019	[redacted], 1991	*in vivo:* Wistar rat, ♂ / ♀	Glyphosate technical (Batch: 60, Purity: 96.8 %)	valid, Category 2a	>5000 mg/kg bw
CA 5.2.2/020	[redacted] 1990	*in vivo:* CD rat, ♂ / ♀	Glyphosate technical (Batch: 0190 A, 98.1 %)	valid, Category 2a	>8000 mg/kg bw
CA 5.2.2/021	[redacted], 1989	*in vivo:* Sprague-Dawley rat, ♂ / ♀	Glyphosate technical (PMG) (Batch: 206-Jak-25-1, Purity: 98.6 %)	valid, Category 2a	>2000 mg/kg bw
CA 5.2.2/022	[redacted] 1989	*in vivo:* Wistar rat, ♂ / ♀	Glyphosate technical (Purity: 62 % (IPA))##	invalid, Category 4b	>2000 mg/kg bw
CA 5.2.2/023	[redacted], 1988	*in vivo:* New Zealand White rabbit, ♂ / ♀	Glyphosate (Batch: XLI-55, Purity: 97.76 %)	valid, Category 2a	>5000 mg/kg bw
CA 5.2.2/024	[redacted], 1987	*in vivo:* New Zealand White rabbit, ♂ / ♀	MON 8722 (Batch: XLG-256, Purity: 70.7 %)##	valid, Category 2a	>5000 mg/kg bw
CA 5.2.2/025	[redacted], 1987	*in vivo:* New Zealand White rabbit, ♂ / ♀	MON-8750 (Batch: XLG-255, Purity: 90.8 %)##	valid, Category 2a	>5000 mg/kg bw
CA 5.2.2/026	[redacted], 1983	*in vivo:* Albino NWS rabbit, ♂ / ♀	Glyphosate technical (Batch: R & D sample (8.7.83.), Purity: 95 %)	supportive, Category 3a	>2000 mg/kg bw
CA 5.2.2/027	[redacted] 1981	in vivo: New Zealand White (Isf: (NZW) rabbit, ♂ / ♀	MON 0139 (Batch: SSRT-11012)##	supportive, Category 2a	>5000 mg/kg bw

This study was performed at the [redacted]
This study was performed with a glyphosate salt

1. Information on the study

Data point	CA 5.2.2/001
Report author	[redacted]
Report year	2011
Report title	Glyphosate Technical - Acute Dermal Toxicity Study in Rats - Final Report Amendment 1
Report No	10/218-002P
Document No	Not reported
Guidelines followed in study	OECD 402 (1987), US EPA OPPTS 870.1200 (1998), EC 440/2008 B.3 (2008)
Deviations from current test guideline (OECD 402, 2017)	No stepwise approach was used, five rats of both sexes instead of two animals, preferred females, were used per dose. Age of animals was not reported. After treatment the first observation was perfomed after 1 hour instead of during the first 30 minutes.
Previous evaluation	Yes, accepted in RAR (2015)

GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

In an acute dermal toxicity study, a group of one male and one female and a second group of four male and four female RjHan:(WI) Wistar rats were treated with a single administration of glyphosate technical (96.3 % w/w glyphosate technical) at 5000 mg/kg bw by dermal application to a shaved area of the back (approximately 10 % area of the total body surface). The application period was 24-hours, followed by a 14-day observation period.

Clinical observations along with a check of viability and mortality were performed on all animals at 1 and 5 hours after dosing and daily for 14 days thereafter. Body weight was measured prior to dosing on Day 0 and on Days 7 and 14. Rats were killed and given a gross macroscopic examination at the end of the 2-week observation period (Day 14).

No mortality occurred. No adverse clinical signs were observed after treatment with the test item or during the 14-day observation period. The body weights were within the range commonly recorded for this strain and age. There was no treatment related macroscopic findings observed in any animals.

The acute dermal LD_{50} of glyphosate technical after a single dermal administration to male and female RjHan:(WI) Wistar rats, observed over a period of 14 days was

LD_{50}, dermal, rat > 5000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate Technical
Description:	Dry white powder
Lot/Batch #:	569753
Purity:	96.3 % w/w glyphosate technical
Stability of test compound:	Stable under storage conditions (< 30 °C), recertification date: 31 August 2011

2. Vehicle and/or positive control: Water

3. Test animals:

Species:	Rat
Strain:	RjHan:(WI) Wistar
Source:	[redacted]
Sex:	Males and females (nulliparous and nonpregnant)
Age:	Young adults
Weight at dosing:	Males: 228 – 259 g; females: 220 – 231 g
Acclimatisation period:	6 days
Diet:	ssniff® SM R/M-Z+H "Autoclavable complete feed for rats and mice – breeding and maintenance" produced by ssniff Spezialdiäten GmbH, D-59494 Soest Germany, *ad libitum*

Water:	Tap water, *ad libitum*	
Housing:	Individually in Type II polypropylene / polycarbonate cages with Lignocel bedding for laboratory animals	
Environmental conditions:	Temperature:	20.7 – 24.0 °C
	Humidity:	39 – 65 %
	Air changes:	15 – 20 air changes per hour
	Photoperiod:	12-hour light / dark cycle (light 6:00 AM to 6:00 PM)

B: Study design and methods

In life dates: 2010-10-06 to 2010-10-20 (Start of treatment to final sacrifice)

Animal assignment and treatment:
A group of one male and one female and a second group of four male and four female RjHan:(WI) Wistar rats were treated with a single administration of glyphosate technical (96.3 % w/w glyphosate technical) at 5000 mg/kg bw by dermal application. The application period was 24-hours, followed by a 14-day observation period.

The backs of the animals were shaved (approximately 10 % area of the total body surface) approximately 24-hours prior to the treatment. Only those animals without injury or irritation on the skin were used in the test. On Day 0, the test item was moistened with water and applied uniformly over the skin. Sterile gauze pads were placed on the skin of rats at the site of application. These gauze pads were kept in contact with the skin by a patch with adhesive hypoallergenic plaster. The entire trunk of the animal was then wrapped with semi occlusive plastic wrap for 24-hours. At the end of the 24-hour exposure period, residual test item was removed, using body temperature water.

A clinical examination was performed on the day of treatment, at 1 and 5 hours after the application of the test item, and once each day for 14 days thereafter. The body weight of all animals was recorded on Day 0 (beginning of the experiment) and on Days 7 and 14. All animals were anaesthetised with Euthasol® 40 % and exsanguinated. After examination of the external appearance, the cranial, thoracic and abdominal cavities were opened and the appearance of the tissues and organs were observed. Any gross macroscopic findings were recorded.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

There were no clinical signs noted in any animals throughout the study. No treatment related skin irritation was observed in any animal throughout the study.

C. BODY WEIGHT

There were no effects on body weight and body weight gain during the observation period (see table below).

Table 5.2.2-1 Glyphosate technical: Acute Dermal Toxicity study in Rats ([redacted] 2011): Body weight and body weight gain

Dose level	5000 mg/kg bw							
Sex	Males			Females			Males	Females
Day	0	7	14	0	7	14	0 - 14	0 - 14
Animal No.§	Body weight [g]						Body weight gain [g]	
1946 (1951)	228	262	304	225	230	248	76	23
1947 (1952)	259	306	357	225	252	259	98	34
1948 (1953)	240	284	337	220	238	243	97	23
1949 (1954)	244	296	337	231	243	258	93	27
1950 (1955)	238	276	319	220	245	266	81	46
Mean	**241.8**	**284.8**	**330.8**	**224.2**	**241.6**	**254.8**	**89.0**	**30.6**
± SD	**± 11.3**	**± 17.1**	**± 20.1**	**± 4.5**	**± 8.2**	**± 9.2**	**± 9.9**	**± 9.7**

§ = animal No. of males and (females)

D. NECROPSY

No macroscopic findings were recorded at the scheduled necropsy.

III. CONCLUSIONS

The acute dermal LD_{50} of glyphosate technical after a single dermal administration to male and female Wistar rats, observed over a period of 14 days was greater than 5000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD 402 (2017). Therefore, the outcome can be reported as valid. The acute dermal LD_{50} is above 5000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point	CA 5.2.2/002
Report author	[redacted]
Report year	2010
Report title	Acute Dermal Toxicity Study of Glyphosate TC in CD Rats
Report No	24876
Document No	Not reported
Guidelines followed in study	OECD 402 (1987), US EPA OPPTS 870.1200 (1998), EC method B.3. (92/69/EEC)
Deviations from current test guideline (OECD 402, 2017)	No stepwise approach was used, five rats of both sexes instead of two animals, preferred females, were used per dose. Age of male animals (7 weeks) was outside of the range specified in the guideline (at least 8 – 10 weeks).

Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid#
Category study in AIR 5 dossier (L docs)	Category 2a

#: Following the feedback from the Assessment Group Glyphosate in March 2020, the Notifier is aware of concerns regarding work conducted at this specific contract laboratory ([redacted]). For the sake of completeness, this study is included in the dossier submission since it was part of the data package of previous EU evaluation processes.

2. Full summary

The test substance, glyphosate technical, was evaluated for its acute dermal toxicity potential in rats when administered as single dose of 2000 mg/kg bw. No mortality occurred during the study and no clinical signs or skin reactions were observed. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. Hence, the acute dermal LD_{50} was determined to be

LD_{50}, dermal, rat > 2000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate technical grade
Description:	White solid powder
Lot/Batch #:	2009051501
Purity:	96.4 %
Stability of test compound:	Expiry date: 2011-05-15

2. Vehicle and/or positive control: *Aqua ad iniectabilia* [water for injection]

3. Test animals:

Species:	Rat
Strain:	CD / Crl:CD(SD)
Source:	[redacted]
Age:	Males: approx. 7 weeks; females: approx. 9 weeks
Sex:	Males and females
Weight at dosing:	Males: 233 – 249 g; females: 211 – 229 g
Acclimation period:	At least 5 days
Diet/Food:	ssniff® R/M-H V1534 (ssniff Spezialdiäten GmbH, Germany), *ad libitum* except for approx. 16 h before administration
Water:	Tap water, *ad libitum*
Housing:	Individual housing in Makrolon cages (type III plus) with granulated textured wood as bedding material
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 40 – 70 % Air changes: Not reported Photoperiod: 12-hour light / dark cycle

B: Study design and methods

In life dates: 2009-10-29 to 2009-11-12 (Start of treatment to final sacrifice)

Animal assignment and treatment:

One dose level group of five male and five female rats was examined in a limit test. The dose level of 2000 mg/kg bw was applied once as a suspension in *aqua ad iniectabilia* for 24-hours on the shaved intact dorsal skin of the rats (approximately 10 % of total body surface). The administration volume was 10 mL/kg bw. The test substance was applied to 8 layers of gauze. The gauze was covered with a plastic sheet and secured with adhesive plaster on the application site. At the end of the exposure period no residual test item had to be removed. Observations for mortality and clinical/behavioural signs of toxicity were made before, immediately, 5, 15, 30 and 60 min, as well as 3, 6 and 24-hours after administration and at least once daily thereafter for 14 days. Individual body weights were recorded before administration of the test item and thereafter in weekly intervals up to the end of the study. The skin was observed for the development of erythema and oedema. At the end of the experiments, all animals were sacrificed, dissected and inspected macroscopically, and all abnormalities were recorded.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

A single dermal administration of 2000 mg/kg bw to five male and five female rats did not reveal any clinical signs of toxicity. No skin reactions were observed.

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance (see Table below).

Table 5.2.2-2 Acute Dermal Toxicity Study of Glyphosate TC in CD Rats ([redacted] 2010): Body weight and body weight gain

Dose level	2000 mg/kg bw							
Sex	Males			Females			Males	Females
Day	0	7	14	0	7	14	0 – 14	0 – 14
Animal No.§	Body weight [g]						Body weight gain [%]	
1 M (6 F)	243	311	349	212	251	256	43.6	20.8
2 M (7 F)	236	309	353	226	265	282	49.6	24.8
3 M (8 F)	233	288	321	211	242	251	37.8	19.0
4 M (9 F)	246	318	359	212	245	249	45.9	17.5
5 M (10 F)	249	329	381	229	269	287	53.0	25.3
Mean	**241.4**	**311.0**	**352.6**	**218.0**	**254.4**	**265.0**	**46.0**	**21.5**
± SD	**6.7**	**15.0**	**21.6**	**8.7**	**12.0**	**18.1**	**-**	**-**

§ = animal No. of males and (females); - = not calculated in report

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute dermal LD_{50} of the test material (glyphosate technical) after a single dermal administration to male and female CD rats, observed over a period of 14 days was greater than 2000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD 402 (2017). Therefore, the outcome can be reported as valid. The acute dermal LD_{50} is above 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point	CA 5.2.2/003
Report author	[redacted]
Report year	2010
Report title	Acute Dermal Toxicity Study of Glyphosate TC in CD Rats
Report No	24604
Document No	Not reported
Guidelines followed in study	OECD 402 (1987), US EPA OPPTS 870.1200 (1998), EC method B.3. (92/69/EEC)
Deviations from current test guideline (OECD 402, 2017)	No stepwise approach was used, five rats of both sexes instead of two animals, preferred females, were used per dose. Age of male animals (7 weeks) was outside of the range specified in the guideline (at least 8 - 10 weeks).
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid#
Category study in AIR 5 dossier (L docs)	Category 2a
#: Following the feedback from the Assessment Group Glyphosate in March 2020, the Notifier is aware of concerns regarding work conducted at this specific contract laboratory ([redacted]). For the sake of completeness, this study is included in the dossier submission since it was part of the data package of previous EU evaluation processes.	

2. Full summary

The test substance, glyphosate technical, was evaluated for its acute dermal toxicity potential in albino rats when administered as single dose of 2000 mg/kg bw. No mortality occurred during the study, and no clinical signs or skin reactions were observed. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. Hence, the acute dermal LD_{50} was determined to be

LD_{50}, dermal, rat > 2000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate technical grade
Description:	White powder
Lot/Batch #:	20090506
Purity:	97.3 %
Stability of test compound:	Expiry date: May 2011

2. Vehicle and/or positive control: *Aqua ad iniectabilia* [water for injection]

3. Test animals:

Species:	Rat
Strain:	CD / Crl:CD(SD)
Source:	[redacted]
Age:	Males: approx. 7 weeks; females: approx. 9 weeks
Sex:	Males and females
Weight at dosing:	Males: 278 – 292 g; females: 202 – 225 g
Acclimation period:	At least 5 days
Diet/Food:	ssniff® R/M-H V1534 (ssniff Spezialdiäten GmbH, Germany), *ad libitum* except for approx. 16 h before administration
Water:	Tap water, *ad libitum*
Housing:	Individual housing in Makrolon cages (type III plus) with granulated textured wood as bedding material
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 40 – 70 % Air changes: Not reported Photoperiod: 12-hour light / dark cycle

B: Study design and methods

In life dates: 2009-11-12 to 2009-11-26 (Start of treatment to final sacrifice)

Animal assignment and treatment:

One dose level group of five male and five female rats was examined in a limit test. The dose level of 2000 mg/kg bw was applied once as a suspension in *aqua ad iniectabilia* for 24-hours on the shaved intact dorsal skin of the rats (approximately 10 % of total body surface). The administration volume was 10 mL/kg bw. The test substance was applied to 8 layers of gauze. The gauze was covered with a plastic sheet and secured with adhesive plaster on the application site. At the end of the exposure period no residual test item had to be removed. Observations for mortality and clinical/behavioural signs of toxicity were made before, immediately, 5, 15, 30 and 60 min, as well as 3, 6 and 24-hours after administration and at least once daily thereafter for 14 days. Individual body weights were recorded before administration of the test item and thereafter in weekly intervals up to the end of the study. The skin was observed for the development of erythema and oedema. At the end of the experiments, all animals were sacrificed, dissected and inspected macroscopically, and all abnormalities were recorded.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

A single dermal administration of 2000 mg/kg bw to five male and five female rats did not reveal any clinical signs of toxicity. No skin reactions were observed.

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance (see Table below).

Table 5.2.2-3 Acute Dermal Toxicity Study of Glyphosate TC in CD Rats ([redacted] 2010): Body weight and body weight gain

Dose level	2000 mg/kg bw							
Sex	Males			Females			Males	Females
Day	0	7	14	0	7	14	0 – 14	0 – 14
Animal No.§	Body weight [g]						Body weight gain [%]	
1 M (6 F)	291	358	384	202	223	237	32.0	17.3
2 M (7 F)	278	331	346	215	242	262	24.5	21.9
3 M (8 F)	281	347	374	208	228	229	33.1	10.1
4 M (9 F)	282	353	371	217	239	252	31.6	16.1
5 M (10 F)	292	367	390	225	249	256	33.6	13.8
Mean	**284.8**	**351.2**	**373.0**	**213.4**	**236.2**	**247.2**	**30.9**	**15.8**
± SD	**6.3**	**13.5**	**16.9**	**8.8**	**10.6**	**13.7**	**-**	**-**

§ = animal No. of males and (females); - = not calculated

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute dermal LD_{50} of the test material (glyphosate technical) after a single dermal administration to male and female CD rats, observed over a period of 14 days was greater than 2000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD 402 (2017). Therefore, the outcome can be reported as valid. The acute dermal LD_{50} is above 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point	CA 5.2.2/004
Report author	[redacted]
Report year	2009
Report title	Glyphosate: Acute Dermal Toxicity Study in Rats
Report No	12171-08
Document No	Not reported
Guidelines followed in study	US EPA OPPTS 870.1200 (1998)
Deviations from current test guideline (OECD 402, 2017)	No stepwise approach was used, five rats of both sexes instead of two animals, preferred females, were used per dose. Humidity was in the range of 30 – 86 % instead of 30 – 70 %. Weight of two female animals was outside of the range specified in the guideline (200 – 300 g).
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The test substance, glyphosate technical, was evaluated for its dermal toxicity potential and relative skin irritancy when a single dose of 5050 mg/kg bw was applied to the intact skin of albino rats. No mortality occurred during the study, and no clinical signs or skin reactions were observed. There was no effect on body weight gain in animals surviving to termination, with the exception of two animals that lost or failed to gain weight during Day 7 – 14 of the study. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. Hence, the dermal LD_{50} was determined to be

LD_{50}, dermal, rat > 5050 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate Tech Grade Mixed 5-Batch
Description:	White powder
Lot/Batch #:	080704-1 thru 5
Purity:	96.71 % (analysis dated 2009-01-08); 96.40 % (analysis dated 2008-10-17)
Stability of test compound:	No data given in the report; however, since the purity of the test material analysed after the test was conducted is consistent with the purity before the test was conducted, the test material is believed to be stable for the duration of the test.

2. Vehicle and/or positive control: Deionised water

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	Approx. 10 weeks
Sex:	Males and females (nulliparous and non-pregnant)
Weight at dosing:	Males: 299 – 348 g; females: 189 – 207 g
Acclimation period:	5 days
Diet/Food:	Formulab #5008 (PMI Feeds Inc.), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individual housing in suspended, wire bottom, stainless steel cages
Environmental conditions:	Temperature: 19 – 22 °C Humidity: 30 – 86 % Air changes: 10 – 12 / hour Photoperiod: 12-hour light / dark cycle

B: Study design and methods

In life dates: 2008-12-04 to 2008-12-18 (Start of treatment to final sacrifice)

Animal assignment and treatment:

In a limit test, one dose level of 5050 mg/kg bw was examined using five male and five female rats. The test substance was moistened with water and applied once for 24-hours on the shaved intact dorsal skin of the rats (approximately 10 % of the total body surface). The area of application was covered with surgical gauze patch and secured with non-irritating adhesive tape. After 24-hours the tape was removed, and the skin was washed with water and a cloth to remove excess test material. Observations for mortality and clinical/behavioural signs of toxicity were made at least three times on the day of dosing (Day 0) and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and on Days 7 and 14. Observations for evidence of dermal irritation were made at approximately 60 minutes after removal of wrappings, and on Days 4, 7, 11 and 14. On Day 14 after dosing, animals were sacrificed, subjected to gross necropsy, and all abnormalities were recorded.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

A single dermal administration of 5050 mg/kg bw to five male and five female rats did not reveal any clinical signs of toxicity. No skin reactions were observed.

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance, with the exception of two animals that lost or failed to gain weight during Day 7 – 14 of the study. A summary of the body weights is given in the table below.

Table 5.2.2-4 Glyphosate: Acute Dermal Toxicity Study in Rats ([redacted], 2009): Body weight and body weight gain

Dose level	**5050 mg/kg bw**							
Sex	**Males**			**Females**			**Males**	**Females**
Day	**0**	**7**	**14**	**0**	**7**	**14**	**0 - 14**	**0 - 14**
Animal No.§	**Body weight [g]**						**Body weight gain [g]**	
1 M (6 F)	348	356	379	190	204	245	31	55
2 M (7 F)	340	355	387	207	215	217	47	10
3 M (8 F)	319	327	345	189	201	203	26	14
4 M (9 F)	299	318	318	205	218	220	19	15
5 M (10 F)	310	327	340	206	222	219	30	13
Mean	**323.2**	**336.6**	**353.8**	**199.4**	**212.0**	**220.8**	**30.6**	**21.4**
± SD	**± 20.5**	**± 17.6**	**28.7**	**± 9.1**	**± 9.1**	**± 15.2**	**± 10.3**	**± 18.9**

§ = animal No. of males and (females)
Note: Mean and standard deviation as well as body weight gain were not given in the study report. Values were calculated retrospectively.

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute dermal LD_{50} of the test material (glyphosate technical) after a single dermal administration to male and female SD rats, observed over a period of 14 days was greater than 5050 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD 402 (2017). Therefore, the outcome can be reported as valid. The acute dermal LD_{50} is above 5050 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point	CA 5.2.2/005
Report author	[redacted]
Report year	2009
Report title	Glyphosate Technical: Acute dermal toxicity study in rats
Report No	C22875
Document No	Not reported
Guidelines followed in study	OECD 402 (1987), Commission Regulation (EC) No 440/2008 (2008) method B.3
Deviations from current test guideline (OECD 402, 2017)	No stepwise approach was used, five rats of both sexes instead of two animals, preferred females, were used per dose. Weight of female animals (189.8 – 208.6 g) was outside of the range specified in the guideline (200 – 300 g).
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The test substance, glyphosate technical, was evaluated for its acute dermal toxicity potential when applied dermally to an area of clipped skin of male and female HanRcc: WIST (SPF) rats at a dose level of 2000 mg/kg bw. No mortality occurred during the study. No clinical signs were observed during the course of the study. No local dermal signs were observed in any of the treated male animals. In four female animals, slight erythema, scaling, and scabs were observed. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute dermal LD_{50} was calculated to be

LD_{50}, dermal, rat > 2000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate Technical
Description:	Solid
Lot/Batch #:	GI-1045
Purity:	96.66 % Expiry date: July 2010
Stability of test compound:	Stable under storage conditions (20 ± 5 °C, light protected). Stable in purified water for 2 days.

2. Vehicle and/or positive control:

Purified (deionised, PURLAB Option-R unit-treated) water

3. Test animals:

Species:	Rat
Strain:	HanRcc: WIST (SPF)
Source:	[redacted]
Age:	Males: 9 weeks; females: 11 weeks
Sex:	Males and females
Weight at dosing:	Males: 238.9 – 258.3 g; females 189.8 – 208.6 g
Acclimation period:	7 days
Diet/Food:	Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet, batch no. 61/08 (Provimi Kliba AG, 4303 Kaiseraugst / Switzerland), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in Makrolon type-3 cages with standard softwood bedding ("Lignocel", Schill AG, 4132 Muttenz / Switzerland) during treatment and observation period.
Environmental conditions:	Temperature: 22 ± 3´ °C Humidity: 30 – 70 % Air changes: 10 – 15 / hour Photoperiod: 12-hour light / dark cycle

B: Study design and methods

In life dates: 2009-01-20 to 2009-02-03 (Start of treatment to final sacrifice)

Animal assignment and treatment:

Single dose of 2000 mg/kg bw of test substance (glyphosate technical) was applied dermally to an area of clipped skin (approximately 10 % of total body surface area) of five male and five female young adult rats. The treatment area was covered with a semi-occlusive dressing. Application volume was 4 mL/kg bw. Twenty-four hours after the application the dressing was removed and the skin was flushed with lukewarm tap water and dapped off with disposable paper towels. The animals were evaluated for effects on the day of dosing (Day 0) at 30 minutes and at 1, 2, 3 and 5 hours after application and once daily during Days 1 – 14. Clinical observations, dermal findings, body weights and gross post mortem examinations were recorded.

II. RESULTS AND DISCUSSION

A. MORTALITY

No deaths occurred during the study.

B. CLINICAL OBSERVATIONS

No systemic clinical signs were observed during the course of the study.

C. BODY WEIGHT

A slight body weight loss (0.3 – 0.8 %) was observed in two females between acclimatisation and treatment start. The animals recovered until the end of the study. In spite of this body weight loss, the body weights of all animals were considered to be within the range commonly recorded for this strain and age. A summary of the body weights is given in the table below.

Table 5.2.2-5 Glyphosate Technical: Acute Dermal Toxicity Study in Rats ([redacted] 2009): Body weight and body weight gain

Dose level	2000 mg/kg bw							
Sex	Males			Females			Males	Females
Day	0	7	14	0	7	14	0 – 14	0 – 14
Animal No.§	Body weight [g]						Body weight gain [g]	
1 M (6 F)	250.9	268.6	297.6	203.5	201.8	215.5	46.7	12.0
2 M (7 F)	238.9	264.6	289.7	200.4	205.6	214.5	50.8	14.1
3 M (8 F)	253.6	270.7	301.9	189.8	194.0	205.0	48.3	15.2
4 M (9 F)	252.4	281.5	311.5	195.8	195.2	203.5	59.1	7.7
5 M (10 F)	258.3	288.5	318.0	208.6	213.0	223.0	59.7	14.4
Mean	**250.8**	**274.8**	**303.7**	**199.6**	**201.9**	**212.3**	**52.9**	**12.7**
± SD	**± 7.2**	**± 9.9**	**± 11.2**	**± 7.2**	**± 7.8**	**± 8.1**	**± 6.1**	**± 3.0**

§ = animal No. of males and (females)
Note: Body weight gain and mean and SD body weight gain were not given in the study report. Values were calculated retrospectively.

D. NECROPSY

No macroscopic findings were recorded at necropsy.

E. SKIN OBSERVATIONS

No local dermal signs were observed in any of the treated male animals. After removal of the patch, a very slight erythema was noted in four females on Day 4 and persisted up to Days 6, 11 or 12. Scaling was observed in the same four females on Day 4 and persisted up to Days 10, 11 and 12. Scabs were recorded in two females on Day 9 that persisted to Day 11 (see table below).

Table 5.2.2-6 Glyphosate Technical: Acute Dermal Toxicity Study in Rats ([redacted] 2009): Skin observation data

Dose level		2000 mg/kg bw										
Day		0* – 2	3	4	5	6	7	8	9	10	11	12 – 14
Sex	Skin observations											
M	Erythema	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)
	Scaling	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)
	Scabs	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)
F	Erythema	(0/5)	1 (4/5)	1 (4/5)	1 (4/5)	1 (3/5)	1 (3/5)	1 (3/5)	1 (3/5)	1 (3/5)	1 (1/5)	(0/5)
	Scaling	(0/5)	√ (4/5)	√ (4/5)	√ (4/5)	√ (4/5)	√ (4/5)	√ (4/5)	√ (4/5)	√ (3/5)	√ (2/5)	(0/5)
	Scabs	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	√ (2/5)	√ (2/5)	√ (2/5)	(0/5)	(0/5)

1 = slight erythema, √ = scaling or scabs noted.
* Examinations on Day 0 were performed within the first 30 minutes and 1, 2, 3, and 5 hours after exposure.

III. CONCLUSIONS

The acute dermal LD_{50} of the test material (glyphosate technical) after a single dermal administration to male and female Wistar rats, observed over a period of 14 days was greater than 2000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD 402 (2017). Therefore, the outcome can be reported as valid. The acute dermal LD_{50} is above 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point	CA 5.2.2/006
Report author	[redacted]
Report year	2009
Report title	Acute Dermal Toxicity Study of Glyphosate TC in CD Rats
Report No	23912
Document No	Not reported
Guidelines followed in study	OECD 402 (1987), US EPA OPPTS 870.1200 (1998), EC method B.3. (92/69/EEC)
Deviations from current test guideline (OECD 402, 2017)	No stepwise approach was used, five rats of both sexes instead of two animals, preferred females, were used per dose. Age of male animals (7 weeks) was outside of the range specified in the guideline (at least 8 – 10 weeks).
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid#

Category study in AIR 5 dossier (L docs)	Category 2a

#: Following the feedback from the Assessment Group Glyphosate in March 2020, the Notifier is aware of concerns regarding work conducted at this specific contract laboratory ([redacted]). For the sake of completeness, this study is included in the dossier submission since it was part of the data package of previous EU evaluation processes.

2. Full summary

The test substance, glyphosate technical, was evaluated for its acute dermal toxicity potential in rats when administered as single dose of 2000 mg/kg bw. No mortality occurred during the study, and no clinical signs or skin reactions were observed. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. Hence, the acute dermal LD_{50} was determined to be

LD_{50}, dermal, rat > 2000 mg/kg bw

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate technical grade
Description:	White solid powder
Lot/Batch #:	20080801
Purity:	98.8 %
Stability of test compound:	Expiry date: 2010-08-01

2. Vehicle and/or positive control: *Aqua ad iniectabilia* [water for injection]

3. Test animals:

Species:	Rat
Strain:	CD / Crl:CD(SD)
Source:	[redacted]
Age:	Males: 51 days; females: 65 days
Sex:	Males and females
Weight at dosing:	Males: 224 – 234 g; females: 200 – 217 g
Acclimation period:	At least 5 days
Diet/Food:	ssniff® R/M-H V1534 (ssniff Spezialdiäten GmbH, Germany), *ad libitum* except for approx. 16 h before administration
Water:	Tap water, *ad libitum*
Housing:	Individual housing in Makrolon cages (type III plus) with granulated textured wood as bedding material
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 40 – 70 % Air changes: 12 – 18 / hour Photoperiod: 12-hour light / dark cycle

B: Study design and methods

In life dates: 2009-02-18 to 2009-03-04 (Start of treatment to final sacrifice)

Animal assignment and treatment:

One dose level group of five male and five female rats was examined in a limit test. The dose level of 2000 mg/kg bw was applied once as a suspension in *aqua ad iniectabilia* for 24-hours on the shaved intact dorsal skin of the rats (approximately 10 % of total body surface). The administration volume was 10 mL/kg bw. The test substance was applied to 8 layers of gauze. The gauze was covered with a plastic sheet and secured with adhesive plaster on the application site. At the end of the exposure period no residual test item had to be removed. Observations for mortality and clinical/behavioural signs of toxicity were made before, immediately, 5, 15, 30 and 60 min, as well as 3, 6 and 24-hours after administration and at least once daily thereafter for 14 days. Individual body weights were recorded before administration of the test item and thereafter in weekly intervals up to the end of the study. The skin was observed for the development of erythema and oedema. At the end of the experiments, all animals were sacrificed, dissected and inspected macroscopically, and all abnormalities were recorded.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

A single dermal administration of 2000 mg/kg bw to five male and five female rats did not reveal any clinical signs of toxicity. No skin reactions were observed.

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance.

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute dermal LD_{50} of the test material (glyphosate technical) after a single dermal administration to male and female CD rats, observed over a period of 14 days was greater than 2000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD 402 (2017). Therefore, the outcome can be reported as valid. The acute dermal LD_{50} is above 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point	CA 5.2.2/007
Report author	[redacted]
Report year	2008
Report title	Acute Dermal Toxicity Study in Wistar Hannover Rats for Glyphosate Technical
Report No	[redacted]-3996.310.456.07
Document No	Not reported
Guidelines followed in study	OECD 402 (1987)
Deviations from current test guideline (OECD 402, 2017)	No stepwise approach was used, five rats of both sexes instead of two animals, preferred females, were used per dose. One female rat weighed less than 200 g on the day of test item application.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The test substance, glyphosate technical, was evaluated for its acute dermal toxicity potential in albino rats when administered as single dose of 2000 mg/kg bw. No mortality occurred during the study, and no clinical signs or skin reactions were observed. All animals gained the expected body weight, except for two females on the second week of the observation period. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. Hence, the acute dermal LD_{50} was determined to be

LD_{50}, dermal, rat > 2000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate Technical
Description:	Solid
Lot/Batch #:	20070606
Purity:	98.05 %
Stability of test compound:	No data given in the report

2. Vehicle and/or positive control: Deionised water

3. Test animals:

Species:	Rat
Strain:	Wistar Hannover
Source:	[redacted]
Age:	9 – 11 weeks

Sex:	Males and females (nulliparous and non-pregnant)
Weight at dosing:	Males: 266 – 298 g; females: 199 – 213 g
Acclimation period:	7 days
Diet/Food:	Autoclaved Nuvilab CR-1 pellet diet type for rodents (Nuvital Nutrients Ltda., Curitiba - PR, Brazil), *ad libitum*
Water:	Filtered drinking water, *ad libitum*
Housing:	Individual housing in polypropylene rodents cages with wire mesh tops and bedding material
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 30 – 70 % Air changes: At least 10 / hour Photoperiod: 12-hour light / dark cycle

B: Study design and methods

In life dates: 2007-09-11 to 2008-06-11

Animal assignment and treatment:

One dose level group of five male and five female rats was examined in a limit test. The dose level of 2000 mg/kg bw was moistened with deionised water and applied once for 24-hours on the shaved intact dorsal skin of the rats (approximately 10 % of the total body surface). The test item was held in contact with the skin with a porous gauze dressing and non-irritating tape. The test site was further covered using adhesive tape (semi-occlusive dressing). At the end of the exposure period the dressing was removed, and residual test substance was wiped off with deionised water. Observations for mortality and clinical/behavioural signs of toxicity were made once within the first 30 minutes after dosing, three times more during the first 4 hours after dosing, and daily thereafter for a period of 14 days. Individual body weights were determined before the application of the test item (Day 0) and on Days 7 and 14. On Day 14 after dosing, each animal was sacrificed. All study animals were subjected to gross necropsy and all abnormalities were recorded.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

A single dermal administration of 2000 mg/kg bw to five male and five female rats did not reveal any clinical signs of toxicity. No skin reactions were observed.

C. BODY WEIGHT

All animals gained the expected body weight, except for two females in the second week of the observation. A summary of the body weights is given in the table below.

Table 5.2.2-7 Acute Dermal Toxicity Study in Wistar Hannover Rats for Glyphosate Technical ([redacted], 2008): Body weight and body weight gain

Dose level	2000 mg/kg bw								
Sex	Males			Females			Males	Females	
Day	0	7	14	0	7	14	0 – 14	7 – 14	0 – 14
Animal No.§	Body weight [g]						Body weight gain [%]		
49 M (56 F)	289	309	326	203	204	213	37	9	10
50 M (57 F)	266	279	293	199	204	211	27	4	12
51 M (58 F)	298	319	343	213	216	214	45	-2	1
53 M (59 F)	297	321	339	202	207	210	42	3	8
54 M (60 F)	298	322	346	211	219	219	48	0	8
Mean	**289.6**	**310.0**	**329.4**	**205.6**	**210.0**	**213.4**	**39.80**	**3.40**	**7.80**
± SD	**± 13.72**	**± 18.08**	**± 21.73**	**± 6.07**	**± 7.04**	**± 3.51**	**± 8.23**	**± 4.62**	**± 4.15**

§ = animal No. of males and (females)

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute dermal LD_{50} of the test material (glyphosate technical) after a single dermal administration to male and female Wistar rats, observed over a period of 14 days was greater than 2000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD 402 (2017). Therefore, the outcome can be reported as valid. The acute dermal LD50 is above 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point	CA 5.2.2/008
Report author	[redacted]
Report year	2007
Report title	Glyphosate Technical (NUP 05068): Acute dermal toxicity study in rats
Report No	B02283
Document No	Not reported
Guidelines followed in study	OECD 402 (1987), EC method B.3. (92/69/EEC), JMAFF, Guidelines for Preparation of Study Results, Acute Dermal Toxicity Studies,

	Guideline 2-1-2. Notification 12 NohSan No. 8147, as partly revised in 16-Shouan-9260, on 16 March 2005; English translation by AGIS on 17 Oct 2005
Deviations from current test guideline (OECD 402, 2017)	No stepwise approach was used, five rats of both sexes instead of two animals, preferred females, were used per dose. Body weight of two female animals (194.8 and 199.9 g) was outside of the range specified in the guideline (200 – 300 g).
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Glyphosate technical (NUP 05068) diluted in vehicle (PEG 300) was applied dermally at 2000 mg/kg bw (with an application volume of 6 mL/kg bw) to five male and five female rats. The application period was 24-hours. The animals were examined daily for mortality, viability, and clinical signs. Body weights were recorded on Days 0 (prior to administration), 7, and 14. No deaths occurred during the study. No clinical signs nor body weight changes were observed during the course of the study. No macroscopic findings were observed at necropsy. The Glyphosate Technical (NUP 05068) dermal LD_{50} (rat) is

LD_{50}, dermal, rat > 2000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate Technical (NUP 05068)
Description:	Solid
Lot/Batch #:	200609062
Purity:	95.1 %
Stability of test compound:	Stable under storage conditions.

2. Vehicle and/or positive control: Polyethylene glycol 300 (PEG 300)

3. Test animals:

Species:	Rat
Strain:	HanRcc:WIST (SPF)
Source:	[redacted]
Age:	Males: 8 weeks; females: 11 weeks
Sex:	Males and females
Weight at dosing:	Males: 233.0 – 260.2 g; females: 194.8 – 216.4 g
Acclimation period:	6 days

Diet/Food:	Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet, batch no. 67/06 (Provimi Kliba AG, CH-4303 Kaiseraugst, Switzerland), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in Makrolon type-3 cages with standard softwood bedding ("Lignocel", Schill AG, CH-4132 Muttenz, Switzerland)
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 30 – 70 % Air changes: 10 – 15 / hour Photoperiod: 12-hour light / dark cycle

B: Study design and methods

In life dates: 2006-12-19 to 2007-01-02 (Start of treatment to final sacrifice)

Animal assignment and treatment:

One day before treatment, the backs of the animals were clipped with an electric clipper, exposing an area of approximately 10 % of the total body surface. Only those animals without injury or irritation on the skin were used in the test. On Day 0, the test item was applied at a dose of 2000 mg/kg bw with an application volume of 6 mL/kg bw in vehicle (PEG 300) on the intact skin and covered with a semi-occlusive dressing. The dressing was wrapped around the abdomen and fixed with an elastic adhesive bandage. Twenty-four hours after the application, the dressing was removed, and the skin was flushed with lukewarm tap water and dried with disposable paper towels. Animals were examined for clinical signs at approximately 30 minutes, 1, 2, 3 and 5 hours after treatment on Day 0 and once daily during Days 1 – 14. Local signs were noted once daily from Day 1 – 14. Mortality was recorded at approximately 30 minutes, 1, 2, 3 and 5 hours after administration on Day 0 and twice daily during Days 1 – 14. Body weights were recorded on Day 0 prior to administration and on Days 7 and 14. The fur of all animals was shaved on Days 3 and 8 just after the assessment of the reaction to facilitate the skin reading for the next day. All animals were sacrificed at the end of the observation and examined macroscopically.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

No systemic or local signs of toxicity were observed during the study period.

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance.

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute dermal LD_{50} of the test material (glyphosate technical) after a single dermal administration to

male and female Wistar rats, observed over a period of 14 days was greater than 2000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD 402 (2017). Therefore, the outcome can be reported as valid. The acute dermal LD_{50} is above 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point	CA 5.2.2/009
Report author	[redacted]
Report year	2007
Report title	Glyphosate technical material: Acute dermal toxicity study in rats
Report No	B02766
Document No	Not reported
Guidelines followed in study	OECD 402 (1987), US EPA OPPTS 870.1200 (1998), JMAFF 12 NohSan 8147 (2000)
Deviations from current test guideline (OECD 402, 2017)	A group of one rat per sex and a second group of four rats per sex were treated in a sequential manner, instead of two animals, preferred females, per dose. Weight of three female animals was outside of the range specified in the guideline (200 – 300 g).
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

In an acute dermal toxicity study, a group of one male and one female and a second group of four male and four female HanRcc:WIST (SPF) rats were treated with glyphosate technical material (96.1 % w/w glyphosate technical) at 5000 mg/kg bw by dermal application. The test item was moistened with purified water before application. The application period was 24-hours.

The animals were examined daily during the acclimatization period and mortality, viability, and clinical signs were recorded. All animals were examined for clinical signs once at approximately 30 minutes, 1, 2, 3 and 5 hours after treatment on Day 0 and once daily during Days 1 to 14. Local signs were noted once daily from Day 1 to 14. Mortality/viability was recorded once at approximately 30 minutes, 1, 2, 3 and 5 hours after treatment on Day 0 (with the clinical signs) and twice daily during Days 1 – 14. Body weights were recorded on Day 0 (prior to administration) and on Days 7 and 14. All animals were necropsied and examined macroscopically.

No deaths occurred and no systemic signs or local signs of irritation were noted during the course of the

study. The body weights of the animals were within the range commonly recorded for this strain and age. No macroscopic findings were observed at necropsy.

The acute dermal LD_{50} of glyphosate technical material after a single dermal administration to male and female HanRcc:WIST (SPF) rats, observed over a period of 14 days was

LD_{50}, dermal, rat > 5000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate technical material
Description:	White powder
Lot/Batch number:	0507
Purity:	96.1 % w/w
Stability of test compound:	Re-analysis date August 2008. Stable under storage conditions (room temperature range 20±5 °C, protected from light and humidity).

2. Vehicle and/or positive control: Purified water

3. Test animals:

Species:	Rat
Strain:	HanRcc:WIST (SPF)
Source:	[redacted]
Sex:	Males and females
Age:	Males: 8 weeks; females: 11 weeks
Weight at dosing:	Males: 222.7 – 247.0 g; females: 191.3 – 204.2 g
Acclimation period:	7 days
Diet/Food:	Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet (Provimi Kliba AG, CH-4303 Kaiseraugst, Switzerland), *ad libitum*
Water:	Community tap water, *ad libitum*
Housing:	Individually in Makrolon type-3 cages with standard softwood bedding ("Lignocel", Schill AG, CH-4132 Muttenz, Switzerland)
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 30 – 70 % Air changes: 10 – 15 air changes per hour Photoperiod: 12-hour light / dark cycle

B: Study design and methods

In life dates: 2006-12-07 to 2006-12-22 (Start of treatment to final sacrifice)

Animal assignment and treatment:

A group of one male and one female and a second group of four male and four female HanRcc:WIST (SPF) rats were treated with glyphosate technical material (96.1 % w/w glyphosate technical) at 5000 mg/kg bw by dermal application. One day before treatment, the backs of the animals were clipped with an electric clipper, exposing an area of approximately 10 % of the total body surface. Only those animals without

injury or irritation on the skin were used in the test. The test item was moistened with purified water before application (0.5 – 0.6 mL). The dry paste was applied evenly on the intact skin of the clipped area and covered with a semi-occlusive dressing. The dressing was wrapped around the abdomen and anchored with tape. The area of skin covered by the test item was approximately 8 cm^2 for the males and females. Twenty-four hours after application the dressing was removed, and the skin was flushed with lukewarm tap water and dried with disposable paper towels. Thereafter, the reaction sites were assessed.

A single animal of each sex was treated first. As no deaths, severe local effects, or severe systemic symptoms were observed after the 24-hour exposure, the test was completed using the four remaining male and female animals for an exposure period of 24-hours.

The fur of all males and females was shaved, on Day 5 (female no. 2), on Days 4 and 8 (male no. 1), on Days 3 and 7 (males nos. 3 – 6 and females nos. 7 – 10) just after the assessment of the reaction to facilitate the skin reading for the next day. The animals were checked daily for mortality/viability during the acclimatization period, at approximately 30 minutes, 1, 2, 3 and 5 hours after administration on Day 0 (with the clinical signs) and twice daily during Days 1 to 14. Clinical observations were recorded daily during the acclimatisation period, at approximately 30 minutes, 1, 2, 3 and 5 hours after administration on Day 0 and once daily during Days 1 – 14. The animals were examined daily for local signs at the application site. Body weights were recorded on Days 0 (prior to administration), 7, and 14. All animals were sacrificed at the end of the observation period and macroscopic examinations were performed. No organs or tissues were retained.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities.

B. CLINICAL OBSERVATIONS
No systemic signs or local signs of irritation were noted during the course of the study.

C. BODY WEIGHT
The body weights of the animals were within the range commonly recorded for this strain and age.

D. NECROPSY
No macroscopic findings were recorded at the scheduled necropsy.

III. CONCLUSIONS

The acute dermal LD_{50} of glyphosate technical after a single dermal administration to male and female Wistar rats, observed over a period of 14 days was greater than 5000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD 402 (2017). Therefore, the outcome can be reported as valid. The acute dermal LD50 is above 5000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point	CA 5.2.2/010
Report author	[redacted]
Report year	2005
Report title	Glyphosate Acid Technical: Acute Dermal Toxicity Study in Rats – Limit Test
Report No	15275
Document No	Not reported
Guidelines followed in study	OECD 402 (1987), US EPA OPPTS 870.1200 (1998), JMAFF 59 NohSan No. 4200 (1985)
Deviations from current test guideline (OECD 402, 2017)	No stepwise approach was used, five rats of both sexes instead of two animals, preferred females, were used per dose. Weight of female animals (193 – 200 g) was outside of the range specified in the guideline (200 – 300 g).
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The test substance, glyphosate acid technical, was evaluated for its acute dermal toxicity potential in rats when administered as single dose of 5000 mg/kg bw. No mortality occurred during the study and no clinical signs or skin reactions were observed. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. Hence, the acute dermal LD_{50} was determined to be

LD_{50}, dermal, rat > 5000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate Acid Technical
Description:	White crystalline powder
Lot/Batch #:	040205
Purity:	97.23 %
Stability of test compound:	Test substance was expected to be stable for the duration of testing.

2. Vehicle and/or positive control: Distilled water

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley derived, albino

Source:	[redacted]
Age:	8 weeks
Sex:	Males and females
Weight at dosing:	Males: 231 – 264 g; females: 193 – 200 g
Acclimation period:	8 days
Diet/Food:	Purina Rodent Chow #5012, *ad libitum*
Water:	Filtered tap water, *ad libitum*
Housing:	Individual housing in suspended stainless steel cages with mesh floors. Litter paper was placed beneath the cage and was changed at least three times per week.
Environmental conditions:	Temperature: 19 – 23 °C
	Humidity: Not reported
	Air changes: Not reported
	Photoperiod: 12-hour light/ dark cycle

B: Study design and methods

In life dates: 2004-05-05 to 2004-05-19

Animal assignment and treatment:

One dose level group of five male and five female rats was examined in a limit test. The dose level of 5000 mg/kg bw was applied once as a dry paste (50 % w/w mixture in distilled water) for 24-hours on the shaved intact dorsal skin of the rats (approximately 10 % of total body surface). The test substance was held in contact with the skin with a gauze pad and Durapore tape. After 24-hours exposure the pads were removed and the test sites were gently cleansed of any residual test substance. Observations for mortality and clinical/behavioural signs of toxicity were made during the first several hours post-dosing and at least once daily thereafter for 14 days after dosing. Individual body weights were recorded just prior to dosing and on Days 7 and 14. On Day 14 after dosing, each animal was sacrificed. All study animals were subjected to gross necropsy and all abnormalities were recorded.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

A single dermal administration of 5000 mg/kg bw to five male and five female rats did not reveal any clinical signs of toxicity. No skin reactions were observed.

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance.

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute dermal LD_{50} of glyphosate acid technical after a single dermal administration to male and female SD rats, observed over a period of 14 days was greater than 5000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD 402 (2017). Therefore, the outcome can be reported as valid. The acute dermal LD_{50} is above 5000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point	CA 5.2.2/011
Report author	[redacted]
Report year	1996
Report title	Glyphosate Acid: Acute Dermal Toxicity in the Rat
Report No	[redacted]/P/4664
Document No	Not reported
Guidelines followed in study	OECD 402 (1987), US EPA 81-2, EC method B.3. (92/69/EEC, 1992)
Deviations from current test guideline (OECD 402, 2017)	No stepwise approach was used, five rats of both sexes instead of two animals, preferred females, were used per dose. Age of animals was not reported.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

In an acute dermal toxicity study, a group of five male and five female, young adult Alpk:AP_fSD (Wistar-derived) rats were given a single dermal application of 2000 mg/kg bw of glyphosate acid in deionised water and observed for 14 days.

No mortality occurred during the study. There were no significant signs of systemic toxicity and practically no signs of skin irritation. One male showed slight erythema on Days 1 and 2, and one female had small scabs from Day 2 to 7. Most animals had exceeded their initial weight by the end of the study. There were no treatment-related findings at examination *post mortem*.

The acute dermal LD_{50} of glyphosate acid was determined to be

LD_{50}, dermal, rat > 2000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate acid
Description:	White solid
Lot/Batch number:	P24
Purity:	95.6 % w/w
Stability of test compound:	The test substance was used within the expiry date.

2. Vehicle and/or positive control: Deionised water

3. Test animals:

Species:	Rat
Strain:	Alpk:AP$_f$SD (Wistar-derived)
Source:	[redacted]
Sex:	Males and females
Age:	Young adult
Weight at dosing:	Males: 250 – 274 g; females: 203 – 216 g
Acclimation period:	At least 6 days
Diet/Food:	Diet (PCD), supplied by Special Diet Services Limited, Witham, Essex, UK, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in multiple rat racks suitable for animals of this strain and the weight range expected during the course of the study.
Environmental conditions:	Temperature: 21 ± 2 °C Humidity: 40 – 70 % Air changes: Approximately 25 – 30 / hour Photoperiod: 12-hour light / dark cycle

B: Study design and methods

In life dates: 1995-03-16 to 1996-03-30

Animal assignment and treatment:

A group of five male and five female, young adult Alpk:AP$_f$SD (Wistar-derived) rats were given a single dermal application of 2000 mg/kg bw of glyphosate acid. Sixteen to 32 hours before application, the hair was removed by clipping from an area on the dorso-lumbar region of each rat (approximately 10 cm × 5 cm). The appropriate amount of test substance was moistened to a dry paste with 0.6 - 0.8 mL of deionised water. On the day of treatment (Day 0), approximately half the application site was covered by test substance (equivalent to 20.0 – 21.9 mg/cm^2 for males and 16.2 – 17.3 mg/cm^2 for females). The application site was covered with a 4-ply gauze patch (approximately 7 cm × 7 cm) and kept in place for 24-hours using an occlusive dressing. The gauze patch was covered by a piece of plastic film (7 cm × 7 cm), held in place using adhesive bandage (25 cm × 7.5 cm) secured by two pieces of PVC tape. At the end of the 24-hour contact period, the dressings were carefully removed and the skin cleansed of any residual test substance using clean warm water.

Prior to the start of the study, all rats were examined to ensure that they were physically normal and exhibited normal activity. The animals were observed for signs of systemic toxicity once between one and four hours of dosing. Subsequent observations were made daily, up to Day 14.

The animals were weighed immediately before dosing (Day 0) and on Days 2, 4, 7 and 14. All animals were subjected to an examination *post mortem*. This involved an external observation and a careful examination of all thoracic and abdominal viscera.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities.

B. CLINICAL OBSERVATIONS

There were no significant signs of systemic toxicity (only urinary incontinence in three of five females due to bandaging, which is not considered to be of toxicological significance). The skin of all animals was stained cream by the test substance, but this did not prevent the assessment of erythema. There were practically no signs of skin irritation. One male showed slight erythema on Days 1 and 2, and one female had small scabs from Day 2 to 7 (see table below).

Table 5.2.2-8 Glyphosate Acid: Acute Dermal Toxicity study in the rat ([redacted] 1996): Skin observation data

Dose level		2000 mg/kg bw								
Day		**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8 – 14**
Sex	**Skin observations**									
M	Skin stained	(0/5)	(5/5)	(5/5)	(5/5)	(5/5)	(5/5)	(5/5)	(0/5)	(0/5)
	Erythema	(0/5)	1 (1/5)	1 (1/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)
	Scabs	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)
F	Skin stained	(0/5)	(5/5)	(5/5)	(5/5)	(1/5)	(1/5)	(1/5)	(0/5)	(0/5)
	Erythema	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)
	Scabs	(0/5)	(0/5)	2 (1/5)	2 (1/5)	2 (1/5)	2 (1/5)	2 (1/5)	2 (1/5)	(0/5)
	Signs of urinary incontenence	(0/5)	(3/5)	(3/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)

1 = slight erythema, 2 = small scattered

C. BODY WEIGHT

Two males and three females lost weight initially, but all had exceeded their initial weight by Day 4, and except for one female, continued to gain weight throughout the remainder of the study. One female lost weight slightly from Day 4. A summary of the body weights is given in the table below.

Table 5.2.2-9 Glyphosate Acid: Acute Dermal Toxicity study in the rat ([redacted] 1996): Body weight and body weight gain

Dose level	**2000 mg/kg bw**											
Sex	**Males**					**Females**					**Males**	**Females**
Day	**0**	**2**	**4**	**7**	**14**	**0**	**2**	**4**	**7**	**14**	**0 – 14**	**0 – 14**
Animal No.§	**Body weight [g]**										**Body weight gain [g]**	
6 M (51 F)	250	248	274	297	337	205	205	209	233	246	87	41

Table 5.2.2-9 Glyphosate Acid: Acute Dermal Toxicity study in the rat [redacted] 1996): Body weight and body weight gain

7 M (52 F)	253	257	282	305	353	216	197	218	217	215	100	71
8 M (53 F)	274	274	291	314	352	211	214	222	247	258	78	47
9 M (54 F)	262	268	286	306	346	207	205	216	230	236	84	29
10 M (55 F)	256	252	270	286	327	203	201	204	228	232	74	29
Mean	**259.0**	**259.8**	**280.6**	**301.6**	**343.0**	**208.4**	**204.4**	**213.8**	**231.0**	**237.4**	**84.0**	**29.0**
± SD	**± 9.5**	**± 10.9**	**± 8.6**	**± 10.6**	**± 11.0**	**± 5.2**	**± 6.3**	**± 7.2**	**± 10.8**	**± 16.1**	**± 10.8**	**± 18.5**

§ = animal No. of males and (females)
Note: Body weight gain values were not given in the study report. Values were calculated retrospectively.

D. NECROPSY

Red mottled lungs were seen in one male. This is a common spontaneous finding in rats of this age and strain and is considered not to be treatment-related (see table below).

Table 5.2.2-10 Glyphosate Acid: Acute Dermal Toxicity study in the rat ([redacted], 1996): Necropsy data

Dose level	**2000 mg/kg bw**	
Sex	**Males**	**Females**
Animals examined	5	5
NAD	4	5
Lung		
- mottled: (moderate) red	1§	0

NAD = Number of animals without abnormalities
§ Note that this finding is described as occurring for a female in the text of the report, but indicated for animal No. 10, a male, in Table 4 of the report.

III. CONCLUSIONS

The acute dermal LD_{50} of glyphosate acid after a single dermal administration to male and female Wistar rats, observed over a period of 14 days was greater than 2000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD 402 (2017). Therefore, the outcome can be reported as valid. The acute dermal LD_{50} is above 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point	CA 5.2.2/012
Report author	[redacted]
Report year	1995
Report title	HR-001: Acute dermal toxicity study in rats
Report No	94-0154
Document No	Not reported
Guidelines followed in study	OECD 402 (1987), US EPA FIFRA Guideline Subdivision F Acute Dermal Toxicity 81-2 (1984), JMAFF 59 NohSan No. 4200 (1985)
Deviations from current test guideline (OECD 402, 2017)	No stepwise approach was used, five rats of both sexes instead of two animals, preferred females, were used per dose. Age of rats (7 weeks) and weight (178 – 198 g) of female animals was outside of the range specified in the guideline (at least 8 – 10 weeks and 200 – 300 g, respectively). No observation during the first 30 minutes after dosing.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Glyphosate technical was singly administered to specific pathogen free SD rats (Crj:CD) in order to investigate its acute dermal toxicity at a dose level of 2000 mg/kg bw using five males and five females. There were no deaths in either sex. Neither clinical signs nor gross abnormalities at necropsy were noted in any animals. No body weight losses were recorded at 7 and 14 days after the administration when compared with the body weights of the day of administration. Based on the results mentioned above, the acute dermal LD_{50} was determined to be

LD_{50}, dermal, rat > 2000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate technical (Code HR-001)
Description:	White crystal
Lot/Batch #:	940908-1
Purity:	95.68 %
Stability of test compound:	No data given in the report.

2. Vehicle and/or positive control:

Deionised water

3. Test animals:

Species:	Rat
Strain:	Specific pathogen free SD rats (Crj:CD)
Source:	[redacted]
Age:	7 weeks
Sex:	Males and females
Weight at dosing:	Males: 248 – 268 g; females: 178 – 198 g
Acclimation period:	9 days
Diet/Food:	Certified Pellet Diet MF (Oriental Yeast Co., Tokyo, Japan), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individual housing in suspended, wire mesh bottom, stainless steel cages
Environmental conditions:	Temperature: 24.0 - 24.5 °C Humidity: 53 – 55 % Air changes: 12 times/hour Photoperiod: 12-hour light/dark cycle (light 7:00 AM to 7:00 PM)

B: Study design and methods

In life dates: 1995-02-09 – 1995-02-23 (Start of treatment to final sacrifice)

Animal assignment and treatment:

The test material was finely ground with a mortal and pestle, moistened with deionised water, and applied onto the shaved skin of five male and five female specific pathogen free SD rats (Crj:CD) at a dose level of 2000 mg/kg bw by a closed patch for 24-hours. A negative control group received 0.5 mL of deionised water by the same procedure. After removal of closed patch, the test substance remaining in contact with the skin site was washed off with lukewarm water. Mortality and clinical signs were recorded 1, 3, and 6 hours after administration and at least once daily thereafter until the termination of the 14-day observation period. All animals were weighed on the day of administration and on Days 7 and 14 after administration. The surviving animals were sacrificed after completion of the observation period (Day 14) and examined for gross abnormalities. All animals were subjected to necropsy.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

No clinical signs were noted in any animals of control and 2000 mg/kg bw groups.

C. BODY WEIGHT

All animals gained their body weights on Days 7 and 14 after administration.

D. NECROPSY

There was no macroscopic abnormality in any surviving animals at final necropsy after completion of the observation period.

III. CONCLUSIONS

The acute dermal LD_{50} of glyphosate technical after a single dermal administration to male and female SD rats, observed over a period of 14 days was greater than 2000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD 402 (2017). Therefore, the outcome can be reported as valid. The acute dermal LD_{50} is above 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point	CA 5.2.2/013
Report author	[redacted]
Report year	1995
Report title	Final report for "Oral and dermal LD50 tests with Sanachem Glyphosate acid technical in rats, limit test"
Report No	00917
Document No	Not reported
Guidelines followed in study	OECD 402 (1987), US EPA 81-2 (1984)
Deviations from current test guideline (OECD 402, 2017)	No stepwise approach was used, five rats of both sexes instead of two animals, preferred females, were used per dose. Age of animals was not reported. Body weight of male animals was outside of the range specified in the guideline (200 – 300 g). Body weights were only recorded once prior to start of study and not once a week during the study. Time of observation on the day of application was not specified according to the test guideline. Individual animal data were not provided.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The acute dermal toxicity potential of the test material, glyphosate acid technical (Batch: 1073; Purity: 97.6 %), was investigated in groups of five male and five female Sprague-Dawley rats. The test substance was applied at doses of 2000 mg/kg bw (limit test) to clipped dorsal and ventral skin using cotton seed oil

as a vehicle and covered by occlusive dressing for 24-hours. After exposure residual test substance was removed with water and the rats were observed for 14 days.

Mortality and clinical signs of toxicity were monitored on the day of administration and daily thereafter. All animals were subjected to gross necropsy.

No mortality and clinical signs occurred during the study. Gross necropsy revealed splenomegaly and centrilobular hepatic congestion. The acute dermal LD_{50} was

LD_{50}, dermal, rat > 2000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate acid technical
Description:	Fine white granular powder
Lot/Batch #:	1073
Purity:	97.6 % (certificate of analysis)
Stability of test compound:	Expiry date: August 1996

2. Vehicle and/or positive control: Cotton seed oil (500 mg/mL)

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	Young adult (not further specified)
Sex:	Male and female
Weight at dosing:	Males: 150.2 – 237.2 g; females: 205.6 – 260.9 g
Acclimation period:	At least 5 days
Diet/Food:	Rodent pelleted feed, *ad libitum*
Water:	Not specified, *ad libitum*
Housing:	Groups of 5/sex in standard rodent polycarbonate cages
Environmental conditions:	Temperature: 19 – 21 °C Humidity: 62 – 73 % Air changes: Not specified Photocycle: 12-hour light / dark cycle

B: Study design and methods

Study dates: 1995-02-23 to 1995-03-13

Animal assignment and treatment:

Approximately 24-hours before the application of the test material, the fur was removed by clipping the dorsal and ventral area of five male and five female rats.

The test substance as a dilution in cotton seed oil was administered dermally (approximately 10 % of the

total body surface area) in a single application at a dose level of 2000 mg/kg bw (limit test). The treatment volume ranged between 0.73 and 1.10 mL, spread in a thin uniform film. The test substance was held in contact with the skin with a porous gauze dressing and non-irritating tape. The test site was further covered by Elastoplast to retain the gauze dressing and to ensure that animals could not ingest the test substance. After 24-hours the treated skin was wiped with water to remove any residual test material.

Observations for mortality and clinical signs of toxicity were made immediately (1 – 2 hours) after treatment, and thereafter at least once a day. The animals were sacrificed at the end of the 14-day observation period and subjected to a gross necropsy.

II. RESULTS AND DISUSSION

A. MORTALITY
No mortality occurred.

B. CLINICAL OBSERVATIONS
No clinical signs of toxicity were observed during the 14–day observation period.

C. BODY WEIGHT
Body weights were not reported.

D. NECROPSY
Splenomegaly and centrilobular hepatic congestion were observed in male and female animals.

III. CONCLUSIONS

The acute dermal LD_{50} of glyphosate acid technical after a single dermal administration to male and female SD rats, observed over a period of 14 days was greater than 2000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
Except to minor deviations, the study is in accordance to the OECD 402 (2017). Therefore, the study is considered acceptable and the outcome can be reported as valid. The acute dermal LD_{50} is above 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point	CA 5.2.2/014
Report author	[redacted]
Report year	1995
Report title	Final report for "Oral and dermal LD_{50} tests with Sanachem Glyphosate 62 % IPA in rats, limit test"
Report No	00926
Document No	Not reported
Guidelines followed in study	OECD 402 (1987), US EPA 81-2 (1984)
Deviations from current test guideline (OECD 402, 2017)	No stepwise approach was used, five rats of both sexes instead of two animals, preferred females, were used per dose. Age of animals was not reported. Body weight of male and female animals was outside of the range specified in the guideline (200 – 300 g). Body weights were only recorded once prior to start of study and not once a week during the study. Time of observation on the day of application was not specified according to the test guideline. Individual animal data were not provided.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The acute dermal toxicity potential of the test material, glyphosate salt (Batch: 940950; Purity: 61.8 %), was investigated in five male and five female Sprague-Dawley rats. The test substance was applied undiluted to clipped dorsal and ventral skin at doses of 2000 mg/kg bw (limit test) and covered by occlusive dressing for 24-hours. After exposure the test substance was removed using water and the rats were observed for 14 days.

Mortality and clinical signs of toxicity were monitored on the day of administration and daily thereafter. All animals were subjected to gross necropsy.

No mortality and clinical symptoms occurred during the study. The gross necropsy revealed severe lung congestion, splenomegaly, hepatomegaly with centrilobular congestion, and subcapsular renal petechiae in all animals. The acute dermal LD_{50} was

LD_{50}, dermal, rat > 2000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification: Glyphosate 62 % IPA
Description: Light greenish viscous liquid
Lot/Batch #: 940950

Purity:	61.8 %
Stability of test compound:	Expiry date: August 1996

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	Young adult (not further specified)
Sex:	Male and female
Weight at dosing:	Male: 153.0 – 212.2 g; female: 197.2 – 248.7 g
Acclimation period:	At least 5 days
Diet/Food:	Pelleted feed, *ad libitum*
Water:	Not specified, *ad libitum*
Housing:	Groups of 5/sex in standard rodent polycarbonate cages
Environmental conditions:	Temperature: 19 – 21 °C Humidity: 62 – 73 % Air changes: Not specified Photocycle: 12-hour light / dark cycle

B: Study design and methods

Study dates: 1995-02-23 to 1995-03-13

Animal assignment and treatment:

Approximately 24-hours before the application of the test material, the fur was removed by clipping the dorsal and ventral area of five male and five female rats.

The undiluted test substance was administered dermally (approximately 10 % of the total body surface area) in a single application at a dose level of 2000 mg/kg bw. The treatment volume ranged between 0.59 and 0.78 mL. The test substance was held in contact with the skin with a porous gauze dressing and non-irritating tape. The test site was further covered by Elastoplast to retain the gauze dressing and to ensure that animals could not ingest the test substance. After 24-hours the treated skin was wiped with water to remove any residual test material.

Observations for mortality and clinical signs of toxicity were made immediately (1 – 2 hours) after treatment, and thereafter at least once a day. The animals were sacrificed at the end of the 14–day observation period and subjected to a gross necropsy.

II. RESULTS AND DISUSSION

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of toxicity were observed during the 14–day observation period.

C. BODY WEIGHT

Body weights were not reported.

D. NECROPSY

Severe lung congestion, splenomegaly, hepatomegaly with centrilobular congestion, and subcapsular renal petechiae were observed in all male and female animals.

III. CONCLUSIONS

The acute dermal LD_{50} of glyphosate salt after a single dermal administration to male and female SD rats, observed over a period of 14 days was greater than 2000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
Except to minor deviations, the study is in accordance to the OECD 402 (2017). Therefore, the study is considered acceptable and the outcome can be reported as valid. The acute dermal LD_{50} is above 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

2. Information on the study

Data point	CA 5.2.2/015
Report author	[redacted]
Report year	1994
Report title	Acute dermal toxicity of glyphosate technical in the rat
Report No	T1586.3.A
Document No	Not reported
Guidelines followed in study	No final conclusion possible.
GLP	No final conclusion possible.
Previous evaluation	Yes, accepted in RAR (2015)
Short description of study design and observations	Purity of test compound: 95 % Vehicle: suspended (50 % w/w) in natrosol (1 % w/w in water) Test animals: 5 rats (Sprague-Dawley)/sex Dose level: 2000 mg/kg bw
Short description of results	No findings LD_{50} >2000 mg/kg bw (limit test)
Reasons for why the study is not considered relevant/reliable or not considered as key study	Full study report is not available to the applicant. Only a short summary is provided in the Monograph, B5, 2002, *Category 4b*
Reasons why the study report is not available for submission	The notifier has not access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance" (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL
Category study in AIR 5 dossier (L docs)	Category 4b

1. Information on the study

Data point	[redacted]
Report author	Snell, K.
Report year	1994
Report title	Glyphosate: Acute dermal toxicity (limit test) in the rat
Report No	710/15
Document No	Not reported
Guidelines followed in study	No final conclusion possible.
GLP	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Short description of study design and observations	Purity of test compound: 95 % Vehicle: none Test animals: 5 rats (Sprague-Dawley) /sex Dose level: 2000 mg/kg bw
Short description of results	No findings LD_{50} >2000 mg/kg bw (limit test)
Reasons for why the study is not considered relevant/reliable or not considered as key study	Full study report is not available to the applicant. Only a short summary is provided in the Monograph, B5, 2002, *Category 4b*
Reasons why the study report is not available for submission	The notifier has not access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance" (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL
Category study in AIR 5 dossier (L docs)	Category 4b

1. Information on the study

Data point	CA 5.2.2/017
Report author	[redacted]
Report year	1994
Report title	Glyphosate (Alkaloida, Tiszavaszári): Acute Dermal Toxicity in Rats
Report No	GHA-94-402/R
Document No	Not reported
Guidelines followed in study	OECD 402 (1981)
Deviations from current test guideline (OECD 402, 2017)	No stepwise approach was used, five rats of both sexes instead of two animals, preferred females, were used per dose. Additional five rats per sex served as a control group. Age of animals was not reported. Body weight of female animals was outside of the range specified in the guideline (200 – 300 g). Size of exposed skin area and the amount of vehicle used was not reported.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The acute dermal toxicity potential of the test material, glyphosate technical (Batch: 36300892; Purity: 99.6 %), was investigated in five male and five female LATI/Wistar rats per dose group. The test substance was applied at doses of 0 and 2000 mg/kg bw (limit test) to clipped dorsal skin and covered by occlusive dressing for 24-hours. After exposure the test substance was removed with water and the rats were observed for 14 days.

Mortality and clinical signs of toxicity were monitored on the day of administration and daily thereafter. All animals were subjected to gross necropsy.

No mortality and clinical symptoms occurred during the study. No differences were noted in body weight. The gross necropsy demonstrated no gross changes attributable to the test material. The acute dermal LD_{50} was

LD_{50}, dermal, rat > 2000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate technical
Description:	White or almost white crystalline powder
Lot/Batch #:	36300892
Purity:	99.6 %
Stability of test compound:	Expiry date: 1994-09-01

2. Vehicle and/or positive control: Water

3. Test animals:

Species:	Rat
Strain:	LATI/Wistar
Source:	[redacted]
Age:	Not specified
Sex:	Male and female
Weight at dosing:	Male: 210 – 215 g; female: 150 – 155 g
Acclimation period:	22 days
Diet/Food:	Altromin rodent chow, *ad libitum*
Water:	Daily changed tap water in bottles, *ad libitum*
Housing:	Macrolon III. box
Environmental conditions:	Temperature: 20 ± 2 °C Humidity: 45 – 70 % Air changes: 10 / hour Photocycle: 12-hour light / dark cycle

B: Study design and methods

In life dates: 1994-01-19 to 1994-02-02

Animal assignment and treatment:

Before the application of the test material, the fur was removed by clipping the dorsal skin of five male and five female rats.

The test substance was administered dermally in a single application at a dose level of 0 and 2000 mg/kg bw (limit test) at a concentration of 33.3 % in an aqueous solution. The test substance was held in contact with the skin by means of non-absorbent binder of polyethylene.

After 24-hours, the binders were removed, and the treated skin was wiped with water to remove any residual test material.

Observations for mortality and clinical signs of toxicity were made immediately after dosing, at 1 and 4 hours postdose, and once daily thereafter for 14 consecutive days. The animals were sacrificed at the end of the 14-day observation period. Gross changes in the thoracic and abdominal organs as well as selected organ weights were recorded.

II. RESULTS AND DISUSSION

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of toxicity were observed during the 14–day observation period.

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance.

D. NECROPSY

No gross changes attributable to the test material were observed in treated and control animals. The absolute and relative organ weights revealed no differences attributable to the test material.

III. CONCLUSIONS

The acute dermal LD_{50} of glyphosate technical after a single dermal administration to male and female Wistar rats, observed over a period of 14 days was greater than 2000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
Except to minor deviations, the study is in accordance to the OECD 402 (2017). Therefore, the study is considered acceptable and the outcome can be reported as valid. The acute dermal LD_{50} is greater than 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point	CA 5.2.2/018
Report author	[redacted]
Report year	1992
Report title	Glyphosate technical: Acute dermal toxicity (limit test) in the rat
Report No	134/38
Document No	Not reported
Guidelines followed in study	OECD 402 (1981)
Deviations from current test guideline (OECD 402, 2017)	No stepwise approach was used, five rats of both sexes instead of two animals, preferred females, were used per dose. Purity of test substance was not reported.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The acute dermal toxicity potential of the test material, glyphosate technical, was investigated in five male and five female Sprague-Dawley rats per dose. The test substance was applied to clipped dorsal skin at doses of 2000 mg/kg bw. After 24-hours exposure duration the test substance was removed and the rats were observed for 14 days. No mortality, clinical symptoms and local skin reactions occurred during the study, and the gross necropsy conducted at termination of the study demonstrated no observable abnormalities. All animals showed expected gain in body weight. The acute dermal LD_{50} was

LD_{50}, dermal, rat > 2000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate technical
Description:	White powder
Lot/Batch #:	L3258
Purity:	Not specified
Stability of test compound:	Not specified

2. Vehicle and/or positive control: Distilled water

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	10 – 14 weeks

Sex:	Males and females
Weight at dosing:	Males: 219 – 247 g; females: 200 – 215 g
Acclimation period:	At least 5 days
Diet/Food:	Rat and Mouse Expanded Diet No. 1 (Special Diet Services Limited, Witham, Essex, UK, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Solid-floor polypropylene cages with softwood sawdust bedding; individually housed during the exposure. For the remainder of the study, housed five animals per sex per cage in polypropylene grid-floor cages suspended over trays lined with absorbent paper.
Environmental conditions:	Temperature: 19 – 22 °C Humidity: 52 – 63 % Air changes: 15 per hour Photoperiod: 12 hour light / dark cycle

B: Study design and methods

In life dates: 1992-01-06 to 1992-01-20

Animal assignment and treatment:

On the day before application of the test material, a group of five male and five female rats per dose was prepared by clipping the backs and flanks free of hair (approximately 10 % of the total body surface area).

Glyphosate technical was administered dermally in a single application under semi-occlusion at a dose level of 2000 mg/kg bw. On Day 0 the undiluted test material was applied onto the shorn skin of rats, previously moisted with distilled water, surgical gauze was placed over the treatment area, and the area was semi-occluded with a piece of self-adhesive bandage (HYPERTIE). The bandage was further secured with a piece of BLENDERM wrapped around each end.

After 24-hours the bandage was removed and the treated skin was wiped with cotton wool moistened with distilled water to remove any residual test material.

Observations for mortality and clinical signs of toxicity were made 0.5, 1, 2 and 4 hours after dosing and subsequently once daily for 14 days. The rats were weighed immediately prior to dosing, 7 and 14 days after dosing. The animals were sacrificed at the end of the 14-day observation period and subjected to a gross necropsy.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of toxicity were observed during the 14 days observation period. No treatment related skin irritation was observed in any animal throughout the study.

C. BODY WEIGHT

All animals gained weight over the study period (see table below).

Table 5.2.2-11 Glyphosate technical: Acute Dermal Toxicity (Limit test) in the Rat ([redacted] 1992): Mean body weight

Dose level	2000 mg/kg bw									
Sex	Males			Females			Males		Females	
Day	0	7	14	0	7	14	0 – 7	0 – 14	0 – 7	0 – 14
	Body weight [g]						Body weight gain [g]			
Mean	233.8	283.8	334.0	208.0	225.8	246.8	50.0	100.2	17.8	38.8
± SD	± 11.3	± 19.6	± 27.5	± 6.6	± 8.3	± 9.6	± 15.0	± 21.0	± 5.9	± 7.7

Note: Body weight and body weight gain values were not given in the study report. Values were calculated retrospectively.

D. NECROPSY

No abnormalities were observed during necropsy.

III. CONCLUSIONS

The acute dermal LD_{50} of glyphosate technical after a single dermal administration to male and female SD rats, observed over a period of 14 days was greater than 2000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in accordance to the OECD 402 (2017). Therefore, the outcome can be reported as valid. The acute dermal LD_{50} is above 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point	CA 5.2.2/019
Report author	[redacted]
Report year	1991
Report title	Acute dermal toxicity study with glyphosate technical (FSG 03090 h/05 March 90) in Wistar rats
Report No	ES.876.ADR
Document No	Not reported
Guidelines followed in study	OECD 402 (1987)
Deviations from current test guideline (OECD 402, 2017)	No stepwise approach was used, five rats of both sexes instead of two animals, preferred females, were used per dose. Two high doses (above limit dose of 2000 mg/kg bw) were selected (2500 and 5000 mg/kg bw). Weight of male (150 - 200 g) and female animals (150 - 180 g) was outside of the range specified in the guideline (200 - 300 g). Time of observation on the day of application was not specified according to the test guideline.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes

Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The acute dermal toxicity potential of the test material, glyphosate technical, was investigated in five male and five female Wistar rats per dose. The test substance was applied to clipped dorsal skin at doses of 2500 and 5000 mg/kg bw. After 24-hours exposure duration the test substance was removed and the rats were observed for 14 days. No mortality, clinical symptoms and local skin reactions occurred during the study, and the gross necropsy conducted at termination of the study demonstrated no observable abnormalities. Some of the animals showed body weight losses. The acute dermal LD_{50} was

LD_{50}, dermal, rat > 5000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate Technical
Description:	Solid white coloured crystals, odourless
Lot/Batch #:	60
Purity:	96.8 %
Stability of test compound:	More than two years (declared), expiry date: July 1992

2. Vehicle and/or positive control: Distilled water

3. Test animals:

Species:	Rat
Strain:	Wistar
Source:	Toxicology Department. [redacted]
Age:	14 weeks
Sex:	Males and females
Weight at dosing:	Males: 150 – 200 g; females: 150 – 1 80 g
Acclimation period:	At least 1 week
Diet/Food:	Standard 'Gold Mohur' brand pelleted rat feed (M/s Lipton India Ltd., Bangalore, India), *ad libitum*
Water:	Deep bore well water passed through activated charcoal filter and exposed to UV rays, *ad libitum*
Housing:	Individual polypropylene cages with steam sterilized clean paddy husk bedding
Environmental conditions:	Temperature: 23 ± 2 °C Humidity: 68 ± 6 % Air changes: 10 – 15 / hour Photoperiod: 12-hour light / dark cycle

B: Study design and methods

In life dates: October 1990 (no details given)

Animal assignment and treatment:

Approximately 24-hours before application of the test material, a group of five male and five female rats per dose was prepared by clipping the backs free of hair (approximately 10 % of the total body surface area).

Glyphosate Technical was administered dermally in a single application under occlusion at a dose level of 2500 and 5000 mg/kg bw. On test day one (Day 0) the test material was made into a slurry with distilled water on aluminium foil (non-occlusive covering). The test material preparation along with foil was applied to the prepared area of the skin of rats and fixed with tape. After 24-hours the tape was removed and the skin was rinsed with luke warm water, washed with 1 % Labklin, and rinsed with luke warm water. Skin was wiped dry with a cotton hand towel.

Observations for mortality and clinical signs of toxicity were made four times during Day 0 and daily during Days 1 – 14. The rats were weighed immediately prior to dosing, 7 and 14 days after dosing. The animals were sacrificed at the end of the 14-day observation period and subjected to a gross necropsy.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

Single dermal administrations of 2500 or 5000 mg/kg bw to five male and five female rats did not reveal any clinical signs of toxicity. No skin reactions were observed.

C. BODY WEIGHT

Body weight gains, with the exception of some animals which lost weight, were acceptable. One rat in the 2500 mg/kg bw dose group and five rats in 5000 mg/kg bw dose group had lost body weight at Day 7. At Day 14 one rat each in 2500 and 5000 mg/kg bw dose group had lost body weight, while the remaining rats had gained body weight. A summary of the body weights is given in the table below.

Table 5.2.2-12 Acute Dermal Toxicity Study with glyphosate technical (FSG 03090 h/05 March 90) in Wistar rats ([redacted] 1991): Body weight and body weight gain

Dose level	**2500 mg/kg bw**							
Sex	**Males**			**Females**			**Males**	**Females**
Day	**0**	**7**	**14**	**0**	**7**	**14**	**0 – 14**	**0 – 14**
Animal No.§	**Body weight [g]**						**Body weight gain [g]**	
41 M (46 F)	190	192	216	184	188	180	26	-4
42 M (47 F)	150	150	166	168	172	184	16	16
43 M (48 F)	180	184	194	182	184	200	14	18
44 M (49 F)	200	222	252	170	174	174	52	4
45 M (50 F)	170	160	176	166	170	188	6	22
Mean	**178.0**	**181.6**	**200.8**	**174.0**	**177.6**	**185.2**	**22.8**	**11.2**
± SD	**± 19.2**	**± 28.3**	**± 34.4**	**± 8.4**	**± 7.9**	**± 9.8**	**± 17.8**	**± 10.8**
Dose level	**5000 mg/kg bw**							
Sex	**Males**			**Females**			**Males**	**Females**
Day	**0**	**7**	**14**	**0**	**7**	**14**	**0 – 14**	**0 – 14**

Table 5.2.2-12 Acute Dermal Toxicity Study with glyphosate technical (FSG 03090 h/05 March 90) in Wistar rats [redacted] 1991): Body weight and body weight gain

Animal No.§	Body weight [g]						Body weight gain [g]	
51 M (56 F)	154	140	170	156	160	164	16	8
52 M (57 F)	178	184	210	154	164	170	32	16
53 M (58 F)	174	154	180	170	172	180	6	10
54 M (59 F)	182	178	192	176	172	180	10	4
55 M (60 F)	174	174	190	170	160	168	16	-2
Mean	**172.4**	**166.0**	**188.4**	**165.2**	**165.6**	**172.4**	**16.0**	**7.2**
± SD	**± 10.8**	**± 18.4**	**± 14.9**	**± 9.7**	**± 6.1**	**± 7.3**	**± 9.9**	**± 6.7**

§ = animal No. of males and (females). All animal numbers are preceeded by "A 03", and have been truncated to the last two digits in this table (i.e. animal A 0341 is listed as 41 in this table).
Note: Mean and standard deviation as well as body weight gain data were not given in the study report. Values were calculated retrospectively.

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute dermal LD_{50} of glyphosate technical after a single dermal administration to male and female Wistar rats, observed over a period of 14 days was greater than 5000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD 402 (2017). Therefore, the outcome can be reported as valid. The acute dermal LD_{50} is above 5000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point	CA 5.2.2/020
Report author	[redacted]
Report year	1990
Report title	Acute dermal toxicity study in the rat: Glyphosate technical.
Report No	AGC-900823A
Document No	Not reported
Guidelines followed in study	OECD 402 (1981), EC method B3 (84/449/EEC, 1984)
Deviations from current test guideline (OECD 402, 2019)	No stepwise approach was used, five rats of both sexes instead of two animals, preferred females, were used per dose. Three high doses (above limit dose) were selected. Age of animals was not reported. Body weight is not within the interval of ± 20 % of the mean weight.

	Temperature was in the range of 13 – 25 °C instead of 22 ± 3 °C. Individual animal data were not provided for some endpoints.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The acute dermal toxicity potential of the test material, glyphosate technical, was investigated following single application to groups of 10 rats (five males, five females). The test substance was applied to shaved skin at doses of 3000, 5000 and 8000 mg/kg bw. A dry gauze pad was used as the control substance. After 24-hours contact period the test substance was removed and the rats were observed for 14 days. There were no mortality, clinical signs of toxicity, or necropsy findings. Changes of body weight were in the normal range. The acute dermal LD_{50} was

LD_{50}, dermal, rat > 8000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate Technical
Description:	Not specified
Lot/Batch #:	0190 A
Purity:	98.1 %
Stability of test compound:	Stable at room temperature (declared)

2. Vehicle and/or positive control: 0.9 % saline

3. Test animals:

Species:	Rat
Strain:	CD
Source:	[redacted]
Age:	Young adults (not further specified)
Sex:	Males and females (nulliparous and non-pregnant)
Weight at dosing:	Male: 220 – 301 g; female: 200 – 225 g
Acclimation period:	5 days
Diet/Food:	Standard rat diet pellets (Redmills, Goresbridge, Co. Kilkenny, Ireland), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individual polypropylene cages with stainless steel lids and autorclaved shaving bedding

Environmental conditions:	Temperature:	13 – 25 °C
	Humidity:	43 – 63 %
	Air changes:	Not specified
	Photoperiod:	12-hour light / dark cycle

B: Study design and methods

In life dates: 1990-03-15 to 1990-08-23

Animal assignment and treatment:

Approximately 24-hours before application of the test material, a group of five male and five female rats per dose group was prepared by shaving the backs free of hair. Only animals with healthy intact skin were used.

Glyphosate technical was administered by direct application to the skin at dose levels of 3000, 5000 and 8000 mg/kg bw. Each animal received a single administration of glyphosate technical. The test material was moistened using 0.9 % saline and applied to the gauze pad. The gauze pad was held in place by an adhesive tape covered with self-sealing plastic. A final covering was made with adhesive tape which was wrapped completely around the rat. After 24-hours the dressing was removed and residual test substance was washed off with water. A dry gauze pad was applied to the control group which was otherwise treated identically to the test group.

Observations for mortality and clinical signs of toxicity were made 20 – 30 minutes, 60 – 70 minutes and 3 – 3.5 hours post dosing and daily thereafter. For each animal body weight was recorded before dosing (Day 0), on Day 7 and on Day 14. The animals were sacrifice at the end of the 14-day observation period and subjected to gross necropsy.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

Single dermal administrations of 3000, 5000 and 8000 mg/kg bw to five male and five female rats did not reveal any clinical signs of toxicity.

C. BODY WEIGHT

All animals gained their body weights on Days 7 and 14 after administration. Thus, body weight gain was unaffected by the administration of the test substance.

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute dermal LD_{50} of glyphosate technical after a single dermal administration to male and female CD rats, observed over a period of 14 days was greater than 8000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD 402 (2017). Therefore, the outcome can be reported as valid. The acute dermal LD_{50} is above 8000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point	CA 5.2.2/021
Report author	[redacted]
Report year	1989
Report title	Glyphosate Technical: Acute dermal toxicity (limit) test in rats
Report No	5884
Document No	Not reported
Guidelines followed in study	OECD, EEC, EPA[1]
Deviations from current test guideline (OECD 402, 2017)	No stepwise approach was used, five rats of both sexes instead of two animals, preferred females, were used per dose. Purity of test substance as well as amount of vehicle used (i.e. water) were not reported. From the batch number a purity of 98.6 % was concluded. Weight of female animals was outside of the range specified in the guideline (200 – 300 g).
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

[1] Guideline numbers are not specified in the report, however the study is compliant with OECD 402, EEC B3, and EPA 81-2 with the exception of a slightly different test item application procedure.

2. Full summary

The acute dermal toxicity potential of the test material, glyphosate technical, was investigated in five male and five female Sprague-Dawley rats. The test substance was administered dermally in a single application under occlusion at a dose level of 2000 mg/kg bw. Clinical signs noted at 30 minutes to 1 day after dosing included piloerection and reduced activity. Scab formation was noted at the test sites 2 – 14 days after dosing. No mortality occurred during the study, and the gross necropsy conducted at termination of the study demonstrated no observable abnormalities. Body weight gains with the exception of one animal, which lost weight, were acceptable. The acute dermal LD_{50} was

LD_{50}, dermal, rat > 2000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate technical (PMG)
Description:	White powder
Lot/Batch #:	206-Jak-25-1
Purity:	Not specified in the study report Batch 206-JaK-25-1 reported with 98.6 %, see [redacted] 1991(see CA 5.2.1/25).
Stability of test compound:	No data given in the report

2. Vehicle and/or positive control: Water

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	8 - 10 weeks
Sex:	Males and females (nulliparous and non-pregnant)
Weight at dosing:	Males: 212 - 240 g; females: 188 - 234 g
Acclimation period:	6 days
Diet/Food:	Expanded Rat and Mouse Maintenance Diet (Special Diets Services), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Polypropylene cages with mesh floors in groups of five animals per sex per cage; individually housed during exposure (24-hours)
Environmental conditions:	Temperature: 19 °C and 22 °C (min. and max. average) Humidity: 49 % (average) Air changes: Not specified Photoperiod: 12-hour light/dark cycle (light 07:00 – 19:00)

B: Study design and methods

In life dates: 1989-06-06 to 1989-06-21 (Start of treatment to study completion)

Animal assignment and treatment:

A group of five male and five female rats was prepared by clipping the backs free of hair, approximately 24-hours before application of the test material. Care was taken to avoid abrading the skin. Glyphosate technical was administered dermally in a single application under occlusion at a dose level of 2000 mg/kg bw.

The test material, moistened with water, was applied evenly onto gauze dressing, which was applied to the shaved back of each rat. At least 10 % of the body surface was in contact with the test material. The trunk of the rat was then encircled with a strip of non-irritating tape. After 24-hours the tape was removed, and the skin was wiped with a water-dampened tissue to remove excess test material.

The rats were observed frequently on the day of dosing and once daily for 14 days following dosing. They were weighed immediately prior to dosing, 7 days after dosing and at sacrifice at the end of the 14-day observation period.

At the end of the observation period and sacrifice by carbon dioxide asphyxiation, each animal was subjected to a gross post mortem examination.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

Clinical signs noted 30 minutes to 1 day after dosing included piloerection and reduced activity. Scab formation was noted at test sites 2 to 14 days after dosing (see table below).

Table 5.2.2-13 Glyphosate technical: Acute Dermal Toxicity (limit) test in Rats ([redacted], 1989): Clinical observation data

Sex	Observation	Animals affected / Time (after dosing)								
		1 m	30 m	1 h	2 h	1 d	2 d	3 d	4 d	5 - 14 d
Males	NAD	5/5					5/5	5/5	5/5	5/5
	Pilorection		5/5	5/5	5/5	5/5				
	Reduced activity		5/5	5/5	5/5					
	Scab formation						1/5*	1/5*	1/5*	1/5*
Females	NAD	5/5					5/5	5/5	5/5	5/5
	Pilorection		5/5	5/5	5/5	5/5				
	Reduced activity		5/5	5/5	5/5					
	Scab formation						2/5*	2/5*	2/5*	2/5*

NAD = No abnormalities detected; m = minute; h = hour; d = day
* = Animals NAD but scab formation noted at test sites

C. BODY WEIGHT

Body weight gains with the exception of one animal, which lost weight, were acceptable. A summary of the body weights is given in the table below.

Table 5.2.2-14 Glyphosate technical: Acute Dermal Toxicity (limit) test in Rats ([redacted]., 1989): Body weight and body weight gain

Dose level	2000 mg/kg bw							
Sex	Males			Females			Males	Females
Day	0	7	14	0	7	14	0 - 14	0 - 14
Animal No.§	Body weight [g]						Body weight gain [g]	
11 M (16 F)	232	268	300	234	259	229	68	-5
12 M (17 F)	240	289	336	188	213	278	96	90
13 M (18 F)	212	252	283	204	216	243	71	39
14 M (19 F)	237	285	313	207	226	248	76	41
15 M (20 F)	224	271	320	208	227	241	96	33
Mean	**229**	**273**	**310**	**208**	**228**	**248**	**81**	**40**
± SD	**± 11**	**± 15**	**± 20**	**± 17**	**± 18**	**± 18**	**± 14**	**± 34**

§ = animal No. of males and (females)

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute dermal LD_{50} of glyphosate technical after a single dermal administration to male and female SD rats, observed over a period of 14 days was greater than 2000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD 402 (2017). Therefore, the outcome can be reported as valid. The acute dermal LD_{50} is above 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point	CA 5.2.2/022
Report author	[redacted]
Report year	1989
Report title	Acute dermal toxicity study with glyphosate technical (isopropylamine salt 62 % in water equivalent to 46 % of Nphosphonomethylglycine acid) in rats
Report No	238061
Document No	[redacted]425
Guidelines followed in study	No final conclusion possible.
GLP	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Short description of study design and observations	Purity of test compound: 62 % (IPA) Vehicle: none Test animals: 5 rats (Wistar)/sex Dose level: 2000 mg/kg bw
Short description of results	Erythema maculate (1 ♂), scales (1 ♀) LD_{50} > 2000 mg/kg bw (limit test)
Reasons for why the study is not considered relevant/reliable or not considered as key study	Full study report is not available to the applicant. Only a short summary is provided in the Monograph, B5, 2000, *Category 4b*
Reasons why the study report is not available for submission	The notifier has not access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance" (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL.
Category study in AIR 5 dossier (L docs)	Category 4b

1. Information on the study

Data point	CA 5.2.2/023
Report author	[redacted]
Report year	1988
Report title	Acute dermal toxicity study of glyphosate batch/lot/NBR No. XLI-55 in New Zealand White rabbits
Report No	88.2053.008
Document No	Not reported
Guidelines followed in study	US EPA Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals [Acute Dermal Toxicity, 81-2] (1984)
Deviations from current test guideline (OECD 402, 2017)	No stepwise approach was used, five rabbits of both sexes instead of two animals, preferred female rats, were used per dose. High dose (above suggested limit dose of 2000 mg/kg bw) was selected. Age of animals was not reported. Even though there was just one obvious abnormality, individual animal data showing necropsy findings were not reported.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The acute dermal toxicity potential of the test material glyphosate was investigated following single application to 10 rabbits (five males, five females). The test substance was applied to clipped skin at a dose of 5000 mg/kg bw. After 24-hours contact period, the test substance was removed and the rabbits were observed for 14 days. One female rabbit was found dead on Day 13. The female that died exhibited diarrhea and anorexia. No internal abnormalities were noted during gross examination. Therefore, this death was not considered treatment related. Further, anorexia (one male, one female), diarrhea (one male, one female) and soft stool (one female) were noted in the animals which survived to study termination. Test substance application caused no adverse effect on mean body weight in either sex. One male had a white caseous substance adhered to the lungs, which was not considered treatment-related. The acute dermal LD_{50} was

LD_{50}, dermal, rabbit > 5000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate
Description:	White powder
Lot/Batch #:	XLI-55
Purity:	97.76 %
Stability of test compound:	Not specified

2. Vehicle and/or positive control: Physiological saline

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Young adults (not further specified)
Sex:	Males and females (nulliparous and non-pregnant)
Weight at dosing:	Males: 2.36 – 2.49 kg; females: 2.34 – 2.97 kg
Acclimation period:	At least 5 days
Diet/Food:	NIH 09 Rabbit Ration, certified feed (Zeigler Brothers, Inc., Gardners, PA, US), *ad libitum*
Water:	Fresh tap water, *ad libitum*
Housing:	Individual wire mesh cages
Environmental conditions:	Temperature: 20 – 23.9 °C Humidity: 40 – 60 % Air changes: Not specified Photoperiod: 12-hour light / dark cycle

B: Study design and methods

In life dates: 1988-04-11 to 1988-04-25

Animal assignment and treatment:

Approximately 24-hours before application of the test material, five male and five female rabbits were prepared by clipping the backs (from the shoulders to the hindquarters, approximately 10 % of the total body surface) free of hair. Only animals with healthy intact skin were used.

Glyphosate was moistened with physiological saline (approximately 1 mL/g of test substance) and applied topically to the clipped area at a dose level of 5000 mg/kg bw. Each test site was covered with gauze and occluded with a layer of plastic wrap and stockinette sleeve held in place with tape. After 24-hours the binders were removed and the exposure sites were gently wiped with gauze.

Observations for mortality and clinical signs of toxicity were made three times on the day of administration and twice daily thereafter. For each animal body weight was recorded before dosing (Day 0), on Day 7 and on Day 14. The animals were sacrifice at the end of the 14-day observation period and subjected to gross necropsy.

II. RESULTS AND DISCUSSION

A. MORTALITY

One female rabbit was found dead on Day 13 following test substance administration. All other rabbits survived to study termination on Day 14.

B. CLINICAL OBSERVATIONS

The female rabbit that died exhibited diarrhea and/or anorexia on Days 8 – 12. These observations are consistent with mucoid enteropathy, a condition occasionally noted in stock laboratory rabbits. Anorexia (one male, one female), diarrhea (one male, one female) and soft stool (one female) were noted in the

animals that survived to study termination. Clinical observations are summarised in the table below.

Table 5.2.2-15: **Acute Dermal Toxicity Study of Glyphosate batch/lot/NBR No. XLI-55 in New Zealand White rabbits ([redacted], 1988): Clinical observation data**

Dose level (mg/kg bw)	5000	
Sex	**Males**	**Females**
Clinical observation	**Incidence***	
Anorexia	1/5[a] (Day 1 – 8)	1/5 (Day 8 – 12)
Diarrhea	1/5 (Day 5 – 8)	1/5 (Day 9 – 12)
Soft stools	-	1/5 (Day 11, 12) 1/4 (Day 13)

* = number of observed clinical signs/number of surviving animals, point in time (day) in parenthesis
- = not observed

C. BODY WEIGHT

All surviving animals maintained or gained weight over the study period with the exception of one male rabbit, which had slight weight loss. A summary of the body weights is given in the table below.

Table 5.2.2-16 Acute Dermal Toxicity Study of Glyphosate batch/lot/NBR No. XLI-55 in New Zealand White rabbits ([redacted] 1988): Body weight and body weight gain

Dose level	5000 mg/kg bw							
Sex	**Males**			**Females**			**Males**	**Females**
Day	**0**	**7**	**14**	**0**	**7**	**14**	**0 – 14**	**0 – 14**
Animal No.[§]	**Body weight [g]**						**Body weight gain [g]**	
1169 (1245)	2.49	2.69	2.40	2.34	2.49	2.64	-0.09	0.30
1173 (1247)	2.46	2.60	2.75	2.60	2.71	2.77	0.29	0.17
1174 (1250)	2.44	2.51	2.69	2.74	3.05	3.21	0.25	0.47
1175 (1251)	2.38	2.36	2.61	2.69	2.73	2.89	0.23	0.20
1184 (1254)	2.36	2.07	2.43	2.97	2.82	-[a]	0.07	-
Mean	**2.43**	**2.45**	**2.58**	**2.67**	**2.76**	**2.88**	**0.15**	**0.29**
± SD	**± 0.05**	**± 0.24**	**± 0.16**	**± 0.23**	**± 0.20**	**± 0.24**	**± 0.16**	**± 0.14**

§ = animal No. of males and (females)
a = Animal found dead on Day 13 (final body weight 2.45 kg).
Note: Body weight gain data were not given in the study report. Values were calculated retrospectively.

D. NECROPSY

No abnormalities were noted during gross necropsy examination of the rabbit that died. One male rabbit that was sacrificed at study termination had a white caseous substance adhered to the lungs. In the study report this finding is not discussed. Nevertheless, rabbits have been observed to develop a disease showing caseous necrosis after bacterial infections. The finding is not considered treatment-related.

III. CONCLUSIONS

The acute dermal LD_{50} of glyphosate technical after a single dermal administration to male and female New Zealand White rabbits, observed over a period of 14 days was greater than 5000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD 402 (2017). Therefore, the outcome can be reported as valid. The acute dermal LD_{50} is above 5000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point	CA 5.2.2/024
Report author	[redacted]
Report year	1987
Report title	Acute dermal toxicity study of MON 8722 in New Zealand White rabbits
Report No	9307A
Document No	Not reported
Guidelines followed in study	US EPA Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals [Acute Dermal Toxicity, 81-2] (1982)
Deviations from current test guideline (OECD 402, 2017)	No stepwise approach was used, five rabbits of both sexes instead of two animals, preferred female rats, were used per dose. High dose (above suggested limit dose of 2000 mg/kg bw) was selected. Age of animals was not reported. Observation time on the day of application not specified as recommended in the test guideline. Even though there were only a few sporadic clinical observations and no obvious abnormalities, individual animal data showing clinical signs of toxicity findings and time course of onset were not reported.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The acute dermal toxicity potential of the test material MON 8722 was investigated following single application to 10 rabbits (five males and five females). The test substance was applied to clipped skin at a dose of 5000 mg/kg bw. After 24-hours contact period the test substance was removed and the rabbits were observed for 14 days. No mortality occurred. Soft stools were transiently noted in one animal per sex. Test substance administration did not produce an adverse effect in weight gain. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute dermal LD_{50} was

LD_{50}, dermal, rabbit > 5000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	MON 8722
Description:	White powder
Lot/Batch #:	XLG-256
Purity:	70.7 %
Stability of test compound:	Not specified

2. Vehicle and/or positive control: Physiological saline

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Young adults (not further specified)
Sex:	Males and females (multiparous and non-pregnant)
Weight at dosing:	Males: 2.31 – 2.64 kg; females: 2.18 – 2.67 kg
Acclimation period:	At least 5 days
Diet/Food:	NIH 09 Rabbit Ration, certified feed (Zeigler Brothers, Inc., Gardners, PA, US), *ad libitum*
Water:	Fresh tap water, *ad libitum*
Housing:	Individual wire mesh cages
Environmental conditions:	Temperature: 20 – 23.9 °C Humidity: 40 – 60 % Air changes: Not specified Photoperiod: 12-hour light / dark cycle

B: Study design and methods

In life dates: 1986-11-07 to 1986-11-21

Animal assignment and treatment:

Approximately 24-hours before application of the test material, five male and five female rabbits were prepared by clipping the backs (from the shoulders to the hindquarters, approximately 10 % of the total body surface) free of hair. Only animals with healthy intact skin were used.

MON 8750 was mixed with physiological saline (1 mL/g) and applied topically to the clipped area at a dose level of 5000 mg/kg bw. Each test site was wrapped with occlusive binders consisting of a layer of plastic wrap and stockinette sleeve held in place with the tape. After 24-hours the binders were removed and the exposure sites were gently wiped with gauze.

Observations for mortality and clinical signs of toxicity were made frequently on the day of administration and twice daily thereafter. For each animal body weight was recorded before dosing (Day 0), on Day 7 and on Day 14. The animals were sacrificed at the end of the 14-day observation period and subjected to gross necropsy.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortality occurred. All rabbits survived to study termination on Day 14.

B. CLINICAL OBSERVATIONS

Soft stools were transiently noted in one animal per sex. All animals appeared normal at study termination.

C. BODY WEIGHT

Body weight development was not affected by the treatment in either sex.

D. NECROPSY

No macroscopic pathologic abnormalities were noted during gross necropsy examination in any animal.

III. CONCLUSIONS

The acute dermal LD_{50} of glyphosate salt after a single dermal administration to male and female New Zealand White rabbits, observed over a period of 14 days was greater than 5000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD 402 (2017). Therefore, the outcome can be reported as valid. The acute dermal LD_{50} is above 5000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point	CA 5.2.2/025
Report author	[redacted]
Report year	1987
Report title	Acute dermal toxicity study of MON-8750 in New Zealand White rabbits
Report No	9308A
Document No	Not reported
Guidelines followed in study	US EPA OPP 81-2, Acute Dermal Toxicity (1982)
Deviations from current test guideline (OECD 402, 2017)	No stepwise approach was used, five rabbits of both sexes instead of two animals, preferred female rats, were used per dose. High dose (above suggested limit dose of 2000 mg/kg bw) was selected. Age of animals was not reported. Observation time on the day of application not specified as recommended in the test guideline. Even though there were only a few sporadic clinical observations and no obvious abnormalities, individual animal data showing clinical signs of toxicity findings and time course of onset were not reported.

Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The acute dermal toxicity potential of the test material MON 8750 was investigated following single application to 10 rabbits (five males, five females). The test substance was applied to clipped skin at a dose of 5000 mg/kg bw. After 24-hours contact period, the test substance was removed and the rabbits were observed for 14 days. One female rabbit was found dead on Day 3, however, this death was not considered treatment related. The female that died exhibited anorexia and decreased activity. Further, diarrhea and soft stools were noted in three male rabbits and three female rabbits. All surviving animals maintained or gained weight over the study period, with the exception of two males that had slight weight loss. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute dermal LD_{50} was

LD_{50}, dermal, rabbit > 5000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	MON 8750
Description:	White powder
Lot/Batch #:	XLG-255
Purity:	90.8 %
Stability of test compound:	Not specified

2. Vehicle and/or positive control: Physiological saline

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Young adults (not further specified)
Sex:	Males and females (nulliparous and non-pregnant)
Weight at dosing:	Males: 2.26 - 2.57 kg; females: 2.20 - 2.59 kg
Acclimation period:	At least 5 days
Diet/Food:	NIH 09 Rabbit Ration, certified feed (Zeigler Brothers, Inc., Gardners, PA, US), *ad libitum*
Water:	Fresh tap water, *ad libitum*
Housing:	Individual wire mesh cages

Environmental conditions:	Temperature:	20 - 23.9 °C
	Humidity:	40 - 60 %
	Air changes:	Not specified
	Photoperiod:	12-hour light/dark cycle

B: Study design and methods

In life dates: 1986-11-07 to 1986-11-21

Animal assignment and treatment:

Approximately 24-hours before application of the test material, five male and five female rabbits were prepared by clipping the backs (from the shoulders to the hindquarters, approximately 10 % of the total body surface) free of hair. Only animals with healthy intact skin were used.

MON 8750 was mixed with physiological saline (1 mL/g) and applied topically to the clipped area at a dose level of 5000 mg/kg bw. Each test site was wrapped with occlusive binders consisting of a layer of plastic wrap and stockinette sleeve held in place with tape. After 24-hours, the binders were removed and the exposure sites were gently wiped with gauze.

Observations for mortality and clinical signs of toxicity were made frequently on the day of administration and twice daily thereafter. For each animal bodyweight was recorded before dosing (Day 0), on Day 7, and on Day 14. The animals were sacrifice at the end of the 14-day observation period and subjected to gross necropsy.

II. RESULTS AND DISCUSSION

A. MORTALITY

One female rabbit was found dead on Day 3. All other rabbits survived to study termination on Day 14.

B. CLINICAL OBSERVATIONS

The female rabbit that died exhibited anorexia and decreased activity prior to death. Diarrhea and soft stools were sporadically noted in three males and three females during the observation period.

C. BODY WEIGHT

All surviving animals maintained or gained weight over the study period with the exception of two male rabbits, which had slight weight loss. A summary of the body weights is given in Table 5.2.2-17.

Table 5.2.2-17 Acute Dermal Toxicity Study of MON-8750 in New Zealand White rabbits ([redacted]., 1987): Body weight and body weight gain

Dose level	5000 mg/kg bw							
Sex	Males			Females			Males	Females
Day	0	7	14	0	7	14	0 - 14	0 - 14
Animal No.[§]	Body weight [g]						Body weight gain [g]	
3325 (3400)	2.26	2.42	2.56	2.37	2.54	2.67	0.30	0.30
3326 (3401)	2.36	2.47	2.49	2.59	2.74	2.87	0.13	0.28
3327 (3402)	2.29	2.39	2.30	2.20	2.43	2.61	0.01	0.41
3328 (3403)	2.37	2.55	2.70	2.38	-[a]	-	0.33	-
3329 (3404)	2.57	2.58	2.56	2.48	2.53	2.76	-0.01	0.28
Mean	**2.37**	**2.48**	**2.52**	**2.40**	**2.56**	**2.73**	**0.15**	**0.32**

Table 5.2.2-17 Acute Dermal Toxicity Study of MON-8750 in New Zealand White rabbits ([redacted], 1987): Body weight and body weight gain

± SD	**± 0.12**	**± 0.08**	**± 0.15**	**± 0.14**	**± 0.13**	**± 0.11**	**± 0.16**	**± 0.06**

§ = animal No. of males and (females)
a = Animal found dead on Day 3
Note: Body weight gain data were not given in the study report. Values were calculated retrospectively.

D. NECROPSY

No abnormalities were noted during gross necropsy examination in any animal.

III. CONCLUSIONS

The acute dermal LD_{50} of glyphosate salt after a single dermal administration to male and female NEW Zealand White rabbits, observed over a period of 14 days was greater than 5000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in accordance with the current OECD 402 (2017). Therefore, the outcome can be reported as valid. The acute dermal LD_{50} is above 5000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point	CA 5.2.2/026
Report author	[redacted]
Report year	1983
Report title	The acute dermal toxicity (LD_{50}) to rabbits with glyphosate (tech) of Excel Industries Ltd.
Report No	Not reported
Document No	Not reported
Guidelines followed in study	None
Deviations from current test guideline (OECD 402, 2019)	Two rabbits of both sexes were used. The following information were not given: age of animals, housing conditions, size of exposed skin area (i.e. at least 10 % of total body surface area), type and amount of vehicle. Body weights of animals after administration of the test substance were not determined. Animals were not subjected to gross necropsy. Individual animal data showing signs of toxicity were not reported.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facility (GLP was not compulsory at the time the study was performed)
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

The acute dermal toxicity potential of the test material, glyphosate technical, was investigated following single application to four rabbits (two males, two females). The test substance was applied to shaved skin at a dose of 2000 mg/kg bw. After 24-hours contact period the test substance was removed and the rabbits were observed for 14 days. No mortality occurred. Slight erythema at the test site of treatment was observed for 24-hours. The acute dermal LD_{50} was

LD_{50}, dermal, rabbit > 2000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate Technical
Description:	White amorphous powder
Lot/Batch #:	R & D sample (8.7.83.)
Purity:	95 %
Stability of test compound:	Not specified

2. Vehicle and/ or positive control: No information provided

3. Test animals:

Species:	Rabbit
Strain:	Albino NWS
Source:	[redacted]
Age:	Not specified
Sex:	Males and females
Weight at dosing:	1.5 – 2.5 kg
Acclimation period:	Not specified
Diet/Food:	Lucerne grass, carrots, germinated grains with wheat bran
Water:	Not specified
Housing:	Individual
Environmental conditions:	Temperature: not specified Humidity: not specified Air changes: not specified Photoperiod: Regular lighting conditions

B: Study design and methods

In life dates: No information provided

Animal assignment and treatment:

Glyphosate technical was applied topically to shaved skin area of 2 female and 2 male rabbits at a dose level of 2000 mg/kg bw. Additional 2 rabbits per sex served as a control group. A gauze patch was secured over each treated area by means of adhesive tape. After 24-hours the patchs were removed and the test site was washed with warm water.

The animals were observed for 15 days for toxic symptoms and mortality.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

Single dermal administration of 2000 mg/kg bw to 2 male and 2 female rabbits did not reveal any clinical signs of toxicity. Slight erythema was observed at the site of treatment for 24-hours.

C. BODY WEIGHT

Body weights were not reported.

D. NECROPSY

No necropsy was conducted.

III. CONCLUSIONS

The acute dermal LD_{50} of glyphosate technical after single dermal administration to male and female Albino NWS rabbits, observed over a period of 15 days was estimated to be greater than 2000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
Due to deviations of the study and the fact that it was not performed according to any guideline and not according to GLP, the study provided supplementary information on acute dermal toxicity of glyphosate, only.
The available study report in pdf format was of poor quality, so that not all information could be finally verified.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point	CA 5.2.2/027
Report author	[redacted]
Report year	1981
Report title	Acute dermal toxicity of MON 0139 to rabbits
Report No	800258
Document No	Not reported
Guidelines followed in study	None
Deviations from current test guideline (OECD 402, 2017)	No stepwise approach was used, five rabbits of both sexes instead of two animals, preferred female rats, were used per dose. High dose (above suggested limit dose of 2000 mg/kg bw) was selected. The following data are not reported: Purity of the test material, age of animals, environmental conditions, type of diet, acclimation period, area of the skin surface covered with the test material, dosing volume, individual animal data (body weights, signs of toxicity, necropsy findings). The skin was abraded with a hypodermic needle prior to test material administration.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities (GLP was not compulsory at the time the study was performed)
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The acute dermal toxicity potential of the test material MON 0139 was investigated following single application to 10 rabbits (five males, five females). The test substance was applied to clipped and abraded skin at a dose of 5000 mg/kg bw. After 24-hours contact period the test substance was removed and the rabbits were observed for 14 days. No deaths were observed in animals of either sex. No clinical signs of toxicity were observed. At necropsy, one male animal had fur stained with diarrheal feces, which was not attributed to toxicity of the test material. The acute dermal LD_{50} was

LD_{50}, dermal, rabbit > 5000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	MON 0139
Description:	Amber liquid
Lot/Batch #:	SSRT-11012
Purity:	Not specified
Stability of test compound:	Not specified

2. Vehicle and/or positive control: none

3. Test animals:

Species:	Rabbit	
Strain:	New Zealand White (Isf: (NZW)	
Source:	[redacted]	
Age:	Young adult (not further specified)	
Sex:	Males and females	
Weight at dosing:	1.99 – 2.56 kg	
Acclimation period:	Not specified	
Diet/Food:	Not specified, *ad libitum*	
Water:	Not specified, *ad libitum*	
Housing:	Individually housed	
Environmental conditions:	Temperature:	not specified
	Humidity:	not specified
	Air changes:	not specified
	Photoperiod:	not specified

B: Study design and methods

In life dates: 1980-08-25 to 1980-09-08

Animal assignment and treatment:

Five male and five female rabbits were prepared by clipping the skin on the dorsal surface and abrading with a hypodermic needle. MON 0139 was applied topically to the dorsal surface at a dose level of 5000 mg/kg bw. The test material was held in place by means of an occlusive wrap of latex rubber secured by bandaging and elastic tape. After 24-hours the occlusive wraps were removed and the excess material was wiped from the animal.

Observations for mortality and clinical signs of toxicity were made three times during the first eight hours following test material administration and twice daily thereafter. For each animal body weight was recorded on the day of exposure (Day 0), on Day 7 and on Day 14. The animals were sacrifice at the end of the 14-day observation period and subjected to gross necropsy.

II. RESULTS AND DISCUSSION

A. MORTALITY

No deaths were observed in animals of either sex.

B. CLINICAL OBSERVATIONS

No clinical signs of toxicity were observed in any of the test animals during this study.

C. BODY WEIGHT

Mean body weight gains were acceptable.

D. NECROPSY

One male animal had fur stained with diarrheal feces. This was not attributed to toxicity of the test material. No abnormalities were noted for the remaining nine animals.

III. CONCLUSIONS

The acute dermal LD_{50} of glyphosate salt after a single dermal administration to male and female New Zealand White rabbits, observed over a period of 14 days was greater than 5000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
Due to the deviations of the study and the fact that it was not performed according to GLP, the study provides supplementary information on acute dermal toxicity of glyphosate, only.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

CA 5.2.3 Inhalation

The acute inhalation toxicity of glyphosate was evaluated in 22 studies performed in rats including 16 studies performed with glyphosate acid and six studies with glyphosate salts (IPA (isopropylamine), ammonium, and potassium salts).

In most of the studies, LC_{50} value was above the limit dose of 5 mg/L air or 2 mg/L air per 4 hours. In several studies the limit dose of 5 mg/L was difficult to achieve. Occasionally mortality was observed and occurred in three of 22 studies including two studies with glyphosate acid and one study with glyphosate salt. In the two studies performed with glyphosate acid, one male rat died at a dose level above 5 mg/L air (CA 5.2.3/001) and two males and two females died at a dose level of 4.43 mg/L air (CA 5.2.3/012), respectively. In one of the studies performed with glyphosate salt, one female rat died at the maximum attainable concentration of 1.3 mg/L air (CA 5.2.3/021).
Clinical signs were not consistently observed throughout the studies. In five of the 22 studies no findings were observed. Clinical signs observed included decreased activity (CA 5.2.3/001, CA 5.2.6/006, CA 5.2.3/011), loss of hair (CA 5.2.3/021), hunched posture (CA 5.2.3/005, CA 5.2.3/011, CA 5.2.3/014, CA 5.2.3/016), piloerection, ruffled/wet fur (CA 5.2.3/005, CA 5.2.3/006 CA 5.2.3/013, CA 5.2.3/014, CA 5.2.3/016, CA 5.2.3/019), slight tremor/dyspnoea/ataxia (CA 5.2.3/002 and CA 5.2.3/003, CA 5.2.3/004, CA 5.2.3/012), salivation (CA 5.2.3/008), ocular/nasal discharge or material around the eyes/nose (CA 5.2.3/010, CA 5.2.3/011, CA 5.2.3/013, CA 5.2.3/020, CA 5.2.3/021), increased respiratory rate (CA 5.2.3/001, CA 5.2.3/005), decreased respiratory rate (CA 5.2.3/014), congested or irregular breathing/breathing effects (CA 5.2.3/008, CA 5.2.3/010, CA 5.2.3/012) and a slight decrease in body weight (CA 5.2.3/008, CA 5.2.3/021). In one study performed with glyphosate salt (IPA), lung dark area or multiple dark foci were observed in several rats (CA 5.2.3/016).

In conclusion glyphosate and its salt is of low inhalation toxicity upon single application and does not need to be classified for acute oral toxicity according to the CLP Regulation (EU) No. 1272/2008.

Table 5.2.3-1: Studies on acute inhalation toxicity with glyphosate

Annex Point	Study	Study type	Substance(s)	Reference list- related category§	Result [LC_{50}]	Main effect
CA 5.2.3/001	[redacted], 2011	*in vivo:* Wistar RjHan:WI rats, ♂ / ♀, dose: 5.04 mg/L, 4 h nose-only exposure (MMAD: 3.65 µm)	Glyphosate technical (Purity: 96.9 %)	Valid, Category 2a	>5.04 mg/L	Mortality: 1♂ on day 4. Laboured and noisy respiration, respiratory rate increase, gasping respiration, sneezing, decreased activity and thin body appearance observed until day 3.
CA 5.2.3/002	[redacted], 2010	*in vivo:* CD/Crl:CD (SD) rats, ♂ / ♀, 4 h nose-only exposure (MMAD: 4.633 µm)	Glyphosate technical (Purity: 97.3 %)	Supportive#, Category 2a	>5.18 mg/L	Slight tremor and slight dyspnoea through 3 hours after exposure
CA 5.2.3/003	[redacted], 2010	*in vivo:* CD / Crl:CD(SD) rats, ♂ / ♀; 4 h nose-only exposure (MMAD: 4.197 µm)	Glyphosate technical (Purity: 96.4 %)	Supportive#, Category 2a	>5.02 mg/L	Slight ataxia, slight tremor and slight dyspnoea through 3 hours after exposure
CA 5.2.3/004	[redacted], 2009	*in vivo:* CD / Crl:CD(SD) rats, ♂ / ♀, 4 h nose-only exposure (No MMAD calculated)	Glyphosate technical (Purity: 98.8 %)	Supportive#, Category 2a	>5.12 mg/L	Slight dyspnoea and ataxia through 1 hours after exposure
CA 5.2.3/005	[redacted], 2009	*in vivo:* HsdRccHan™: WIST rats, ♂ / ♀, 4 h nose-only exposure (MMAD: 5.25 µm)	Glyphosate technical (Purity: 96.66 %)	Supportive, Category 2a	>5.04 mg/L	Increased respiratory rate, hunched posture, pilo-erection and wet fur through 1 hours after exposure
CA 5.2.3/006	[redacted], 2009	*in vivo:* Sprague-Dawley rats, ♂ / ♀, 4 h nose-only exposure (MMAD: 2.6 µm)	Glyphosate technical (Purity: 96.71 %)	Valid, Category 2a	>2.24 mg/L	Piloerection and activity decrease until day 4
CA 5.2.3/007	[redacted], 2008	*in vivo:* Wistar Hannover rats, ♂ / ♀, 4 h nose-only exposure (MMAD: 18.555-19.901 µm)	Glyphosate technical (Purity: 98.05 %)	Invalid, Category 3b	>5.211 mg/L	Wheeze and Dyspnoea until day 6
CA 5.2.3/008	[redacted], 2007	*in vivo:* HanRcc:WIST (SPF) rats, ♂ / ♀, 4 h nose-only exposure (MMAD: 2.95 – 3.05 µm)	Glyphosate technical (Purity: 95.1 %)	Valid, Category 2a	>3.252 mg/L, highest attainable concentration	Salivation in males, breathing effects through day 4 in both sexes; body weight loss

Table 5.2.3-1: Studies on acute inhalation toxicity with glyphosate

Annex Point	Study	Study type	Substance(s)	Reference list- related category§	Result [LC_{50}]	Main effect
CA 5.2.3/009	[redacted], 2005	*in vivo:* Sprague-Dawley derived rats, ♂ / ♀, 4 h nose-only exposure (MMAD: 2.5 µm)	Glyphosate technical (Purity: 97.23 %)	Valid, Category 2a	>2.04 mg/L	No findings
CA 5.2.3/010	[redacted], 2004	*in vivo:* Sprague Dawley rats, ♂ / ♀, 4 h nose-only exposure (MMAD: 2.9 and 3.8 µm)	Potassium salt of glyphosate technical (Purity: 57.8 % (= 47.2 % glypho-sate))##	Valid, Category 2a	>5.27 mg/L	2.21 mg/L: congested breathing, dark material around eyes and nose through day 7; 5.27 mg/L: congested breathing, few faeces through day 2
CA 5.2.3/011	[redacted], 1999	*in vivo:* Sprague-Dawley derived rats, ♂ / ♀, 4 h whole body exposure (MMAD: 2.6 µm)	Isopropylamine glyphosate (Purity: 62 %)##	Valid, Category 2a	>2.08 mg/L	During exposure: ocular and nasal discharge, hunched posture and hypoactivity. After exposure no findings
CA 5.2.3/012	[redacted], 1996	*in vivo:* Alpk:APfSD rats, ♂ / ♀, 4 h nose-only exposure (MMAD: 2.91, 3.41, 3.57, 3.03 µm)	Glyphosate technical (Purity: 95.6 %)	Valid, Category 2a	>4.43 mg/L	Mortality: 2♂ & 2♀ at 4.43 mg/L. Irregular breathing, splayed gait, shaking & reduced righting reflex
CA 5.2.3/013	[redacted], 1995	*in vivo:* SPF Fischer F344/DuCrj rats, ♂ / ♀, 4 h whole body exposure (MMAD: 4.8 µm)	Glyphosate technical (Purity: 97.56 %)	Supportive, Category 2a	>5.48 mg/L	Wet and soiled fur (periocular and nasorostral) through Day 5 after exposure
CA 5.2.3/014	[redacted], 1995	*in vivo:* Sprague-Dawley rats, ♂ / ♀, 4 h nose-only exposure (MMAD: 4.4 µm)	Glyphosate technical (Purity: 95 %)	Valid, Category 4a	>5.35 mg/L	Wet fur, hunched posture, piloerection, incidents of decreased respiratory rate, ptosis, brown stained fur (head) through Day 1 after exposure
CA 5.2.3/015	[redacted], 1994	*in vivo:* LATI/Wistar rats, ♂ / ♀, 4 h exposure route not stated (MMAD not measured)	Glyphosate technical (Purity: 97.2 %)	Supportive, Category 2a	>2.876 mg/m³ nominal	No findings
CA 5.2.3/016	[redacted], 1994	*in vivo:* Sprague-Dawley rats, ♂ / ♀, 4 h nose-only	Isopropylamine glyphosate (Purity:	Valid, Category 2a	>4.24 mg/L	Hunched posture, piloerection one hour after exposure.

Table 5.2.3-1: Studies on acute inhalation toxicity with glyphosate

Annex Point	Study	Study type	Substance(s)	Reference list- related category§	Result [LC_{50}]	Main effect
		exposure (MMAD: 1.1 µm)	62.2 %)##			Lung: dark areas or multiple dark foci in 6 of 10 rats
CA 5.2.3/017	[redacted], 1991	*in vivo:* Wistar rats, ♂ / ♀, 4 h whole body exposure (MMAD not measured)	Glyphosate technical (Purity: 96.8 %)	Supportive, Category 3a	>0.644 mg/L (maximum attainable concentration)	No findings
CA 5.2.3/018	[redacted] 1989	*in vivo:* Sprague-Dawley rats, ♂ / ♀, 4 h nose only exposure (MMAD not measured)	Glyphosate technical (Purity: 98.6###)	Supportive, Category 2a	>4.98 mg/L	No findings
CA 5.2.3/019	[redacted], 1989	*in vivo:* Wistar rats, ♂ / ♀, 4 h nose-only exposure (MMAD not measured)	Isopropylamine glyphosate (Purity: 62 %)##	Valid, Category 4a	>6.49 mg/L	Nose bleeding and ruffled fur
CA 5.2.3/020	[redacted], 1988	*in vivo:* Sprague-Dawley rats, ♂ / ♀, 4 h whole body exposure (MMAD: 4.2 µm)	Ammonium-glyphosate technical (MON-8750) (Purity: 85.5 %)##	Valid, Category 2a	>1.9 mg/L highest attainable concentration	Perinasal encrustation through Day 2 after exposure
CA 5.2.3/021	[redacted] 1987	*in vivo:* Sprague-Dawley rats, ♂ / ♀, 4 h whole body exposure (MMAD: 3.1 µm)	Isopropylamine glyphosate (Purity: 53.8 %)##	Supportive, Category 2a	>1.3 mg/L maximum attainable concentration	Mortality (1♀); yellow/brownnasal discharge, local and/or generalized hairloss, slightly decreased body weight through Day 2
CA 5.2.3/022	[redacted], 1983	*in vivo:* rats, ♂ / ♀, 4 h nose and mouth exposure (MMAD: 3.8-4.0 µm)	Glyphosate technical (Glycel 4l SL) (Purity: not specified)	Supportive, Category 3a	>4.5 mg/L	No findings

This study was performed at the [redacted]
This study was performed with a glyphosate salt
Purity provided in study report CA 5.2.1/024
§: The category describes the acceptability/reliability of the study within the AIR 5 submission. This category does not describe hazard classification, for details please refer to the Doc ID: 110054-B-GRG_Jun_2020)

1. Information on the study

Data point:	CA 5.2.3/001
Report author	[redacted]
Report year	2011
Report title	Glyphosate technical: Acute inhalation toxicity study (nose-only) in the rat
Report No	11/054-004P
Document No	Not reported

Guidelines followed in study	OECD 403 (2009): OPPTS 870.1300 (1998): 440/2008 B.2 (2008
Deviations from current test guideline	The humidity value deviated from required range (30 – 70 %) during the animal exposure. Test performed on 5 animals per sex. These deviations did not affect the study outcome.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

In an acute inhalation toxicity study, a group of young adult Wistar RjHan: (WI) strain rats, (5 males and 5 females) was exposed to a target aerosol concentration of 5 mg/L glyphosate technical (96.9 % w/w glyphosate technical). The animals were exposed for 4 hours using a nose-only exposure system, followed by a 14-day observation period. The day of exposure was designated Day 0. Aerosol concentrations were measured gravimetrically. The particle size distribution of the test aerosol was determined regularly during the exposure period.

Clinical observations and bodyweights were recorded throughout the study and at the end of the scheduled period the animals were killed and subjected to a gross examination *post mortem*.

The mean achieved atmosphere concentration was 5.04 mg/L. The MMAD (Mean Mass Aerodynamic Diameter) was 3.65 µm ± 2.24 (GSD [Geometric Standard Deviation]).

One male rat died following a 4-hour exposure to 5.04 mg/L Glyphosate Technical on Day 4.

Wet fur and fur staining were commonly recorded on the day of exposure and on the day after exposure. These observations were considered to be related to the restraint and exposure. Significant clinical signs were recorded on the day of exposure and the following day included laboured and noisy respiration, respiratory rate increase, gasping respiration, sneezing, activity decreased, thin body appearance (weak/wasted). The majority of the animals recovered from Day 3.

Normal bodyweight gain was noted for all surviving animals from Day 1, with the exception of one male where a slight bodyweight loss was recorded during the first week of the observation period.

No macroscopic findings were seen at necropsy. A specific cause of death was not determined for the single male that died.

Under the experimental conditions of this study, a single death occurred in a group of 10 rats exposed to a mean achieved atmosphere of 5.04 mg/L for 4 hours.

The acute LC_{50} of Glyphosate Technical in rats is therefore considered to be greater than 5.04 mg/L air.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate technical
Description:	Technical; dry white powder
Lot/Batch #:	614034 (20100609\Milled)
Purity:	96.9 % w/w Glyphosate technical
Stability of test compound:	Stable under storage conditions (room temperature range <30 °C), recertification date end January 2014

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rat
Strain:	Wistar RjHan:WI
Source:	[redacted]
Age:	8 - 10 weeks
Sex:	Males and females
Weight at dosing:	229 – 386 g
Acclimation period:	At least five days
Diet/Food:	ssniff® SM R/M-Z+H "Autoclavable complete feed for rats and rats – breeding and maintenance" produced by ssniff Spezialdiäten GmbH, D-59494 Soest Germany, *ad libitum* (except during exposure)
Water:	Tap water, *ad libitum* (except during exposure)
Housing:	In groups of 5 (or 2 in the case of sighting exposure), by sex, in solid-floor cages (Type III) with stainless steel mesh lids and softwood flake bedding.
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 30 – 70 % Air changes: 15 – 20 air changes per hour Photoperiod: 12 hours light / 12 hours dark

B. STUDY DESIGN AND METHODS

In life dates: 2011-04-14 to 2011-04-28

Exposure conditions: Prior to animal exposures, test material atmospheres were generated within the exposure chamber. During these technical trials, air-flow settings and test material input rates were adjusted to achieve the required atmospheric characteristics. Exposure conditions during the study are given later in a table of the test atmosphere characteristics of Glyphosate Technical.

Exposure system: The animals were exposed, nose-only, to an atmosphere of the test item using a TSE Rodent Exposure System (TSE Systems GmbH, Bad Homburg, Germany). This system comprises of 2, concentric anodised aluminium chambers and a computer control system incorporating pressure detectors and mass flow controllers.

Fresh aerosol from the generation system was constantly supplied to the inner plenum (distribution

chamber) of the exposure system from where, under positive pressure, it was distributed to the individual exposure ports. The animals were held in polycarbonate restraint tubes located around the chamber which allowed only the animal's nares to enter the exposure port. After passing through the animal's breathing zone, used aerosol entered the outer cylinder from where it was exhausted through a suitable filter system. Atmosphere generation was therefore dynamic.

Airflows and relative pressures within the system were constantly monitored and controlled by the computer system thus ensuring a uniform distribution and constant flow of fresh aerosol to each exposure port (breathing zone). The flow of air through each port was at least 0.7 L/min. This flow rate was considered adequate to minimise re-breathing of the test atmosphere as it is about twice the respiratory minute volume of a rat.

Homogeneity of the test atmosphere within the test chamber and amongst the exposure ports was not specifically determined during this study. However, chambers of this design have been fully validated and have shown to produce evenly distributed atmospheres in the animals' breathing zones.

Exposure procedure: Each rat was individually held in a tapered, polycarbonate restraining tube fitted onto a single tier of the exposure chamber. Only the nose of each animal was exposed to the test atmosphere. Following an equilibration period of at least the theoretical chamber equilibration time (T99), a group of 10 rats (5 male and 5 female) was exposed to a target atmosphere concentration 5 mg/L for a period of at least 4 hours.

Generation of the test atmosphere / chamber description: The test item was aerosolised using a rotating brush powder disperser (Palas GmbH, Karlsruhe, Germany) located at the top of the exposure chamber. Compressed air was supplied by means of an oil-free compressor and passed through a suitable filter system prior to introduction to the dust generator.

Test atmosphere concentration: The test atmosphere was sampled at regular intervals during each exposure period. Samples were taken from an unoccupied exposure port (representing the animal's breathing zone) by pulling a suitable, known volume of test atmosphere through weighed GF10 glass fibre filters. The difference in the pre and post sampling weights, divided by the volume of atmosphere sampled, was equal to the actual achieved test atmosphere concentration.

The nominal concentration was calculated by dividing the mass of test material disseminated into the chamber by the total volume of air that through the chamber during the same period.

Particle size determination: The particle size of the test atmosphere was determined three times during the exposure period using a 7-stage impactor of Mercer style (which employs an inertial separation technique to isolate particles in the discrete aerodynamic size ranges). Samples were taken from an unoccupied exposure port (representing the animal's breathing zone).

The collection substrates and the backup filter were weighed before and after sampling and the weight of test item, collected at each stage, calculated by this difference.
The total amount collected for each stage was used to determine the cumulative amount below each cut-off point size. In this way, the proportion (%) of aerosol less than 0.55, 0.96, 1.55, 2.11, 3.56, 6.66 and 10.55 µm was calculated.

From these data, using software supplied with the impactor (TSE Systems GmbH, Bad Homburg, Germany), the Mass Median Aerodynamic Diameter (MMAD), and Geometric Standard Deviation were calculated. In addition, the proportion (%) of aerosol less than 4 µm (considered to be the inhalable portion) was determined.

Table 5.2.3-2: Glyphosate technical: Acute inhalation toxicity study (nose-only) in the rat ([redacted], 2011): Summary of main study test atmosphere characteristics

Parameter	Target concentration 5 mg/L	
Mean achieved concentration (mg/L)	5.04 ± 0.17	
Nominal (mg/L)	7.71	
Particle size MMAD; GSD	3.65 µm; 2,24	
Inhalable fraction (% < 4 µm)	54.4	
	% by weight in range	
Size range (µm)	Total mass/stage (mg)	Cumulative mass (%)
<0.55	0.35	2.05
0.55 – 0.96	0.30	3.81
0.96 – 1.55	0.91	9.13
1.55 – 2.11	1.90	20.26
2.11 – 3.56	5.43	52.05
3.56 – 6.66	4.69	79.51
6.66 – 10.55	2.06	91.57
>10.55	1.44	100.00
T99 (Minimum Acceptable Equilibration Time)	1 minute	
Chamber volume (inner plenum)	3.85 L	
Air Flow In (Inner Plenum) (L/min)	20.0 - 20.6	
Air Flow Out (Inner Plenum) (L/min)	19.4 – 38.4	
Temperature	21.6 – 24.7 °C	
Humidity	3.9 – 10.2 % (n=3)	
Oxygen Concentration (%)	19.6 – 20.3	
Carbon Dioxide	0.1 – 0.8	

Sighting studies: Two sighting exposures using 2 male and 2 female rats were performed before the main study due to insufficient information about the test item's inhalation toxicity.

Animal assignment and treatment: Five male and 5 females were exposed to a target aerosol concentration of 5 mg/L Glyphosate Technical. The animals were exposed for 4 hours using a nose-only exposure system, followed by a 14 day observation period. The day of exposure was designated Day 0.

Animals were checked hourly during exposure, 1 hour after exposure and twice daily (early and late in the working day) during the 14 days of the observation period for morbidity and/or mortality. All animals were observed for clinical signs at hourly intervals during exposure, as soon as practically possible following removal from restraint at the end of exposure, 1 hour after exposure and subsequently once daily for 14 days. The body weight of each rat was recorded prior to treatment on the day of exposure (day 0) and on Days 1, 3, 7 and 14.

At the end of the 14 day observation period, the animals were sacrificed by exsanguination under anaesthesia and a gross macroscopic examination was performed, which included a detailed examination of the abdominal and thoracic cavities. Special attention was given to the respiratory tract for macroscopic signs of irritancy or local toxicity.

Statistics: The acute inhalation LC_{50} was calculated from the mortality data.

II. RESULTS

A. MORTALITY

One male rat died on Day 4 following a 4-hour exposure to 5.04 mg/L glyphosate technical.

B. CLINICAL OBSERVATIONS

Wet fur and fur staining were commonly recorded on the day of and the day following exposure. These observations were considered to be related to the restraint and exposure procedures and, in isolation, were considered not to be treatment related.

Significant clinical signs were recorded on day of exposure and the following day included laboured and noisy respiration, respiratory rate increased, gasping respiration, sneezing, decreased activity, thin body appearance (weak/wasted).

The majority of animals recovered from Day 3.

The clinical signs are summarized in the table below.

Table 5.2.3-3: Glyphosate technical: Acute inhalation toxicity study (nose-only) in the rat ([redacted], 2011): Clinical observation data

Clinical sign	Sex	No. of animals exposed [1]	Hours During exposure			On removal from chamber (4 hours)	One-hour Post-exposure	Post-exposure Day				
			1	2	3			1	2	3	4	5-14
Wet fur (on/in restraining apparatus)	Male	5	2	5	5	0	0	0	0	0	0	0
	Female	5	3	5	5	0	0	0	0	0	0	0
Wet fur (whole body)	Male	5	0	0	0	5	5	0	0	0	0	0
	Female	5	0	0	0	5	5	0	0	0	0	0
Ruffled coat	Male	5	0	0	0	0	0	2	0	0	0	0
	Female	5	0	0	0	0	0	1	0	0	0	0
Laboured respiration	Male	5	2	3	4	5	5	3[2,4]	1[4]	1[4]	0	0
	Female	5	2	2	4	4	4	1	0	0	0	0
Noisy respiration	Male	5	0	0	0	1	1	2[2]	1[4]	1[4]	0	0
	Female	5	0	0	0	1	1	0	0	0	0	0
Respiratory rate increased	Male	5	0	0	0	0	0	0	0	0	0	0
	Female	5	0	0	1	2	2	0	0	0	0	0
Gasping respiration	Male	5	0	0	0	0	0	1[4]	0	0	0	0
	Female	5	0	0	0	0	0	0	0	0	0	0
Sneezing	Male	5	0	0	0	0	0	1	1	0	0	0
	Female	5	0	0	0	0	0	0	0	0	0	0
Red-Brown staining (Head, Carnium)	Male	5	0	0	0	5	5	1	0	0	0	0
	Female	5	0	0	0	1	1	0	0	0	0	0
Red-Brown staining (Snout)	Male	5	0	0	0	5	5	0	0	0	0	0
	Female	5	0	0	0	5	5	0	0	0	0	0
Red-Brown staining (Nose)	Male	5	0	0	0	0	0	5	1[4]	0	0	0
	Female	5	0	0	0	0	0	3	0	0	0	0
Activity decreased	Male	5	0	0	0	0	0	1[4]	0	0	0	0
	Female	5	0	0	0	0	0	0	0	0	0	0
Weak	Male	5	0	0	0	0	0	3[3]	3[3]	0	0	0
	Female	5	0	0	0	0	0	0	0	0	0	0
Wasted	Male	5	0	0	0	0	0	0	0	1[4]	0	0
	Female	5	0	0	0	0	0	0	0	0	0	0

Table 5.2.3-3: Glyphosate technical: Acute inhalation toxicity study (nose-only) in the rat [redacted], 2011): Clinical observation data

[1] Day 4 after exposure: 1 male was found dead
[2] One male with severe symptom
[3] One male moderate weak and two males slightly weak
[4] Male found dead on Day 4

C. BODY WEIGHT

Normal body weight gain was noted for all surviving animals from Day 1, with the exception of one male where a slight bodyweight loss was recorded during first week of the observation period.

The body weight data are summarized in the table below.

Table 5.2.3-4: Glyphosate technical: Acute inhalation toxicity study (nose-only) in the rat ([redacted] 2011): Body weight data

Main study		Day 0	Day 1	Day 3	Day 7	Day 14
Male	Mean body weight (kg)	379	354	362	395	432
	Standard deviation	7.8	10.5	20.8	19.4	13.0
Female	Mean body weight (kg)	232	223	234	247	261
	Standard deviation	2.3	4.3	2.3	4.4	5.4

Note: Mean and standard deviation were not given in the study report. Values were calculated retrospectively based on individual animal data.

D. NECROPSY

There were no macroscopic abnormalities in animals surviving to scheduled termination. A specific cause of death was not determined for the single male that died in the main study.

III. CONCLUSIONS

Under the experimental conditions of this study, a single death occurred in a group of 10 rats exposed to glyphosate technical to a mean achieved atmosphere of 5.04 mg/L for 4 hours. The acute inhalation LC_{50} of glyphosate technical, after a 4-hour exposure to male and female Wistar RjHan: (WI) strain rats, observed over a period of 14 days is considered to be greater than 5.04 mg/L.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is performed in accordance with the current OECD 403 guideline (2009) and according to GLP. The MMAD and the applied concentration were in the range of the recommended values of the current OECD guideline. Otherwise, only minor deviations to the current valide OECD guideline are present. Therefore, the study is acceptable. Based on the study results, the acute inhalation LC_{50} is >5.04 mg/L air. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.3/002
Report author	[redacted]
Report year	2010
Report title	Acute Inhalation Toxicity Study of Glyphosate TC In Rats
Report No	24603
Document No	Not reported
Guidelines followed in study	EC method B.2, OECD 403 (1981), EPA Health Effects Test Guidelines, OPPTS 870.1300
Deviations from current test guideline (OECD 403, 2009)	MMAD slightly exceeded the recommended MMAD (4.633 µm with a GSD of 3.02 (no smaller MMAD and GSD could be obtained with the test item supplied)). Limit test performed on 5 animals per sex. Body weight measured at Day 0 and once a week afterwards.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive#
Category study in AIR 5 dossier (L docs)	Category 2a

#: Following the feedback from the Assessment Group Glyphosate in March 2020, the Notifier is aware of concerns regarding work conducted at this specific contract laboratory ([redacted]). For the sake of completeness, this study is included in the dossier submission since it was part of the data package of previous EU evaluation processes.

2. Full summary

The test substance, glyphosate technical, was evaluated for its acute inhalation toxicity potential in rats when administered for a single 4-hour period using a dynamic nose-only exposure chamber at an actual concentration of 5.18 mg/L.

No mortality occurred during the study. Clinical signs included slight tremor and slight dyspnoea immediately until 3 hours after end of exposure. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no pathological findings.

The acute inhalation LC_{50} was determined to be > 5.18 mg/L air.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate TC
Description:	White powder
Lot/Batch #:	20090506
Purity:	97.3 %
Stability of test compound:	At room temperature in the dark stable until May 2011

2. Vehicle and/or positive control: none

3. Test animals:

Species:	Rat
Strain:	CD/Crl:CD (SD)
Source:	[redacted]
Age:	approx. 7 – 9 weeks
Sex:	Males and females
Weight at dosing:	♂ 234 – 270 g; ♀ 208 – 244 g
Acclimation period:	At least 5 days
Diet/Food:	ssniff RIM-H V1 534 (ssniff Spezialdiäten GmbH, Soest, Germany), *ad libitum* (except 16 h before exposure)
Water:	tap water, *ad libitum*
Housing:	In groups of 2 – 3 animals per cage in Makrolon type III plus cages with granulated textured wood bedding.
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 55 ± 15 % Air changes: no data 12 hours light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2010-02-02 to 2010-02-16

Test atmosphere generation:

A dust atmosphere was produced from the test material using a rotating brush dust generator and compressed air.

Exposure chamber conditions:

The cylindrical exposure chamber had a volume of approximately 40 L. The actual dust concentration was measured four times gravimetrically with an air sample filter (Minisart SM 17598; 0.45 pm) and pump (Vacuubrand, MZ 2C, Vacuubrand, Germany) controlled by a rotameter. Dust samples were taken once every hour during the exposure. For that purpose, a probe was placed close to the animals' noses in the inhalation chamber and air was sucked through the air sample filter at a constant flow of air of 5 L/min for 1 minute. The filters were weighed before and after sampling on an analytical balance (accuracy 0.1 mg). Chamber airflow rates ranged from 800 to 900 L/h, providing ≥ 12 air changes per hour.

Particle size distribution:

A Malvern Spraytec Lasersystem (Malvern Instruments, Germany) was employed for the determination of the particle size distribution of the particle diameter (volume) in the exposure air. The particle size distribution of the test atmospheres was measured using a cascade impactor two times during the exposure period. The results were as follows:

Table 5.2.3-5: Acute Inhalation Toxicity Study of Glyphosate TC In Rats ([redacted] 2010): Details of test atmosphere

Mean achieved actual concentration (HPLC)	Actual concentration (gravimetric method)	MMAD	GSD	Respirable amount particle size ≤4 µm	
(mg/L air)	(mg/L air)	(µm)		(mg/L air)	(%)
5.18	5.05	4.633	3.02	1.08	20.8

MMAD = mean mass median aerodynamic diameter
GSD = geometric standard deviation

The generated dust had a mass median aerodynamic diameter (MMAD) of 4.633 µm as determined with a cascade impactor. The Geometric Standard Deviation (GSD) of the MMAD was calculated as 3.02. No smaller MMAD and GSD could be obtained with the test item supplied.

Animal assignment and treatment:

A group of five fasted rats per sex received the test material at a dose level of 5.18 mg/L using a dynamic inhalation apparatus (≥ 12 air changes/h) with a nose-only exposure. Observations for mortality and clinical/behavioural signs of toxicity were made at least once per day for 14 days. Individual body weights were recorded just prior to dosing and weekly thereafter. On Day 14 after dosing, each animal was euthanized and all study animals were subjected to gross necropsy.

II. RESULTS

A. MORTALITY

No deaths occurred.

B. CLINICAL OBSERVATIONS

Clinical signs of toxicity included slight tremor and slight dyspnoea immediately until 3 hours after end of exposure. The clinical signs are summarized in the table below.

Table 5.2.3-6: Acute Inhalation Toxicity Study of Glyphosate TC In Rats [redacted] 2010): Clinical observation data

Test days			**1**	**1**	**1**	**1**	**1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Time after administration (in minutes unless otherwise stated)			**0**	**5**	**15**	**30**	**60**	**3h**													
Sex	Animal No.	Clinical signs																			
M	1	tremor	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
		dyspnoea	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	tremor	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
		dyspnoea	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
	3	tremor	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
		dyspnoea	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
	4	tremor	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
		dyspnoea	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
	5	tremor	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
		dyspnoea	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
F	6	tremor	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
		dyspnoea	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 5.2.3-6: Acute Inhalation Toxicity Study of Glyphosate TC In Rats ([redacted] 2010): Clinical observation data

	7	tremor	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
		dyspnoea	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-		
	8	tremor	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
		dyspnoea	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
	9	tremor	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
		dyspnoea	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
	10	tremor	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
		dyspnoea	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-

+: slight; -: none; h: hours

C. BODY WEIGHT

All animals gained the expected body weight.

D. NECROPSY

No pathological findings were noted at necropsy.

III. CONCLUSIONS

The acute inhalation LC_{50} of glyphosate technical after a 4-hour exposure to male and female rats, observed over a period of 14 days was greater than 5.18 mg/L.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was performed in accordance to OECD 403 guideline and GLP. The MMAD and GSD slightly exceed the recommended values of the current OECD guideline. Otherwise, only minor deviations to the current valide OECD guideline are present. Therefore, the study is acceptable. Based on the study results, the acute inhalation LC_{50} is >5 mg/L air after an exposure period of 4 hours. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.3/003
Report author	[redacted]
Report year	2010
Report title	Acute Inhalation Toxicity Study of Glyphosate TC in Rats
Report No	24875
Document No	Not reported
Guidelines followed in study	EC method B.2. (92/69/EEC), OECD 403 (1981) and OPPTS 870.1300

Deviations from current test guideline (OECD 403, 2009)	MMAD slightly exceeded the recommended MMAD (4.197 µm with a GSD of 2.64 (no smaller MMAD and GSD could be obtained with the test item supplied)). Limit test performed on 5 animals per sex. Body weight measured at Day 0 and once a week afterwards. There were several minor deviations from the Study Plan which did not affect the scientific outcome or the validity of the study
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive#
Category study in AIR 5 dossier (L docs)	Category 2a

#: Following the feedback from the Assessment Group Glyphosate in March 2020, the Notifier is aware of concerns regarding work conducted at this specific contract laboratory ([redacted]). For the sake of completeness, this study is included in the dossier submission since it was part of the data package of previous EU evaluation processes.

2. Full summary

The test substance, glyphosate technical, was evaluated for its acute inhalation toxicity potential. The test substance was administered to albino rats for a single 4-hour period using a dynamic nose-only exposure chamber. The exposure concentration, which was determined by HPLC, was 5.02 mg/L air for 4 hours. In the inhalation chamber, close to the animals' noses, the generated dust had a mass median aerodynamic diameter (MMAD) of 4.197 ± 2.64 µm as determined with a cascade impactor. No smaller MMAD could be obtained with the test item. The analysis of the particle size distribution of the particle diameter (volume) in the exposure air was carried out by laser measurement and determined as $d_{[50]}$ = 37.15 µm. The particle size distribution of the particle size of the delivered test item was $d_{[50]}$ = 14.5 µm. The test concentration revealed slight ataxia, slight tremor and slight dyspnoea immediately until 3 hours after the end of exposure. No mortality occurred during the study and no pathological findings were noted at necropsy. All animals gained the expected body weight.

The acute inhalation LC_{50} was determined to be > 5.02 mg/L air (actual concentration).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

	Glyphosate TC
Identification:	Glyphosate technical grade
Description:	White powder
Lot/Batch #:	2009051501
Purity:	96.4 %
Stability of test compound:	May 2011

2. Test animals:

Species:	Rat albino
Strain / Stock:	CD / Crl:CD(SD)
Source:	[redacted]
Age:	Males: approx. 7 weeks Females: approx. 9 weeks
Sex:	5 male and 5 female

Weight at dosing:	Males: 270 – 282 g; Females: 220 – 251 g
Acclimation period:	5 days
Diet/Food:	ssniff® R/M-H V1534 (ssniff Spezialdiäten GmbH), *ad libitum* except for approx. 16 h before dosing
Water:	Tap water, *ad libitum*
Housing:	Animals were kept by sex in groups of 2-3 animals in MAKROLON cages (type III plus) with granulated textured wood as bedding material.
Environmental conditions:	Temperature: 22 ± 3 °C Rel. humidity: 40 – 70 % Air changes: 12/hour 12-hour light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2009-10-15 to 2010-02-18

Animal assignment and treatment:

The study was carried out using a dynamic inhalation apparatus (≥ 12 air changes/h) with a nose-only exposure of the animals (exposure chamber volume 40 L). The test item was generated with a rotating brush dust generator. Exposure started by locating the rats (5 male and 5 female animals) into the exposure chamber after equilibration of the chamber concentration for at least 15 minutes. The dust concentration in the inhalation chamber was determined gravimetrically as well as by HPLC once every hour during exposure. Animals were exposed for 4 hours to an actual concentration of 5.02 mg/L air (determined by HPLC).

A laser measured the size of the individual particles or individual aerosol drops. The particle size distribution for the estimation of the Mass Median Aerodynamic Diameter (MMAD) was carried out twice during the exposure period using a cascade impactor. The median particle size distribution of the test item was determined with a Malvern Sizer.

After completion of exposure, animals were observed for a period of 14 days. Observations for clinical/behavioural signs of toxicity were made at least once daily until symptoms subsided, and thereafter each working day. Observations on mortality were made at least once daily. Individual body weights were determined before the exposure and weekly after exposure. On Day 14 after completion of exposure, all animals were sacrificed, dissected and inspected macroscopically. All gross pathological changes were recorded. No microscopic examination was performed as no pathological findings were noted at necropsy.

II. RESULTS

A. DUST CONCENTRATION AND PARTICLE SIZE DISTRIBUTION

The actual dust concentration of 5.02 mg Glyphosate TC/L air was measured at the animals' nose and was determined by HPLC. The mean actual exposure concentration of Glyphosate TC was as follows:

Table 5.2.3-7: Acute Inhalation Toxicity Study of Glyphosate TC in Rats ([redacted] 2010): Details of test atmosphere

Actual concentration (HPLC) [mg/L air]	Actual concentration (gravimetric method) [mg/L air]	MMAD [µm]	Respirable amount particle size ≤4 µm	
			[mg/L air]	[%]
5.02	4.99	4.197 ± 2.64	1.03	20.5

No smaller MMAD could be obtained with the test item and no higher fraction of respirable particles could be obtained.

Laser measurement revealed the following particle size distribution during the exposure:

Diameter	Actual concentration 5.02 mg/L air
$d_{[10]}$	12.51 µm
$d_{[50]}$	37.15 µm
$d_{[90]}$	86.42 µm

[xx] = percentage of cumulative particle size distribution

The particle size distribution of the delivered test item was $d_{[50]}$ = 14.5 µm.

B. MORTALITY

There were no mortalities during the study.

C. CLINICAL OBSERVATIONS

A 4-hour exposure to Glyphosate TC at the concentration of 5.02 mg/L revealed slight ataxia, slight tremor and slight dyspnoea immediately until 3 hours after the end of exposure. The clinical signs are summarized in the table below.

Table 5.2.3-8: Acute Inhalation Toxicity Study of Glyphosate TC in Rats ([redacted] 2010): Clinical observation data

Test days			**1**	**1**	**1**	**1**	**1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Time after administration (in minutes unless otherwise stated)			**0**	**5**	**15**	**30**	**60**	**3h**													
Sex	Animal No.	Clinical signs																			
M	1	ataxia	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
		tremor	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
		dyspnoea	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	ataxia	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
		tremor	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
		dyspnoea	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
	3	ataxia	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
		tremor	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
		dyspnoea	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
	4	ataxia	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
		tremor	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
		dyspnoea	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
	5	ataxia	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
		tremor	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
		dyspnoea	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
F	6	ataxia	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
		tremor	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
		dyspnoea	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
	7	ataxia	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 5.2.3-8: Acute Inhalation Toxicity Study of Glyphosate TC in Rats ([redacted] 2010): Clinical observation data

		tremor	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
		dyspnoea	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
	8	ataxia	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
		tremor	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
		dyspnoea	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
	9	ataxia	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
		tremor	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
		dyspnoea	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
	10	ataxia	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
		tremor	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
		dyspnoea	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
+: slight; -: none; h: hour																					

D. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance.

E. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute inhalation LC_{50} of glyphosate technical after a 4-hour exposure to males and females rats, observed over a period of 14 days was greater than 5.02 mg/L air.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was performed in accordance to OECD 403 guideline and GLP. The MMAD and GSD slightly exceed the recommended values of the current OECD guideline. Otherwise, only minor deviations to the current valide OECD guideline are present. Therefore, the study is acceptable. Based on the study results, the acute inhalation LC50 is >5 mg/L air after an exposure period of 4 hours. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.3/004
Report author	[redacted]
Report year	2009
Report title	Acute Inhalation Toxicity Study of Glyphosate TC in Rats
Report No	23911
Document No	Not reported
Guidelines followed in study	EC method B.2. (92/69/EEC), OECD 403 (1981) and OPPTS 870.1300

Deviations from current test guideline (OECD 403, 2009)	No MMAD calculated (mean measured particle size: 6.62 µm). Limit test performed on 5 animals per sex. Body weight measured at Day 0 and once a week. There were several minor deviations from the Study Plan which did not affect the scientific outcome or the validity of the study.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive#
Category study in AIR 5 dossier (L docs)	Category 2a

#: Following the feedback from the Assessment Group Glyphosate in March 2020, the Notifier is aware of concerns regarding work conducted at this specific contract laboratory ([redacted]). For the sake of completeness, this study is included in the dossier submission since it was part of the data package of previous EU evaluation processes.

2. Full summary

The test substance, glyphosate technical, was evaluated for its acute inhalation toxicity potential. The test substance was administered to albino rats for a single 4-hour period using a dynamic nose-only exposure chamber. The exposure concentration, which was determined by HPLC, was 5.12 mg/L air for 4 hours. The analysis of the particle size distribution was carried out by laser measurement and determined as $d_{[50]}$ = 6.62 µm. No finer dust concentration of the test item could be generated. The test concentration revealed slight dyspnoea and ataxia in all 5 of 5 male and 5 of 5 female animals immediately until 60 minutes after the end of exposure. No mortality occurred during the study and no pathological findings were noted at necropsy. All animals gained the expected body weight.

The acute inhalation LC_{50} was determined to be > 5.12 mg/L air (actual concentration).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate TC

Identification:	Glyphosate technical grade
Description:	White powder
Lot/Batch #:	20080801
Purity:	98.8 %
Stability of test compound:	2010-08-01

2. Test animals:

Species:	Rat albino
Strain / Stock:	CD / Crl:CD(SD)
Source:	[redacted]
Age:	Males: 52 days Females: 66 days
Sex:	5 male and 5 female
Weight at dosing:	Males: 240 – 267 g; Females: 209 – 216 g
Acclimation period:	5 days

Diet/Food:	ssniff® R/M-H V1534 (ssniff Spezialdiäten GmbH), *ad libitum* except for approx. 16 h before dosing
Water:	Tap water, *ad libitum*
Housing:	Animals were kept by sex in groups of 2-3 animals in MAKROLON cages (type III plus) with granulated textured wood as bedding material.
Environmental conditions:	Temperature: 22 ± 3 °C Rel. humidity: 40 – 70 % Air changes: 12/hour 12-hour light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2009-02-04 to 2009-07-30

Animal assignment and treatment:

The study was carried out using a dynamic inhalation apparatus (≥ 12 air changes/h) with a nose-only exposure of the animals (exposure chamber volume 40 L). The test item was micronized before administration and the dust was generated with a rotating brush dust generator. Exposure started by locating the rats (5 male and 5 female animals) into the exposure chamber after equilibration of the chamber concentration for at least 15 minutes. The dust concentration in the inhalation chamber was determined gravimetrically as well as by HPLC once every hour during exposure. A laser measured the size of the individual particles or individual aerosol drops. Animals were exposed four 4 hours to an actual concentration of 5.12 mg/L air (determined by HPLC).

After completion of exposure, animals were observed for a period of 14 days. Observations for clinical/behavioural signs of toxicity were made at least once daily until symptoms subsided, and thereafter each working day. Observations on mortality were made at least once daily. Individual body weights were determined before the exposure and weekly after exposure. On Day 14 after completion of exposure, all animals were sacrificed, dissected and inspected macroscopically. All gross pathological changes were recorded. No microscopic examination was performed as no pathological findings were noted at necropsy.

II. RESULTS

A. DUST CONCENTRATION AND PARTICLE SIZE DISTRIBUTION

The actual dust concentration of 5.12 mg Glyphosate TC/L air was measured at the animals' nose and was determined by HPLC. The mean actual exposure concentration of Glyphosate TC was as follows (see table below):

Table 5.2.3-9: Acute Inhalation Toxicity Study of Glyphosate TC in Rats ([redacted] 2009): Details of test atmosphere

Nominal concentration (test item/water) [mg/L air]	Actual concentration (HPLC) [mg/L air]	median diameter [µm]	Respirable amount particle size ≤3.98 µm	
			[mg/L air]	[%]
5.0	5.12	6.62	< 0.01	< 0.01

Laser measurement revealed the following particle size distribution during the exposure:

Diameter	Actual concentration 5.12 mg/L air
$d_{[10]}$	5.64 µm
$d_{[50]}$	6.62 µm
$d_{[90]}$	8.10 µm

[xx] = percentage of cumulative particle size distribution

No finer dust concentration of the test item could be generated.

B. MORTALITY

There were no mortalities during the study.

C. CLINICAL OBSERVATIONS

A 4-hour exposure to Glyphosate TC at the concentration of 5.12 mg/L revealed slight dyspnoea and ataxia in all 5 of 5 male and 5 of 5 female animals immediately until 60 minutes after the end of exposure. The clinical signs are summarized in the table below.

Table 5.2.3-10: Acute Inhalation Toxicity Study of Glyphosate TC in Rats [redacted] 2009): Clinical observation data

Test days			**1**	**1**	**1**	**1**	**1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Time after administration (in minutes unless otherwise stated)			**0**	**5**	**15**	**30**	**60**	**3h**													
Sex	Animal No.	Clinical signs																			
M	1	ataxia	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		dyspnoea	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	ataxia	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		dyspnoea	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	3	ataxia	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		dyspnoea	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	4	ataxia	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		dyspnoea	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	5	ataxia	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		dyspnoea	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F	6	ataxia	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		dyspnoea	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	7	ataxia	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		dyspnoea	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	8	ataxia	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		dyspnoea	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	9	ataxia	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		dyspnoea	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	10	ataxia	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		dyspnoea	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+: slight; -: none

D. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance.

E. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute inhalation LC_{50} of glyphosate technical after a 4-hour exposure to male and female rats, observed over a period of 14 days was greated than 5.12 mg/L air.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was performed in accordance to OECD 403 guideline and GLP. The particle size exceeds the recommended values of the current OECD guideline; however, no finer dust could be generated. Otherwise, only minor deviations to the current valide OECD guideline are present. Therefore, the study is acceptable. Based on the study results, the acute inhalation LC_{50} is >5 mg/L air after an exposure period of 4 hours. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.3/005
Report author	[redacted]
Report year	2009
Report title	Glyphosate Tech: Acute Inhalation Toxicity (Nose only) Study in the Rat
Report No	2743/0001
Document No	Not reported
Guidelines followed in study	OECD 403 (1981) Commission Regulation (EC) No 440/2008 (2008), method B.2 (2008)
Deviations from current test guideline (OECD 403, 2009)	MMAD slightly exceeded the recommended MMAD (5.25 µm with a GSD of 3.35 (due to technical limitations)). Limit test performed on 5 animals per sex. Body weight measured at Day 0 and once a week.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The test substance, glyphosate technical, was evaluated for its acute inhalation toxicity potential in male and female HsdRccHan™: WIST rats by exposure to the dose level of 5.04 mg/L via dust atmosphere for 4 hours using a nose-only exposure system. No mortality occurred during the study. Clinical signs included increased respiratory rate, hunched posture, pilo-erection and wet fur. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

The acute inhalation LC_{50} was determined to be > 5.04 mg/L air.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate Tech
Description:	White powder
Lot/Batch #:	GI-1045
Purity:	96.66 %
Stability of test compound:	Expiration in July 2010

2. Vehicle and/or positive control: Not relevant

3. Test animals:

Species:	Rat
Strain:	HsdRccHan™ : WIST
Source:	[redacted]
Age:	Approx. 8 - 12 weeks
Sex:	Male and female
Weight at dosing:	178 – 350 g
Acclimation period:	5 days
Diet/Food:	With the exception of the exposure period, free access to food (Harlan 2014 Rodent Diet, Harlan UK Limited, Oxon, UK) was allowed throughout the study
Water:	With the exception of the exposure period, free access to drinking water was allowed throughout the study
Housing:	Housed in groups of five by sex in solid-floor polupropylene cages with stainless steel lids, furnished with softwood flakes (Datesand Ltd., Cheshire, UK) and provided with environmental enrichment items: wooden chew blocks and cardboard “fun tunnels” (Datesand Ltd., Cheshire, UK)
Environmental conditions:	Temperature: 19 – 25 °C Humidity: 30 – 70 % Air changes: At least 15/hour 12-hour light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2009-05-12 to 2009-06-04

Animal assignment and treatment:

Five male and female rats were exposed to one dose level of dust atmosphere of glyphosate. The single 5 mg/L four-hour exposure was "nose only" at a mean actual concentration of 5.04 ± 0.37 mg/L (nominal concentration was 27.3 mg/L).

Operational conditions (flow rate, oxygen levels, temperature, and humidity in the inhalation systems) were checked throughout the exposure period. All animals were observed for clinical signs at hourly intervals during exposure, immediately on removal from the restraining tubes at the end of exposure, one hour after termination of exposure and subsequently once daily for 14 days. Individual body weights were recorded prior to treatment on the day of exposure and on Days 7 and 14. At the end of the fourteen-day observation period the animals were killed by intravenous overdose of sodium pentobarbitone. All animals were subjected to a full external and internal examination, and any macroscopic abnormalities were recorded. The respiratory tract was subjected to a detailed macroscopic examination for signs of irritancy or local toxicity.

The chamber flow rate was maintained at 45 L/min providing 90 air changes per hour.

II. RESULTS

A. TEST ATMOSPHERE

The particle size analysis of the atmosphere drawn from the animals' breathing zone, was as follows:

Table 5.2.3-11: Glyphosate Tech: Acute Inhalation Toxicity (Nose only) Study in the Rat ([redacted], 2009): Details of test atmosphere

Mean Achieved Atmoshpere Concentration (mg/L)	Mean Mass Median Aerodynamic Diameter (µm)	Inhalable Fraction (% <4 µm)	Geometric Standard Deviation
5.04	5.25	41.1	3.35

It is noted that the achieved particle size is larger than required by the test guidelines.

During characterisation, changes were made to the generation system (addition of particle sizes separator) and grinding techniques in an attempt to increase the inhalable portion of the test material. However, this reduced the achieved concentration, and therefore, also reduced the actual concentration of particles <4 µm. It was, therefore, preferable to expose the animals to a higher concentration of test material, even though this also increased the mean mass median aerodynamic diameter, as this resulted in the animals being exposed to the highest possible concentration of particles <4 µm.

B. MORTALITY

There were no mortalities during the study.

C. CLINICAL OBSERVATIONS

Signs of hunched posture and piloerection are commonly seen in animals for short periods on removal from the chamber following 4-hour inhalation studies. Wet fur is commonly recorded both during and for a short period after exposure. These observations are considered to be associated with the restraint procedure and, in isolation, are not indicative of toxicity.

In addition to the observations considered to be due to the restraint procedure, increased respiratory rate

was noted in all animals during exposure, on removal from the chamber and one-hour post-exposure. The clinical signs are summarized in the table below.

Table 5.2.3-12: Glyphosate Tech: Acute Inhalation Toxicity (Nose only) Study in the Rat ([redacted], 2009): Clinical observation data

Clinical sign	Sex	No. of animals exposed	Exposure Day					Post-exposure Day							
			During exposure (h)			On removal from chamber	Post-exposure (h)								
			1	2	3		1	1	2	3	4	5	6	7	8-14
Hunched posture	Male	5	0	0	0	5	5	0	0	0	0	0	0	0	0
	Female	5	0	0	0	5	5	0	0	0	0	0	0	0	0
Pilo-erection	Male	5	0	0	0	5	5	0	0	0	0	0	0	0	0
	Female	5	0	0	0	5	5	0	0	0	0	0	0	0	0
Increased respiratory rate	Male	5	5	5	5	5	5	0	0	0	0	0	0	0	0
	Female	5	5	5	5	5	5	0	0	0	0	0	0	0	0
Wet fur	Male	5	5	5	5	5	5	0	0	0	0	0	0	0	0
	Female	5	5	5	5	5	5	0	0	0	0	0	0	0	0

D. BODY WEIGHT

Normal body weight development was noted during the course of the study.

E. NECROPSY

No macroscopic abnormalities were detected at necropsy.

III. CONCLUSIONS

The acute inhalation LC_{50} of glyphosate technical after a 4-hour exposure to male and female rats, observed over a period of 14 days was greater than 5.04 mg/L.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was performed in accordance to OECD 403 guideline and GLP. The MMAD and GSD slightly exceed the recommended values of the current OECD guideline. Otherwise, only minor deviations to the current valide OECD guideline are present. Therefore, the study is acceptable. Based on the study results, the acute inhalation LC50 is >5 mg/L air after an exposure period of 4 hours. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.3/005
Report author	[redacted]
Report year	2009
Report title	Glyphosate – Acute Inhalation Toxicity Study in Rats
Report No	12107-08
Document No	Not reported
Guidelines followed in study	US EPA OPPTS 870.1300.
Deviations from current test guideline (OECD 403, 2009)	Humidity was in the range of 33 – 89 % instead of 30 – 70 %. Female weight was outside the protocol range. Limit test performed on 5 animals per sex. Body weight measured at Day 0 and once a week afterwards. These deviations did not affect the outcome of the study.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The test substance, glyphosate technical, was evaluated for its acute inhalation toxicity potential. Five male and five female rats were exposed for 4 hours to an aerosol generated from the undiluted test substance at a level of 2.24 mg/L. The exposure concentration was determined gravimetrically. The analysis of the particle size distribution was carried out by a cascade impactor and the mass median aerodynamic diameter (MMAD) was estimated to be 2.6 µm. There was no mortality during the study. Clinical sings included piloerection and activity decrease, which were no longer evident by Day 4. Body weights were unaffected by the exposure. The gross necropsy revealed no observable abnormalities.

The acute inhalation LC_{50} was determined to be > 2.24 mg/L air (actual concentration).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate

Identification: Glyphosate Tech Grade Mixed 5-Batch

Description: White powder

Lot/Batch #: 080704-1 thru 5

Purity: 96.71 % (analysed 2009-01-08); 96.40 % (analysed 2008-10-17)

Stability of test compound: No data given in the report.

2. Test animals:

Species: Rat albino

Strain / Stock: Sprague-Dawley

Source: [redacted]

Age:	Approx. 7 – 8 weeks
Sex:	5 male and 5 female
Weight at dosing:	Males: 262 – 289 g; Females: 172 – 191 g
Acclimation period:	5 days
Diet/Food:	Formulab #5008 (PMI Feeds Inc.), *ad libitum* except during the exposure period
Water:	Tap water, *ad libitum* except during the exposure period
Housing:	Individual housing in suspended, wire bottom, stainless steel cages.
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 30 – 70 % Air changes: 10 – 12/hour 12-hour light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2008-11-07 to 2008-11-21

Animal assignment and treatment:

The study was carried out using a 500 L nose-only stainless steel, dynamic flow inhalation chamber with 25 ports in 5 rows. Polycarbonate tubes were inserted into 10 designated individual ports. The test substance was ground for 10 hours and dried prior to exposure. The aerosol was generated from the undiluted test substance by a Venturi Aspirator and sprayed directly into the exposure chamber. Exposure started by locating the rats (5 male and 5 female animals) into the exposure chamber. Animals were exposed to the aerosol for a period of 4 hours. The dust concentration in the inhalation chamber was determined gravimetrically twice per hour and nominally at the end of the exposure. Particle size, taken from the breathing zone of the animals, was determined twice during the exposure using a cascade impactor, and the mass median aerodynamic diameter (MMAD) and particle size distribution were calculated.

Observations for mortality and signs of pharmacological and/or toxicological effects were made frequently on the day of exposure and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to the inhalation exposure and on Days 7 and 14. On Day 14 after completion of exposure, all animals were euthanized by an intraperitoneal injection, dissected and inspected macroscopically. All gross pathological changes were recorded. No microscopic examination was performed as no pathological findings were noted at necropsy.

II. RESULTS

A. DUST CONCENTRATION AND PARTICLE SIZE DISTRIBUTION

The exposure concentration was determined to be 2.24 mg/L with an average MMAD of 2.6 µm.

B. MORTALITY

There were no mortalities during the study.

C. CLINICAL OBSERVATIONS

The only prominent in life observations were piloerection and activity decrease. Animals were asymptomatic by Day 4. The clinical signs are summarized in the table below.

Table 5.2.3-13: Glyphosate – Acute Inhalation Toxicity Study in Rats ([redacted] 2009): Clinical observation data

Clinical sign	Sex	No. of animals exposed	Exposure Day					Post-exposure Day							
			Time after exposure begins (h)												
			0.5	1.0	2.5	4.5	6.0	1	2	3	4	5	6	7	8-14
Pilloerection	Male	5	0	0	0	5	5	5	5	0	0	0	0	0	0
	Female	5	0	0	0	5	5	5	5	0	0	0	0	0	0
Activity decrease	Male	5	0	0	0	5	5	5	5	5	0	0	0	0	0
	Female	5	0	0	0	5	5	5	5	5	0	0	0	0	0

D. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance.

E. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute inhalation LC_{50} of glyphosate technical after a 4-hour exposure to male and female rats, observed over a period of 14 days was greater than 2.24 mg/L air.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was performed in accordance to OECD 403 guideline and GLP. The MMAD and the applied concentration were in the range of the recommended values of the current OECD guideline. Otherwise, only minor deviations to the current valide OECD guideline are present. Therefore, the study is acceptable. Based on the study results, the acute inhalation LC50 is > 2.24 mg/L air after an exposure period of 4 hours. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.3/007
Report author	[redacted]
Report year	2008
Report title	Acute Inhalation Toxicity Test of Glyphosate Technical in Rats *(Rattus norvegicus)*
Report No	[redacted]-3996.309.377.07
Document No	Not reported
Guidelines followed in study	OECD guideline 403 (1981)

Deviations from current test guideline (OECD 403, 2009)	MMAD exceeded the recommended MMAD (MMAD ranged from 18.555 to 19.901 µm, only 4.72 to 5.15 % of the particles were within the respirable size range; no explanation provided why the standard could not be achieved). Experimental phase initiation and conclusion dates were updated. Limit test performed on 5 animals per sex. These deviations did not affect the study outcome.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Invalid
Category study in AIR 5 dossier (L docs)	Category 3b

2. Full summary

The test substance, glyphosate technical, was evaluated for its acute inhalation toxicity potential. One group of rats (five/sex) was exposed nose-only for a 4-hour exposure period to the aerosolized test item, using a total airflow of 10 L/min. The aerodynamic particle size distribution determined with a cascade impactor indicated that 4.72 to 5.15 % of the aerosol generated was within the respirable size range. The mass median aerodynamic diameter (MMAD) ranged from 18.555 to 19.901 µm. The mean actual concentration determined gravimetrically was 5.211 mg/L. No mortality occurred during the study and no pathological findings were noted at necropsy. Clinical signs observed during the 14-day observation period included wheeze and dyspnoea. These acute respiratory signs started within the first day and reverted within the fourth day of the observation period. All animals gained the expected body weight, except for the males on the first post-exposure day.

The acute inhalation LC_{50} was determined to be > 5.211 mg/L air (actual concentration).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate Technical

Identification:	Glyphosate Technical
Description:	Solid
Lot/Batch #:	20070606
Purity:	98.05 %
Stability of test compound:	No data given in the report.

2. Test animals:

Species:	Rat albino (*Rattus norvegicus*)
Strain / Stock:	Wistar Hannover
Source:	[redacted]
Age:	Males: 9 weeks; Females: 11 weeks
Sex:	5 males and 5 females
Weight at dosing:	Males: 262 – 291 g; Females: 178 – 208
Acclimation period:	9 days

Diet/Food:	Nuvilab CR-1 pellet diet type for rodents (Nuvital Nutrients Ltda.), *ad libitum*
Water:	Potable drinking water, *ad libitum*
Housing:	Polypropylene rodents cages with autoclaved wood shavings and stainless steel mesh lids containing five rats of each sex per cage.
Environmental conditions:	Temperature: 19 – 25 °C Humidity: 30 – 70 % Air changes: 10 – 15/hour 12-hour light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2008-06-06 to 2008-06-20

Animal assignment and treatment:

The study was carried out using an inhalation chamber with a nose-only exposure of the animals. The test item was aerosolized. Exposure started by locating the rats (5 male and 5 female animals) into the exposure chamber. Animals were exposed to the aerosol at the maximum attainable concentration (5.211 mg/L) for a period of 4 hours. The actual concentration in the inhalation chamber was determined gravimetrically by taking eight equally time-spaced air samples from the breathing zone. Aerodynamic particle size distribution was determined two times using a Seven Stage Cascade Impactor.
After completion of exposure, animals were observed for a period of 14 days. Observations for clinical/behavioural signs of toxicity were made right after the exposure, and thereafter each working day. On Day 14 after completion of exposure, all animals were euthanized in a carbon dioxide chamber, dissected and inspected macroscopically. All gross pathological changes were recorded. No microscopic examination was performed as no pathological findings were noted at necropsy.

II. RESULTS

A. DUST CONCENTRATION AND PARTICLE SIZE DISTRIBUTION

The mean actual concentration was 5.211 mg/L. The actual concentration of the test item in each sample was within the ±15 % interval from the mean actual concentration, indicating that the test atmosphere was held stable over the 4-hour exposure period.

Analysis of the particle size distribution of samples from the breathing zone indicates that 4.72 to 5.15 % of the mass collected from the aerosol were within the respirable size range. The MMAD ranged from 18.555 to 19.901 µm and the geometric standard deviation (GSD) ranged from 2.869 to 2.914.

B. MORTALITY

There were no mortalities during the study.

C. CLINICAL OBSERVATIONS

Clinical signs observed during the 14-day observation period included wheeze and dyspnoea. These acute respiratory signs started within the first day and reverted within the fourth day of the observation period.

The clinical signs are summarized in the table below.

Table 5.2.3-14: Acute Inhalation Toxicity Test of Glyphosate Technical in Rats *(Rattus norvegicus)* ([redacted] 2008): Clinical observation data

Test days			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Sex	Animal No.	Clinical signs															
M	1	wheeze	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
		dyspnoea	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
	2	wheeze	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		dyspnoea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	3	wheeze	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		dyspnoea	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
	4	wheeze	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		dyspnoea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	5	wheeze	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		dyspnoea	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
F	7	wheeze	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		dyspnoea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	8	wheeze	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		dyspnoea	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
	9	wheeze	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		dyspnoea	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
	10	wheeze	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		dyspnoea	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
	11	wheeze	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		dyspnoea	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-

D. BODY WEIGHT

The mean body weight increased for both sexes, except for the males on the first post-exposure day. All animals exceeded their initial body weight by the conclusion of the experimental phase.

E. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute inhalation LC_{50} of glyphosate technical after a 4-hour exposure to male and female rats, observed over a period of 14 days was greater than 5.211 mg/L air.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is not acceptable as the MMAD is far above the recommended values according to OECD 403 guideline. The MMAD ranged from 18.555 to 19.901 µm.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.3/008
Report author	[redacted]
Report year	2007
Report title	Glyphosate Technical (NUP05068) : 4-Hour acute inhalation toxicity study in rats
Report No	B02327
Document No	Not reported
Guidelines followed in study	European Communities, Directive 92/69/EEC, Part B.2 "Acute Toxicity (Inhalation)", published December 29, 1992. - OECD Guidelines for Testing of Chemicals, Section 4, No. 403: "Acute Inhalation Toxicity", adopted May 12, 1981. - U.S. Environmental Protection Agency, Health Effects Test Guidelines OPPTS 870.1300, Acute Inhalation Toxicity, August 1998. - Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF), Guidelines for Preparation of Study Results, Acute Inhalation Toxicity Studies Guideline 2-1-3. Notification 12 NohSan No. 8147, as partly revised in 16-Shouan-9260, on 16 March 2005. English translation by ACIS on 17 Oct 2005
Deviations from current test guideline (OECD 403, 2009)	The reference to the JMAFF inhalation test guideline was altered on request of the Sponsor. However, this did not affect the contents of the JMAFF inhalation test guideline. In the animal room, on brief occasions (for a total of less than 2 hours) the relative humidity was slightly higher than the upper limit of the target range given in the study plan. On the day of inhalation exposure (test day 1), the total aerosol generation period lasted 4 hours and 30 minutes, because a test aerosol was generated also for 30 minutes prior to the beginning of the exposure. This 30-minute pre-exposure aerosol generation period was used for fine-tuning of the settings of the aerosol generation and exposure system for the inhalation exposure. Consequently the nominal test atmosphere concentration was determined for the total of 4 hours and 30 minutes of aerosol generation (30 min pre-exposure aerosol generation without animals being present plus 4 h inhalation exposure of the animals). Limit test performed on 5 animals per sex. The minor deviations from the study plan were considered not to have compromised the quality, integrity or outcome of the study
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

A group of five male and five female albino rats [HanRcc:WIST(SPF)] was exposed by nose-only, flow-past inhalation to glyphosate technical (NUP 05068) at a gravimetrically determined mean aerosol

concentration of 3.252 mg/L air (s.d. ± 0.053, n = 4). This concentration was considered to represent the highest technically achievable aerosol concentration suitable for acute inhalation toxicity testing in rodents.

Two gravimetric measurements of particle size distribution during the exposure produced mass median aerodynamic diameters and geometric standard deviations (GSD) of 2.95 µm (GSD 2.97) and 3.05 µm (GSD 2.73). All animals were observed for clinical signs and mortality during and following the inhalation exposure, i.e. over a 15-day observation period. Body weights were recorded prior to exposure on test day 1, and during the observation period on test days 4, 8 and 15. On day 15, all animals were sacrificed and necropsied. The ranges of temperature, relative humidity, oxygen content, particle size and airflow measured during the exposure were considered to be satisfactory for a study of this type. There were no deaths and no macroscopic pathology findings. Clinical signs consisted of salivation in two male animals, and transient effects on breathing, i.e. deep respiration and/or rattling breath sounds, in these two and another male, as well as two female animals. Two days after the exposure (test day 3) until the scheduled necropsy day (test day 15) all animals were free from clinical signs. Losses in body weight were evident in three of five male animals (mean loss in the affected males –3.0 %) and three of five female animals (mean loss in the affected females –2.1 %), and retardation in body weight gain in one other male animal (+0.8 % weight gain) over the first three days following the inhalation exposure (test days 1 to 4). The effects on body weight were only transient and were followed by normal body weight gain in all animals. The clinical signs and the transient losses in body weight were attributed to the treatment with the test item, although slight physical stress during restraint in the exposure tubes may have contributed to the effect on body weight.

In conclusion, the LC_{50} of glyphosate technical (NUP 05068) obtained in this study was estimated to be greater than 3.252 mg/L air (gravimetrically determined mean aerosol concentration).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate Technical (NUP 05068)
Description:	White powder
Lot/Batch #:	200609062
Purity:	95.1 %
Stability of test compound:	Stable under storage conditions.

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rat
Strain:	HanRcc:WIST (SPF)
Source:	[redacted]
Age:	Males: 9 weeks; Females: 10 weeks
Sex:	5 Males / 5 Females
Weight at dosing:	Males 241.6 – 257.4 g; Females 200.6 – 219.8g
Acclimation period:	5 days
Diet/Food:	Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet, batch no. 67/06 (Provimi Kliba AG, CH-4303 Kaiseraugst/ Switzerland) ad libitum
Water:	Tap water, ad libitum

Housing:	During acclimatization in groups of five per sex in Makrolon type-4 cages with standard softwood bedding.	
Environmental conditions:	Temperature:	19 – 20 °C
	Humidity:	35 – 78 %
	Air changes:	10 – 15/hour
	12-hour light/dark cycle	

B. STUDY DESIGN AND METHODS

In life dates: 2006-12-14 to 2006-12-28

Animal assignment and treatment:

A dust aerosol was generated from the milled and pre-dried test item using a rotating brush aerosol generator (CR 3020, CR Équipements SA, CH-1295 Tannay, Switzerland) connected to amicronising jet mill. No extra diluent air was added. The generated aerosol was discharged into the exposure chamber through a ^{63}Ni charge neutraliser. The achieved mean aerosol concentration of 3.252 mg/L air administered for 4 hours was considered to represent the highest technically achievable concentration suitable for acute inhalation toxicity testing in rodents. An increase in aerosol concentration by an increased supply of test item to the rotating brush of the aerosol generator would have led to complete blockage of the rotating brush (which had happened in a pre-study technical trial not performed under GLP), and consequently to complete blockage of the aerosol generation and exposure system. Two generator cylinders containing test item were needed, in order to generate the highest technically achievable aerosol concentration over a 4-hour and 30-minute aerosol generation period.
The test atmosphere enters the top under slight positive pressure and is distributed to the entrance of each feed tube. It is then delivered through these tubes to the animal's nose. The inhalation exposure system is located inside a ducted extraction cabinet. Test atmosphere samples for the gravimetric measurements of the test item concentration and particle size distribution, and for the measurement of temperature, relative humidity and oxygen concentration, were collected directly from the feed tube in the breathing zone of the animals, at an empty port of the exposure chamber delivering "fresh" test item to the animal's nose. This approach was chosen in order to obtain representative samples of what was delivered to the animals.
The particle size distribution was determined twice during the exposure using a Mercer 7 stage cascade impactor (Model 02-130, In-Tox Products Inc., Albuquerque, New Mexico, U.S.A.).

Representative samples of the test atmosphere were drawn through the impactor with a flow rate of 1.0 L/min and the particles deposited according to their aerodynamic size onto stainless steel slips and the final filter stage (Type HVLP, Polyvinylidenedifluoride membrane, pore size 0.45 µm), on each stage of the impactor. To obtain the mass deposited on each stage of the impactor, the steel slips and the final filter stage were carefully weighed before and after sampling using a Mettler MX5 analytical balance (Mettler AG, CH-8604 Volketswil, Switzerland). The total mass (µg) deposited in the impactor was then calculated by adding together the mass deposited on each of the stainless steel slips and the final filter stage. As the Effective Cut-off Diameters (ECD) represent the lower size limit of the particles collected on each stage, the cumulative percent less than the indicated size was tabulated as a function of the ECD (Aerosol Measurement, K. Willeke & P.A. Baron, editors, page 224, Van Nostrand Reinhold, New York (1993)). This data was used to calculate the mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) using Microsoft Excel software. The target range for the MMAD was 1 to 4 µm, and was achieved.

Mortality was checked once daily during the acclimatisation period, once before exposure on the day of exposure (test day 1), at approximately 1, 2, 3 and 4 hours after exposure start, approximately 1 and 2 hours after the end of exposure on test day 1, and twice daily during the remainder of the observation period. Clinical signs were recorded at approximately 1, 2, 3 and 4 hours after exposure start and approximately 1 hour after the end of exposure on test day 1. In addition, clinical signs were recorded once daily on test

days 2 to 15. Body weights were recorded on test days 1 (before exposure), 4, 8 and 15 (day of necropsy). At the end of the observation period, all animals were sacrificed. All animals were necropsied and were examined for any abnormalities.

II. RESULTS

A. TEST ATMOSPHERE

The nominal test aerosol concentration, the mean and standard deviation of the gravimetrically determined test aerosol concentration, and the Mass Median Aerodynamic Diameters (MMAD) and their Geometric Standard Deviations (GSD) are summarized in Table 5.2.3.9-1.

Table 5.2.3-15: Glyphosate Technical (NUP05068) : 4-Hour acute inhalation toxicity study in rats [redacted] 2007): Details of test atmosphere

Nominal aerosol concentration (mg/L)	Mean achieved concentration (gravimetric) (mg/L) ± standard deviation	Mean Mass Median Aerodynamic Diameter (µm)	Geometric Standard Deviation
6.304	3.252 ± 0.053	2.95 (Impactor 1) 3.05 (Impactor 2)	2.97 (Impactor 1) 2.73 (Impactor 2)

The gravimetrically determined mean aerosol concentration was only 52 % of the nominal aerosol concentration. This was attributed to accumulation of a large proportion of the test item in the aerosol generation and exposure system. The gravimetrically determined aerosol concentration can be regarded as stable over the 4-hour inhalation exposure period. This was evident from the low standard deviation.
The MMADs were within the range of 1 to 4 µm according to OECD 403 guideline. Therefore, satisfactory deposition of the particles can be assumed in the upper and the lower respiratory tract. Hence, the particle size distribution and MMADs obtained were considered to be appropriate for acute inhalation toxicity testing.

B. MORTALITY

There were no mortalities during the study.

C. CLINICAL OBSERVATIONS

The following clinical signs were recorded during and/or after the inhalation exposure, whereby the whole range of the stated severity grades was not necessarily recorded in each affected animal: Salivation, moderate in degree, and deep respiration in two male animals (nos. 3 & 5), and breath sounds [rales], slight to marked in degree, in three male (nos. 1, 3 & 5) and two female animals (nos. 6 & 10).
The findings of salivation and deep respiration were seen at approximately 3 and 4 hours after exposure start, when the animals were restrained in the exposure tubes. Deep respiration was still evident one hour afterwards, at approximately one hour after the end of the exposure period. Breath sounds [rales] were only noticed at approximately one hour after the end of the exposure period and on the day afterwards (test day 2) after the animals had returned to their housing cages. By two days after the inhalation exposure (test day 3) all clinical signs had cleared, and all animals remained free from clinical signs until the scheduled necropsy day (test day 15).
The clinical signs are summarized in the table below.

Table 5.2.3-16: Glyphosate Technical (NUP05068) : 4-Hour acute inhalation toxicity study in rats [redacted] 2007): Clinical observation data

Clinical sign	Sex	No. of animals exposed	Exposure Day –Time after start of exposure (h)					Test Day				
			1	2	3	4	5	2	3	4	5	6-15
Deep respiration	Male	5	0	0	2	2	2	0	0	0	0	0
	Female	5	0	0	0	0	0	0	0	0	0	0
Breath sound (rales)	Male	5	0	0	0	0	3 (marked or severe)	1 (moderate) 2 (marked or severe)	0	0	0	0
	Female	5	0	0	0	0	2 (moderate)	1 (slight) 1 (moderate)	1	0	0	0
Salivation	Male	5	0	0	2	2	0	0	0	0	0	0
	Female	5	0	0	0	0	0	0	0	0	0	0

D. BODY WEIGHT

Losses in body weight were evident in three of five male animals (mean loss in the affected males –3.0 %) and three of five female animals (mean loss in the affected females –2.1 %), and retardation in body weight gain in one other male animal (+0.8 % weight gain) over the first three days following the inhalation exposure (test days 1 to 4). The effects on body weight were only transient and were followed by normal body weight gain in all animals. The body weight data are summarized in the table below.

Table 5.2.3-17: Glyphosate Technical (NUP05068) : 4-Hour acute inhalation toxicity study in rats ([redacted] 2007): Body weight data

Group		Day 1	Day 4	Day 8	Day 15
Male	Mean body weight (g)	252.1	249.2	276.8	312.1
	Standard deviation	7.2	12.1	12.4	13.0
Female	Mean body weight (g)	208.8	207.8	218.5	232.4
	Standard deviation	8.5	9.7	8.7	9.8

E. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute inhalation LC_{50} of glyphosate technical (NUP 05068) after a 4-hour exposure in male and female rats observed for a period of 15 days, was greater than 3.252 mg/L air (gravimetrically determined mean aerosol concentration). This concentration was considered to represent the highest technically achievable aerosol concentration suitable for acute inhalation toxicity testing in rodents.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was performed in accordance to OECD 403 guideline and GLP. The highest technically achievable concentration of 3.252 mg/L air was tested. The MMAD was within the recommended range according to the guideline. Otherwise, only minor deviations to the current valide OECD guideline are present. Therefore the study is acceptable. Based on the study results, the acute inhalation LC50 is >3.252 mg/L air after an exposure period of 4 hours. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.3/009
Report author	[redacted]
Report year	2005
Report title	Glyphosate Acid Technical: Acute Inhalation Toxicity Study in Rats – Limit Test
Report No	15276
Document No	Not reported
Guidelines followed in study	OPPTS 870.1300 (1998), OECD 403 and JMAFF 59 NohSan No. 4200 (1985).
Deviations from current test guideline (OECD 403, 2009)	There were no deviations from the Study Plan. Limit test performed on 5 animals per sex. Body weight measured at Day 0 and once a week afterwards. These deviations did not affect the outcome of the study.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The test substance, glyphosate technical, was evaluated for its acute inhalation toxicity potential. The test substance was administered to albino rats for a single 4-hour period using a dynamic nose-only exposure chamber. The exposure concentration, which was determined gravimetrically, was 2.04 mg/L air for 4 hours. The analysis of the particle size distribution was carried out by a cascade impactor and the mass median aerodynamic diameter (MMAD) was estimated to be 2.5 µm. All animals appeared active and healthy upon removal from the exposure chamber and over the entire 14-day observation period. There were no signs of gross toxicity, adverse pharmacologic effects or abnormal behaviour. No mortality occurred during the study and no pathological findings were noted at necropsy. All animals gained the expected body weight.

The acute inhalation LC_{50} was determined to be >2.04 mg/L air (actual concentration).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate Acid Technical

Identification:	Glyphosate Acid Technical
Description:	White crystalline powder
Lot/Batch #:	040205
Purity:	97.23 %
Stability of test compound:	Test substance was expected to be stable for the duration of testing.

2. Test animals:

Species:	Rat albino
Strain / Stock:	Sprague-Dawley derived
Source:	[redacted]
Age:	9-10 weeks
Sex:	5 male and 5 female
Weight at dosing:	Males: 280 – 318 g; Females: 205 – 224 g
Acclimation period:	13 days
Diet/Food:	Purina Rodent Chow #5012, *ad libitum*
Water:	Filtered tap water, *ad libitum*
Housing:	Individual housing in suspended stainless steel cages with mesh floors. Litter paper was placed beneath the cage and was changed at least three times per week.
Environmental conditions:	Temperature: 19 – 23 °C 12-hour light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2003-05-17 to 2003-05-24

Animal assignment and treatment:

The study was carried out using a nose-only inhalation chamber with an internal volume of approximately 6.7 L and approximately 283 air changes per hour during the study. Animals were individually housed in polycarbonate holding tubes. The test item was micronized before administration and aerosolized using a dust generator which was directly connected to the inhalation chamber. Gravimetric samples were withdrawn at 6 intervals from the breathing zone of the animals to gravimetrically determine the dust concentration in the inhalation chamber. Particle size distribution of the test atmosphere was determined with an Andersen Cascade Impactor. Samples were withdrawn from the breathing zone of the animals at two intervals. Animals were exposed for 4 hours and 1 minute to an actual concentration of 2.04 mg/L air (determined gravimetrically).
Observations for mortality and clinical/behavioural signs of toxicity were made upon removal from the exposure chamber and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to test substance exposure and on Days 7 and 14.
On Day 14 after dosing, each animal was euthanized by an overdose of CO_2. All study animals were subjected to gross necropsy and all abnormalities were recorded.

II. RESULTS

A. DUST CONCENTRATION AND PARTICLE SIZE DISTRIBUTION

The gravimetric and nominal chamber concentrations were 2.04 and 8.99 mg/L, respectively. The mass median aerodynamic diameter was estimated to be 2.5 µm based on the particle size distribution as measured with an Andersen Cascade Impactor.

B. MORTALITY

There were no mortalities during the study.

C. CLINICAL OBSERVATIONS

All animals appeared active and healthy upon removal from the exposure chamber and over the entire 14-day observation period.

D. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance.

E. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute inhalation LC_{50} of glyphosate technical after a 4-hour exposure to male and female rat, observed a period of 14 days was greater than 2.04 mg/L air.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was performed in accordance to OECD 403 guideline and GLP. The MMAD and the applied concentration were in the range of the recommended values of the current OECD guideline. Otherwise, only minor deviations to the current valide OECD guideline are present. Therefore the study is acceptable. Based on the study results, the acute inhalation LC_{50} is > 2.04 mg/L air after an exposure period of 4 hours. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.3/010
Report author	[redacted]
Report year	2004
Report title	An Acute Nose-Only Inhalation Toxicity Study in Rats with MON 78623
Report No	3044.969
Document No	SB-2003-116
Guidelines followed in study	EC method B.2, OECD 403 (1981), EPA Health Effects Test Guidelines, OPPTS 870.1300, JMAFF 12 Nohsan No. 8147
Deviations from current test guideline (OECD 403, 2019)	Limit test performed on 5 animals per sex. Body weight measured at Day 0 and once a week afterwards.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The four-hour nose-only inhalation toxicity of the test substance MON 78623 containing 47.2 % glyphosate (57.8 % potassium salt of glyphosate) was evaluated in Sprague Dawley rats. Two limit tests were performed in which one group of five male and five female rats each received a four-hour nose-only inhalation exposure to a time-weighted average aerosol concentration (analytically determined) of 2.21 or 5.27 mg/L. For the first exposure (2.21 mg/L) the mass median aerodynamic diameter and geometric standard deviation of the sampled particles were 2.9 µm and 2.18, respectively. The percentage of particles ≤ 4.0 µm was determined to be 67 %. Since there was no mortality, a second limit test was conducted at a greater target concentration. For the second exposure (5.27 mg/L) the mass median aerodynamic diameter and geometric standard deviation of the sampled particles were 3.8 µm and 2.20, respectively. The percentage of particles ≤ 4.0 µm was determined to be 53 %. Following each exposure, the limit test rats were observed daily and weighed weekly. A gross necropsy examination was performed on all limit test animals at the time of scheduled euthanasia (day 14).
No mortality occurred for the 2.21 mg/L dose level. The most notable clinical abnormalities observed during the study included transient incidences of congested breathing and dark material around the facial area. Body weight gain was noted for all animals during the test period. No gross internal findings were observed at necropsy on study day 14.

No mortality occurred for the 5.27 mg/L dose level. The most notable clinical abnormalities observed during the study included transient incidences of congested breathing and few feces. Slight body weight loss was noted for two females during the day 0 to 7 body weight interval and for one female during the day 7 to 14 body weight interval. Body weight gain was noted for all other animals during the test period and all animals exceeded their initial body weight at study termination. No gross internal findings were observed at necropsy.

The acute inhalation LC_{50} was calculated to be >5.27 mg/L air.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	MON 78623
Description:	Clear colourless liquid (pipet), light amber liquid (bulk)
Lot/Batch #:	GLP-0306-14124-F
Purity:	47.2 % glyphosate (57.8 % potassium salt of glyphosate)
Stability of test compound:	Expiry June, 2004

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rat
Strain:	Sprague Dawley
Source:	[redacted]
Age:	8-9 weeks
Sex:	Males and females
Weight at dosing:	Males: 276 – 312 g; females: 182 – 210 g
Acclimation period:	At least 5 days
Diet/Food:	PMI Certified Rodent Chow #5002 (PMI Nutrition International), *ad libitum* (except during acclimatization to the exposure tubes and during the exposure)
Water:	Tap water, *ad libitum* (except during acclimatization to the exposure tubes and during the exposure)
Housing:	Individually housed in suspended stainless steel cages
Environmental conditions:	Temperature: 19 – 23 °C Humidity: 31 – 65 % Air changes: 10-15 per hour Light cycle: 12 hours light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2003-10-29 to 2003-12-29

Test atmosphere generation:

The test aerosol was generated with a Master Flex Pump and Pump Head (7523-30 and 77200-60) and a Pistol Spraying System. Conditioned high pressure external air was used in generating the test atmosphere. The aerosol was blown through a 5 L Elutriator, the Multi-Stage 10 L nose-only inhalation chamber and then vented from the chamber to an air treatment system which consisted of a prefilter, a HEPA filter, a charcoal bed and a water scrubbing tower.

Exposure chamber conditions:

Air flow readings were recorded at the initiation of the T99 equilibration period, at approximately 30-minute intervals during each aerosol exposure and at the conclusion of the de-equilibration period. The aerosol concentration was measured at the beginning of each aerosol exposure (after equilibration), at approximate 30-minute intervals during the aerosol exposure, and at the conclusion of each aerosol

exposure (before de-equilibration). Samples of the test article aerosol were collected in the inhalation chamber by gravimetric technique. Both gravimetric and analytical aerosol concentrations were determined. A 5 L sample of the aerosol was drawn from the breathing zone of the animals in the chamber through a preweighed glass fiber filter. For the analytical concentration, the gravimetrically obtained samples were analyzed by liquid chromatography for the non-volatile glyphosate component of the test article. These analyses were performed in order to determine the analytical (actual) concentrations of the aerosol in the chamber for each sampling period. Chamber oxygen content was measured and recorded at approximate 30-minute intervals during each aerosol exposure using a GC-501 Oxygen Detector.

Particle size distribution:

The aerosol aerodynamic particle-size distribution was determined three times during each aerosol exposure using the ITP 7 Stage Cascade Impactor. Each stage of the impactor was fitted with a preweighed glass fiber filter. Five liters per minute of the chamber air were drawn through the impactor and the change in weight of each filter was then determined and recorded. The mean particle-size distribution was subsequently determined using an Excel computer adaptation of the manual method. The Mass Median Aerodynamic Diameter, Geometric Standard Deviation and percentage of particles ≤ 4.0 µm were then determined. The results were as follows:

Table 5.2.3-18: An Acute Nose-Only Inhalation Toxicity Study in Rats with MON 78623 [redacted], 2004): Details of Test Atmosphere

Mean Achieved Actual Concentration (analytical method)	MMAD	GSD	Respirable Amount Particle Size ≤ 4 µm
(mg/L)	(µm)		(%)
2.21	2.9	2.18	67
5.27	3.8	2.20	53

MMAD = mean mass median aerodynamic diameter
GSD = geometric standard deviation

Animal assignment and treatment:

The animals chosen for study use were randomly selected from healthy stock animals using a computerized random numbers table to avoid potential bias. On day 0, the animals chosen for the limit test were weighed, placed in a nose-only exposure tube and allowed to acclimate to the exposure tube for at least one hour. Animals that appeared to have been acclimated to the exposure tube (i.e., minimal struggling and no inversion) were considered to be acceptable. Animals that did not appear to acclimate to the exposure tube were not acceptable. All animals were removed from the exposure tubes and returned to their cages.

The acceptable animals were then placed in exposure tubes and the tubes inserted into the Multi-Stage 10 L nose-only inhalation chamber and the test article aerosolized at the following levels:

Table 5.2.3-19: An Acute Nose-Only Inhalation Toxicity Study in Rats with MON 78623 ([redacted], 2004): Dose Levels

Analytical Exposure Level (mg/L)	No. of Animals	
	Male	Female
2.21	5	5
5.27	5	5

Each aerosol exposure consisted of a 3-minute T99 equilibration period, a 240-minute exposure period and a 3-minute de-equilibration period equal to the T99 equilibration period. After each aerosol exposure,

animals were removed from the exposure tubes and residual test article was removed from the animal's exterior surfaces (where practical) by wiping the haircoat with a towel. The animals were then returned to ad libitum feed and water.

The limit test animals were observed for clinical abnormalities during each aerosol exposure (no positive clinical observations were noted during either exposure), two times on study day 0 (post-exposure) and daily thereafter (days 1-14). Individual body weights were recorded just prior to dosing and weekly thereafter. On Day 14 after dosing, each animal was euthanized and all study animals were subjected to gross necropsy.

II. RESULTS

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

The most notable clinical abnormalities observed for the 2.21 mg/L dose level included transient incidences of congested breathing and dark material around the facial area.

The most notable clinical abnormalities observed for the 5.27 mg/L dose level included transient incidences of congested breathing and few faeces.

The clinical signs are summarized in the table below.

Table 5.2.3-20: An Acute Nose-Only Inhalation Toxicity Study in Rats with MON 78623 [redacted], 2004): Clinical observation data

Clinical sign	Sex	No. of animals exposed	Day of study								
			0	1	2	3	4	5	6	7	8-14
Dose level: 2.21 mg/L											
Congested breathing	Male	5	3	3	0	0	0	0	0	0	0
	Female	5	4	1	0	0	0	0	0	0	0
Dark material around eye(s)	Male	5	1	0	0	1	0	0	0	0	0
	Female	5	3	0	0	0	0	0	0	1	0
Dark material around nose	Male	5	0	0	0	0	0	0	0	0	0
	Female	5	1	0	0	0	0	0	0	0	0
Few faeces	Male	5	0	0	0	0	0	0	0	0	0
	Female	5	0	0	0	0	0	0	0	0	0
Dose level: 5.27 mg/L											
Congested breathing	Male	5	4	1	1	0	0	0	0	0	0
	Female	5	5	0	0	0	0	0	0	0	0
Dark material around eye(s)	Male	5	0	0	0	0	0	0	0	0	0
	Female	5	0	0	0	0	0	0	0	0	0
Dark material around nose	Male	5	0	0	0	0	0	0	0	0	0
	Female	5	0	0	0	0	0	0	0	0	0
Few faeces	Male	5	0	0	0	0	0	0	0	0	0
	Female	5	0	2	0	0	0	0	0	0	0

C. BODY WEIGHT

Body weight gain was noted for all animals for the 2.21 mg/L dose level.

For the 5.27 mg/L dose level, slight body weight loss was noted for two females during the day 0 to 7 body weight interval and for one female during the day 7 to 14 body weight interval. Body weight gain was noted for all other animals and all animals exceeded their initial body weight at study termination.
The body weight data are summarized in the table below.

Table 5.2.3-21: An Acute Nose-Only Inhalation Toxicity Study in Rats with MON 78623 ([redacted], 2004): Body weight data

Dose level: 2.21 mg/L		**Day 0**	**Day 7**	**Day 14**
Male	Mean body weight (g)	298	313	344
	Standard deviation	10.0	17.5	15.6
Female	Mean body weight (g)	187	201	217
	Standard deviation	4.7	7.3	9.5
Dose level: 5.27 mg/L		**Day 0**	**Day 7**	**Day 14**
Male	Mean body weight (g)	284	311	331
	Standard deviation	8.4	11.2	14.9
Female	Mean body weight (g)	201	207	213
	Standard deviation	8.7	14.4	8.1

D. NECROPSY

No gross internal findings were observed at necropsy for the 2.21 mg/L and 5.27 mg/L dose levels on study day 14.

III. CONCLUSIONS

The acute inhalation LC_{50} of the test substance MON 78623 containing 47.2 % glyphosate (57.8 % potassium salt of glyphosate) after a 4-hour exposure to male and female rats, observed over a period of 14 days was greater than 5.27 mg/L.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is performed in accordance with the current OECD 403 guideline (2009) and according to GLP. The test substance contained 47.2 % glyphosate. The MMAD and GSD were in the range of the recommended values of the current OECD guideline. Otherwise, only minor deviations to the current valide OECD guideline are present. Therefore the study is acceptable. Based on the study results, the acute inhalation LC_{50} is >5.27 mg/L air. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.3/011
Report author	[redacted]
Report year	1999
Report title	NUP5a99 62 % glyphosate MUP: Acute inhalation toxicity study in rats – Limit test
Report No	7909
Document No	Not reported
Guidelines followed in study	Health Effects Test Guidelines, OPPTS 870.1300 (1998)
Deviations from current test guideline (OECD 403, 2009)	Limit test performed on 5 animals per sex. Body weight measured at Day 0 and once a week afterwards. These deviations did not affect the outcome of the study.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

After establishing the desired generation procedures during pre-test trials, ten healthy rats were exposed to isopropylamine glyphosate (NUP5a99 (62 % glyphosate MUP)) 2.08 mg/L for 4 hours. Chamber concentration and particle size distributions of the test substance were determined periodically during the exposure period. The animals were observed for mortality, signs of gross toxicity, and behavioral changes at least once daily for 14 days. Body weights were recorded prior to exposure and again on Days 7 and 14 (termination). Necropsies were performed on all animals at terminal sacrifice. All animals survived exposure to the test atmosphere and gained bodyweight over the 14-day observation period. The gravimetric chamber concentration was 2.08 mg/L. Based on graphic analysis of the particle size distribution as measured with an Andersen Cascade Impactor, the mass median aerodynamic diameter was estimated to be 2.6 microns. In-chamber animal observations included ocular and nasal discharge, hunched posture and hypoactivity. Apart from test substance noted on the fur, all animals recovered from the above symptoms upon removal from the exposure chamber and appeared active and healthy throughout the study. Gross necropsy findings at terminal sacrifice were unremarkable. Based on the results of this study, the single exposure

acute inhalation LC_{50} of NUP5a99 62 % glyphosate MUP is >2.08 mg/L air, the maximum achievable concentration.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification: NUP5a99 62 % glyphosate MUP
Description: clear viscous amber liquid
Lot/Batch #: Drum Sample E
Purity: 62 %

Stability of test compound:	No data available
2. Vehicle and/or positive control:	None
3. Test animals:	
Species:	Rat
Strain:	Sprague-Dawley derived, albino
Source:	[redacted]
Age:	Not specified
Sex:	5 males and 5 females
Weight at dosing:	Males: 224 – 256 g; females: 179 – 201 g
Acclimation period:	10 days
Diet/Food:	Purina Rodent Chow #5012
Water:	Tap water, *ad libitum*
Housing:	individually housed in suspended stainless steel caging with mesh floors. Litter paper was placed beneath the cage and was changed at least three times per week.
Environmental conditions:	Temperature: 22 – 24 °C Humidity: not specified Air changes: not specified 12-hour light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: August 6-20, 1999

Test atmosphere generation:

Prior to initiation of the full inhalation study, pre-test trials were conducted to establish generation procedures for achieving as closely as possible the desired chamber concentration (2.0 mg/L) and desired particle size distribution (mass median aerodynamic diameter ≤ 4 /µm). The test atmosphere was generated using 0.25 inch JCO atomizer, FC4 fluid cap and AC1502 air cap (Spraying Systems Inc.). Compressed air was supplied at 30 psi. The test substance was metered to the atomization nozzle through Size 14 Master Flex Tygon tubing, using a Master Flex Pump Model 7520-35.

Exposure chamber conditions:

The animals were placed in a rectangular whole body plexiglass chamber with a volume of 150 liters with prechamber operated under slight negative pressure, and were exposed to the test atmosphere for 4 hours and 15 minutes. The exposure period was extended beyond 4 hours to allow the chamber to reach equilibrium (T_{99}).

Particle size distribution:

An eight-stage Andersen cascade impactor was used to assess the particle size distribution of the test atmosphere. Samples were withdrawn from the breathing zone of the animals at two intervals. The aerodynamic mass median diameter and geometric standard deviation were determined graphically using two-cycle logarithmic probit axes. The mass median aerodynamic diameter was estimated to be 2.6 µm based on the particle size distribution.

Table 5.2.3-22: NUP5a99 62 % glyphosate MUP: Acute inhalation toxicity study in rats – Limit test ([redacted], 1999): Details of test atmosphere

Chamber concentration (mean)*	Standard deviation	MMAD (mean of 2 samples)	GSD (mean of 2 samples)
(mg/L air)		(µm)	
2.08	0.07	2.6	1.72

* The chamber concentration was measured every 30 minutes.
MMAD = mean mass median aerodynamic diameter
GSD = geometric standard deviation

Animal assignment and treatment:

A group of five rats per sex received the test material at a dose level of 2.08 mg/L by whole body exposure. The animals were observed for mortality, signs of gross toxicity, and behavioral changes at least once daily for 14 days. Bodyweights were recorded prior to exposure and again on Days 7 and 14 (termination). Necropsies were performed on all animals at terminal sacrifice.

II. RESULTS

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

In-chamber animal observations included ocular and nasal discharge, hunched posture and hypoactivity. Apart from test substance noted on the fur, all animals recovered from the above symptoms upon removal from the exposure chamber and appeared active and healthy throughout the study.

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance.

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute inhalation LC_{50} of isopropylamine glyphosate (NUP5a99 62 % glyphosate MUP) after a 4-hour exposure to male and female rats, observed over a period of 14 days is greater than 2.08 mg/L air.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was performed similar to OECD 403 guideline and according to GLP. The test substance isopropylamine glyphosate contained 62 % glyphosate MUP. The MMAD and GSD were in the range of the recommended values of the current OECD guideline. Otherwise, only minor deviations to the current valide OECD guideline are present. Therefore the study is acceptable. Based on the study results, the acute inhalation LC_{50} of isopropylamine glyphosate is >2.08 mg/L air after an exposure period of 4 hours. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.3/012
Report author	[redacted]
Report year	1996
Report title	Glyphosate Acid: 4-Hour Acute Inhalation Toxicity Study In Rats
Report No	[redacted]/P/4882
Document No	Not reported
Guidelines followed in study	OECD 403 (1981): OPPTS 870.1300 (1998): 92/69/EEC B.2 (1992) + amendment 93/21/EEC (1993)
Deviations from current test guideline (OECD 403, 2009)	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

In an acute inhalation toxicity study, groups of five male and five female Alpk:APfSD (Wistar-derived) rats were exposed nose-only for a single four-hour period to glyphosate technical at target particulate concentrations of 5 mg/L and 2 mg/L. The particle size distribution of the test atmosphere was analysed at frequent intervals during the exposure period. Following exposure, the animals were retained without treatment for 14 days. Clinical observations and bodyweights were recorded and at the end of the scheduled period, the animals were killed and subjected to an examination *post mortem*.

The achieved test atmosphere had the following characteristics:

Table 5.2.3-23: Glyphosate Acid: 4-Hour Acute Inhalation Toxicity Study In Rats ([redacted] 1996): Details of test atmosphere

Target concentration mg/L	Achieved particulate concentration mg/L	MMAD* µm	GSD+
2	2.47± 0.15	3.57, 3.03	1.94, 1.90
5	4.43 ± 1.297	2.91, 3.41	1.74, 2.04

* Mass Median Aerodynamic Diameter (µm)
+ Geometric Standard Deviation

Two males and two females exposed to 4.43 mg glyphosate technical/L were found dead or were terminated *in extremis* during the observation period, the remaining animals in this group survived until scheduled termination. Clinical signs indicative of moderate toxicity was seen in this group. All surviving animals had regained their initial bodyweight by the end of the study.

Similar but less severe clinical signs were seen in animals exposed to 2.47 mg/L, all animals survived and

showed complete recovery by the end of the study. All animals exposed to 2.47 mg/L survived to scheduled termination.

It was concluded that the acute inhalation LC_{50} of glyphosate technical exceeded 4.43 mg/L air for male and female rats.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:**

Identification:	Glyphosate acid
Description:	Technical; white solid
Lot/Batch #:	P25
Purity:	95.6 % w/w
Stability of test compound:	Confirmed by Sponsor

2. **Vehicle and/or positive control:** None

3. **Test animals:**

Species:	Rat
Strain:	Alpk:APfSD
Source:	[redacted]
Age:	Young adult; 9 – 12 weeks old at delivery
Sex:	Males and females
Weight at dosing:	243 – 365 g (males); 210 – 247 g (females) at the start of exposure
Acclimation period:	At least five days
Diet/Food:	PCD diet (Special Diet Services Limited, Witham, Essex, UK), *ad libitum* except during exposure.
Water:	Mains water, *ad libitum* except during exposure
Housing:	5 per cage, sexes separately, except during exposure, in rat racks suitable for animals of the strain and weight range expected during the study
Environmental conditions:	Temperature: 21 ± 2 °C Humidity: 40 – 70 % Air changes: at least 15/hour Photoperiod: 12-hour light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 1995-11-22 to 1996-03-11

Exposure conditions: Trial generations were carried out prior to the start of the study in order to determine the appropriate generation system and conditions, to determine the appropriate target concentration that could be achieved, or if not, what was the maximum stable attainable concentration, to obtain data on the aerodynamic particle size of the atmosphere generated, to determine an appropriate method of analysis of glyphosate acid. Exposure conditions during the study are given later in a table of the test atmosphere characteristics of glyphosate acid.

Animal assignment and treatment: The study consisted of two main study groups of 5 rats/sex/group exposed nose-only for a single four-hour period to glyphosate acid at target particulate concentrations of 5 mg/L and 2 mg/L. Prior to the start of the study the rats were examined to ensure that they were physically normal and exhibited normal activity. During exposure they were observed frequently and, at the end of the 4-hour exposure period, each rat was given a detailed clinical examination. They were also subjected to detailed clinical observations, daily during a 14-day observation period. The bodyweight of each rat was recorded on day -1, 1, 8 and prior to termination on day 15. All rats were killed on day 15 and subjected to a gross examination *post mortem* involving external observation and careful internal examination of all thoracic and abdominal viscera.

Generation of the test atmosphere / chamber description: Before exposure of the test animals, the atmosphere was shown to have been acceptably stable. The test atmosphere was generated using a modified Wright's dust-feed mechanism. Clean, dry air was passed through the dust feed at a nominal flow rate of 2.5 L/minute (at normal temperature and pressure) and carried the atmosphere to the exposure chamber, having an internal volume of 27.6 litres. Since diluting air was not employed, the flow rate through the exposure chamber was the same as that employed in the generation of the test atmosphere. Air flows were monitored and recorded at approximately 30 minute intervals using variable area flow-meters and were altered as necessary to maintain target concentration. Animals were exposed nose-only to the atmosphere. They were restrained in polycarbonate tubes (Battelle, Switzerland), which were inserted into the Perspex exposure chamber. The chamber was covered with an aluminium cone and stood on an aluminium base.

Test atmosphere concentration: The particulate concentration of the test atmosphere, close to the animals' breathing zone, was measured gravimetrically at frequent intervals during the exposure period. This was done by drawing the test atmosphere, at a known flow rate, for a known time, through a 25 mm diameter, polyvinyl chloride (PVC) GLA 5000 filter housed in a Delrin open-faced filter holder. The filter was weighed before and after the sample was taken. The concentration was calculated as follows:

$$\text{Concentration (mg/L)} = \frac{\text{post wt (mg)} - \text{pre wt (mg)}}{\text{time (minutes) x airflow (L/minute)}}$$

Pre wt = weight of filter prior to sampling
Post wt = weight of filter after sampling

Particle size determination: The aerodynamic particle size distribution of the test atmosphere was measured twice during the exposure period, using a Marple Cascade Impactor, which aerodynamically separates airborne particles into pre-determined size ranges. Using a microcomputer, the data were transformed using a log/probit transform and a linear regression derived from the cumulative data. The linear regression line was then used to calculate the mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD).

Table 5.2.3-24: Glyphosate Acid: 4-Hour Acute Inhalation Toxicity Study In Rats ([redacted] 1996): Summary of acute study test atmosphere characteristics

Parameter	Target concentration 5 mg/L				Target concentration 2 mg/L			
Measured particulate concentration	4.43 ± 1.30 mg/L				2.47 ± 0.15 mg/L			
% total particulate	96.9 ± 4.2				98.5 ± 7.7			
Analysed concentration of glyphosate acid(mg/L)	Mean 4.27 ± 1.15				Mean 2.43 ± 0.19			
Particle size MMAD; GSD	2.91, 3.41µm; 1.74, 2.04				3.57, 3.03µm; 1.94, 1.90			
Size range (µm)	% by weight in range				% by weight in range			
	Run 1 (1hr 35min into exposure)		Run 2 (3hr 29min into exposure)		Run 1 (54min into exposure)		Run 2 (2hr 59min into exposure)	
	Analysed	Gravi-metric	Analysed	Gravi-metric	Analysed	Gravi-metric	Analysed	Grav-imetric

Table 5.2.3-24: Glyphosate Acid: 4-Hour Acute Inhalation Toxicity Study In Rats ([redacted] 1996): Summary of acute study test atmosphere characteristics

Parameter	Target concentration 5 mg/L				Target concentration 2 mg/L			
Particles > 9.8 µm (% w/w)	0.9	0.7	5.1	4.3	3.1	3.1	2.0	2.0
Particles 9.8-6.0 µm (% w/w)	21.1	20.6	26.3	23.8	20.9	23.0	16.7	16.4
Particles 6.0-3.5 µm (% w/w)	34.4	35.5	34.3	31.3	47.9	46.4	37.7	36.8
Particles 3.5-1.55 µm (% w/w)	32.1	32.3	21.3	28.0	19.5	18.8	38.0	36.9
Particles 1.55-0.93 µm (% w/w)	7.5	7.6	9.1	8.6	5.9	5.4	3.6	4.0
Particles 0.93-0.52 µm (% w/w)	2.2	2.3	2.7	2.3	1.9	2.0	1.3	1.6
Particles ≤0.52 µm (% w/w)	1.5	1.0	1.3	1.8	0.8	1.3	0.8	2.4
Flow rate (whole system)	2.5 L/min							
Temperature	14.7 – 21.7 °C							
Humidity	25 – 65 %							

- Percentages are calculated as follows:
Gravimetric: weight trapped at each size range x 100 / Total weight trapped

Statistics: The acute inhalation LC_{50} was estimated.

II. RESULTS

A. MORTALITY

Two males and two females exposed to 4.43 mg/L air were found dead or were terminated in extremis on days 5, 6 or 9 of the study, the remaining animals in this group survived until scheduled termination.

There were no mortalities at 2.47 mg/L air.

Table 5.2.3-25: Glyphosate Acid: 4-Hour Acute Inhalation Toxicity Study In Rats ([redacted] 1996): Mortality / animals treated

Target exposure concentration mg/L	Day number	Cumulative mortality (Number dead / total)		
		Males	Females	Combined
5	5	1/5	0/5	1/10
	6	2/5	1/5	3/10
	9	2/5	2/5	4/10
	14	2/5	2/5	4/10
2	14	0/5	0/5	0/10

B. CLINICAL OBSERVATIONS

Abnormalities generally associated with restraint (wet fur) were seen in all animals during exposure. Clinical changes seen were salivation, irregular breathing and auditory hypoaesthesia, these effects were considered to be related to treatment.

Immediately after exposure, abnormalities generally associated with restraint (hunched posture, piloerection and wet fur) were seen in both males and females. At an exposure concentration of 4.43 mg/L the clinical abnormalities seen in both sexes included breathing irregularities, reduced righting reflex, shaking, splayed gait andwere considered to be indicative of moderate toxicity.

At an exposure concentration of 2.47 mg/L the number of adverse clinical changes observed was reduced in both sexes. Those abnormalities observed were similar to those seen in animals exposed to 4.43 mg/L glyphosate acid.

The clinical condition of most animals appeared to have improved by day 5 of the study, with the exception of 2 males and 2 females exposed to 4.43 mg/L. There was generally an improvement in clinical condition during the remainder of the study.

Table 5.2.3-26: Glyphosate Acid: 4-Hour Acute Inhalation Toxicity Study In Rats [redacted] 1996): Clinical observation data during exposure

Group	Time into exposure (min)	Abnormalities*
4.43 mg/L	30	Most animals: wet fur, changes in breathing rate and depth and reduced response to sound
	60	All animals: salivation Most animals: wet fur, changes in breathing rate and depth and reduced response to sound
	90	All animals: salivation Most animals: wet fur, changes in breathing rate and depth and reduced response to sound
	120	All animals: salivation Most animals: wet fur, changes in breathing rate and depth and reduced response to sound
	150	Some animals: lachrymation All animals: salivation Most animals: wet fur, changes in breathing rate and depth and reduced response to sound
	180	Some animals: lachrymation All animals: salivation Most animals: wet fur, changes in breathing rate and depth and reduced response to sound
	210	Some animals: lachrymation All animals: salivation Most animals: wet fur, changes in breathing rate and depth and reduced response to sound
2.47 mg/L	30	Some animals: wet fur, changes in breathing rate and depth
	60	Some animals: wet fur, changes in breathing rate and depth
	90	Most animals: wet fur, reduced response to sound Some animals: changes in breathing rate and depth
	120	Most animals: wet fur, reduced response to sound Some animals: changes in breathing rate and depth
	150	All animals: wet fur, no response to sound, reduced breathing rate and increased breathing depth or irregular breathing, test substance staining nose Some animals: gasping, lachrymation
	180	All animals: wet fur, no response to sound, reduced breathing rate and increased breathing depth or irregular breathing, test substance staining nose Some animals: gasping, lachrymation
	210	All animals: wet fur, no response to sound, reduced breathing rate and increased breathing depth or irregular breathing, test substance staining nose Some animals: gasping, lachrymation

* No individual animal data provided in the study report.

Table 5.2.3-27: Glyphosate Acid: 4-Hour Acute Inhalation Toxicity Study In Rats [redacted] 1996): Clinical observation data following exposure

Clinical sign	Sex	No. of animals exposed	Day following exposure														
Group 1: 4.43 mg/L			**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
Activity decreased	Male	5	5	1													
	Female	5	5					1									
Breathing depth	Male	5	2														
	Female	5	1								1						
Breathing rate	Male	5	2														
	Female	5	1								1						
Breathing irregular	Male	5	5	5	3	3		1									
	Female	5	4	5	1												
Gasping	Male	5	3														
	Female	5	2					1									
Hunched	Male	5	5	5	5	5		3	3	3	3	3	3	3			
	Female	5	5	5	5	5		5	4	4	4	3	3	3	2		
Found dead (M) / Killed in extremis-toxic (F)	Male	5					1	1									
	Female	5						1			1						
Killed termination	Male	5															3
	Female	5															3
Piloerection	Male	5	5	5	5	5		3	3								
	Female	5	5	5	5	5		5	4								
Abnormal respiratory noise	Male	5	5	5	5	5	4	3	3								
	Female	5	5	4	2	2	5	2	1	1	1						
Reduced righting reflex	Male	5	1														
	Female	5															
Salivation	Male	5	5														
	Female	5	5														
Shaking	Male	5	5														
	Female	5	5														
Splayed gait	Male	5	5	5	5	5		3									
	Female	5	1	1	1												
Sides pinched in	Male	5															
	Female	5							1	1	1						
Reduced stability	Male	5	3														
	Female	5	5														
Signs of urinary incontinence	Male	5		4													
	Female	5		2													
Tail erection	Male	5															
	Female	5	1	1	1	1		1	1								
Thin	Male	5															
	Female	5						1									
Test substance round snout	Male	5	5														
	Female	5	5														
Vocalisation	Male	5					4										
	Female	5					5										
Wet fur	Male	5	5														

Table 5.2.3-27: Glyphosate Acid: 4-Hour Acute Inhalation Toxicity Study In Rats ([redacted] 1996): Clinical observation data following exposure

Clinical sign	Sex	No. of animals exposed	Day following exposure														
	Female	5	5														
Group 2: 2.47 mg/L																	
Activity decreased	Male	5	1														
	Female	5															
Breathing depth	Male	5	4	4	3	1											
	Female	5	2	2													
Breathing rate	Male	5	4	4	2												
	Female	5	3	3													
Breathing irregular	Male	5	2	2	1	2											
	Female	5	2	2	2	2											
Chromodacryorrhea	Male	5	1														
	Female	5															
Head flicking	Male		1														
	Female																
Hunched	Male	5	5	5	5	5	1										
	Female	5	5	5	5	5	1										
Killed termination	Male	5															5
	Female	5															5
Lachrymation	Male		2														
	Female																
Paw flicking	Male		1														
	Female																
Piloerection	Male	5	5	5	5	5	5	3									
	Female	5	5	5	5	5	5	4									
Abnormal respiratory noise	Male	5	5	5	4	3	3	3	2	2	1	1	1	1	1	1	1
	Female	5	4	4	4	2	1	1									
Salivation	Male	5	5														
	Female	5	5														
Scabs	Male	5												1	1	1	1
	Female	5															
Shaking	Male	5	2														
	Female	5															
Reduced stability	Male	5	1														
	Female	5	1														
Test substance round snout	Male	5	5														
	Female	5	5	1													
Wet fur	Male	5	5														
	Female	5	5														

C. BODYWEIGHT

Animals showed a treatment related reduction in bodyweight. At an exposure concentration of 4.43 mg/L all animals had exceeded their initial bodyweight by the end of the study. At an exposure concentration of 2.47 mg/L all animals had exceeded their initial weight by day 8 of the study.

The body weight data are summarized in the table below.

Table 5.2.3-28: Glyphosate Acid: 4-Hour Acute Inhalation Toxicity Study In Rats ([redacted], 1996): Body weight data

Group 1: 4.43 mg/L		Day 1	Day 2	Day 3	Day 8	Day 15
Male	Mean body weight (kg)	350.6	313.8	306.0	342.7	374.7
	Standard deviation	13.2	14.6	29.2	20.5	18.2
Female	Mean body weight (kg)	227.4	208.0	210.0	210.8	236.3
	Standard deviation	14.0	18.7	15.2	31.5	19.6
Group 2: 2.47 mg/L		**Day 1**	**Day 2**	**Day 3**	**Day 8**	**Day 15**
Male	Mean body weight (kg)	252.8	234.2	246.6	281.2	323.0
	Standard deviation	9.8	11.0	11.4	14.2	18.8
Female	Mean body weight (kg)	216.8	203.0	212.6	228.8	237.0
	Standard deviation	3.9	8.0	10.0	8.2	7.0

D. NECROPSY

In the animals exposed to 4.43 mg/L that died or were killed prior to termination, the two males found dead had dark lungs (probably a result of agonal congestion), the lungs of the females were normal.

At scheduled termination, the lungs of rats exposed to 4.43 mg/L were normal. One female exposed to 2.47 mg/L had red spots on the lungs and another female had dark lungs. These findings are considered to be incidental to treatment. Changes at necropsy in a variety of tissues in males exposed to 2.47 mg/L were of low incidence and were considered to be unrelated to treatment.

III. CONCLUSIONS

The acute inhalation LC_{50} of glyphosate technical after a 4-hour exposure to male and female rats, observed over a period of 14 days was greater than 4.43 mg/L air.

Assessment and conclusion

Assessment and conclusion by applicant:
The study is performed in accordance with the current OECD 403 guideline (2009) and according to GLP. The MMAD and the applied concentration were in the range of the recommended values of the current OECD guideline. Therefore, the study is acceptable. Based on the study results, the acute inhalation LC_{50} is > 4.43 mg/L air for male and female rats. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.3/013
Report author	[redacted]
Report year	1995
Report title	HR-001: Acute inhalation toxicity study in rats
Report No	[redacted] 94-0155
Document No	Not reported
Guidelines followed in study	U.S. EPA FIFRA Guideline Subdivision F, OECD 403 (1981)
Deviations from current test guideline (OECD 403, 2009)	MMAD slightly exceeded the recommended MMAD (4.8 ± 0.3 µm with a GSD of 1.7 ± 0.1). Limit test performed on 5 animals per sex. Body weight measured at Day 0 and once a week thereafter.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Five Fischer (F344/DuCrj) rats of each sex were exposed to glyphosate technical mist for 4 hours in a whole body exposure chamber followed by clinical observations for 14 days. Body weight of each animal was measured prior to exposure (day 0) and on days 7 and 14.

After the end of the observation period, all animals were euthanized and necropsied.

Mean actual atmospheric concentration of HR-001 was 5.48 mg/L. Mean values of mass median aerodynamic diameters and geometric standard deviations were 4.8 ± 0.3 and 1.7 ± 0.1 µm. More than 91 % of the test substance consisted of particles with an aerodynamic diameter less than 10 µm or less. Clinical observations of animals during the exposure was impossible due to heavy foggy test substance mist in the chamber. There were no deaths in either sex during exposure or the subsequent 14-day observation period. After the termination of exposure, wetted fur in the perioral and in periocular regions, and red adhesive materials in the periocular and in nasorostral regions were observed. These signs were respectively slight in degree and disappeared by day 4 in males and day 5 in females. All animals gained the expected body weight on day 7 and 14 when compared with those on day 0. No abnormalities were detected in any animal of either sex at final necropsy after the end of observation period.

The acute inhalation LC_{50} was calculated to be >5.48 mg/L air.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification: Glyphosate TC
Description: Whitish crystals
Lot/Batch #: T-941209
Purity: 97.56 %

Stability of test compound:	Not mentioned in the report.

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rat
Strain:	F344/DuCrj
Source:	[redacted]
Age:	8 weeks
Sex:	Males and females
Weight at dosing:	♂ 176 – 187 g; ♀ 138 – 144 g
Acclimation period:	8 days
Diet/Food:	certified pellet diet MF (Lot No. 950109, Oriental Yeast Co., Ltd., Tokyo), *ad libitum*
Water:	tap water, *ad libitum*
Housing:	By group of 5 animals of the same sex in stainless steel wire cages during pre- and post-exposure periods. Individually in stainless steel wire cages during exposure.
Environmental conditions:	Temperature: 22 ± 2 °C Humidity: 55 ± 15 % Air changes: 10/hour 12 hours light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 1995-03-28 to 1995-04-20

Test atmosphere generation:

The dust was generated by a turn-table type dust feeder with compressed air from air compressor. The compressed air was supplied to the dust feeder through an air filter. The air was introduced into the chamber as diluting air after filtering it through a HEPA filter.

Exposure chamber conditions:

The nominal atmospheric concentration of HR-001 was calculating by dividing the total amount of the test substance supplied to the dust feeder during the 4-hour exposure by the total air volume delivered during the exposure.

The actual atmospheric concentration was measured gravimetrically and analysed by high-performance liquid chromatography (HPLC).

Particle size distribution:

The results for the air samples taken for the determination of particle size distribution are given in the table below.

Table 5.2.3-29: HR-001: Acute inhalation toxicity study in rats [redacted] 1995): Particule size distribution

Exposure group	Time of sampling	Analytical concentration	Particule size	
mg/L	(min)	(mg/L)	MMAD[1] (µm)	σg[2]
5.48	60	6.54	5.0	1.6
	120	4.80		
	180	5.11	4.6	1.8
	Mean	5.48	4.8	1.7
	S.D.[3]	0.93	0.3	0.1

[1] MMAD: Mass median aerodynamic diameter
[2] σg: geometric standard deviation
[3] S.D.: Standard deviation

The results revealed a MMAD of 4.8 µm (σg = 1.7) during the exposure period. Thus more than 91 % of the test substance dust consisted of particles of inhalable size.

Animal assignment and treatment:

Groups of 5 male and 5 female specific pathogen free Fisher rats (F344/DuCrj) were exposed (whole-body) continuously for 4 hours to test substance mist containing concentrations of glyphosate at 5.48 mg/L. The flow rate was stable at approximately 100 L/min. Mortality and signs of reaction to treatment were recorded during a subsequent 14-day observation period. All animals were observed for clinical signs at 2 hours after the initiation of exposure, immediately and at 2 hours after the termination of exposure. In addition, animals were observed for lethality at 4 hours after the termination of exposure. All animals were weighed shortly before the exposure and on days 7 and 14. The surviving animals were euthanized on the following day (day 15). All animals were subjected to necropsy.

II. RESULTS

A. MORTALITY

There were no deaths in either sex at the tested concentration of 5.48 mg/L.

B. CLINICAL OBSERVATIONS

No clinical observations were performed during the exposure due to foggy dust in the exposure chamber. No notable serious changes were observed as clinical signs after the exposure period. Wetted and soiled fur in the periocular and nasorostral regions were not considered to be particularly caused by the test substance because the changes were slight in degree and are frequently observed in the acute inhalation toxicity study. The clinical signs are summarized in the table below.

Table 5.2.3-30: HR-001: Acute inhalation toxicity study in rats ([redacted] 1995): Clinical observation data

Clinical sign	Sex	No. of animals exposed	Exposure Day (h)			Post-exposure Day				
			During exposure[1]	After exposure						
			2	0	2	1	2	3	4	5-14
Wetted fur in perioral region	Male	5	-	5	0	0	0	0	0	0
	Female	5	-	5	0	0	0	0	0	0

Table 5.2.3-30: HR-001: Acute inhalation toxicity study in rats ([redacted] 1995): Clinical observation data

Clinical sign	Sex	No. of animals exposed	Exposure Day (h)			Post-exposure Day				
			During exposure[1]	After exposure						
			2	0	2	1	2	3	4	5-14
Red adhesive materials in periocular region	Male	5	-	5	4	4	3	1	0	0
	Female	5	-	1	2	5	1	0	0	0
Wetted fur in periocular region	Male	5	-	5	0	0	0	0	0	0
	Female	5	-	5	0	0	0	0	0	0
Red adhesive materials in nasorostral region	Male	5	-	0	4	4	2	1	0	0
	Female	5	-	0	5	2	1	1	1	0

[1]Animals were not observed due to foggy dust in the chamber

C. BODY WEIGHT

All animals gained weights, reflecting their good healthy conditions.

D. NECROPSY

No abnormalities were observed in any animal of either sex at necropsy.

III. CONCLUSIONS

The acute inhalation LC_{50} of glyphosate technical after a 4-hour exposure to male and female rats, observed over a period of 14 days was greater than 5.48 mg/L.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was performed in accordance to OECD 403 guideline and GLP. The MMAD slightly exceeded the recommended value of the current OECD guideline. Otherwise, only minor deviations to the current valide OECD guideline are present. Therefore the study is acceptable. Based on the study results, the acute inhalation LC_{50} is > 5 mg/L air after an exposure period of 4 hours. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.3/014
Report author	[redacted]
Report year	1994
Report title	Glyphosate: Acute inhalation toxicity study four-hour exposure (nose only) in the rat
Report No	710/16
Document No	Not reported
Guidelines followed in study	OECD 403 (1981)
GLP	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Short description of study design and observations:	One single group of ten Sprague-Dawley strain rats (five males and five females) was exposed for 4 hours to an atmosphere containing 5.35 mg/L of the test material (glyphosate technical, purity: 95 %), using a nose-only exposure system. The generated dust had a mass median aerodynamic diameter (MMAD) of 4.4 µm. The Geometric Standard Deviation (GSD) of the MMAD was not reported. All animals were observed for clinical signs at hourly intervals during the exposure, immediately on removal from the restraining tubes at the end of the exposure, one hour after termination of the exposure and subsequently once daily for 14 days. All rats were weighed before dosing and on days 7 and 14 post exposure. At sacrifice, all animals were subjected to a macroscopic *post mortem* examination.
Short description of results:	No mortality occurred during the study. During exposure, wet fur was common and there were incidents of decreased respiratory rate. On removal from the chamber, hunched posture and piloerection were additionally noted. Three animals showed ptosis and one animal had brown staining of the fur on the head. One hour after completion of exposure, wet fur was no longer evident. On day one following exposure there were still two rats exhibiting hunched posture and/or piloerection. There were no further abnormalities observed for the rest of the study. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no pathological findings. The acute inhalation LC_{50} was calculated to be >5.35 mg/L.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	Study report not available. Monograph (2000): The study is considered acceptable.
Reasons why the study report is not available for submission	The notifier has not access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a “request for administrative assistance” (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL
Category study in AIR 5 dossier (L docs)	Category 4a

1. Information on the study

Data point:	CA 5.2.3/015
Report author	[redacted]
Report year	1994
Report title	Glyphosate (Alkaloida, Tiszavasvari): Acute inhalation toxicity in rats
Report No	GHA-94-403/R
Document No	Not reported
Guidelines followed in study	Not specified
Deviations from current test guideline (OECD 403, 2009)	MMAD not measured. Not tested up to recommended limit concentration (2.876 mg/L). Limit test performed on 5 animals per sex instead of 3. Body weight measured at Day 0 and once a week afterwards. Mode of exposure not stated.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The test substance, glyphosate technical, was evaluated for its acute inhalation toxicity potential in rats when exposed for a single 4-hour period at nominal concentrations of 0, 1.138 and 2.876 mg/L and measured concentrations of 0, 0.653 and 1.538 mg/L, respectively.

No mortality occurred during the study. No clinical signs were observed. There was no effect on body weight gain. The gross necropsy and histopathological evaluation conducted at termination of the study demonstrated no pathological and histopathological findings.

The acute inhalation LC_{50} was calculated to be >2.876 mg/L air (nominal).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification: Glyphosate
Description: White crystalline powder
Lot/Batch #: 36300892
Purity: 97.2 %
Stability of test compound: Not reported

2. Vehicle and/or positive control: Water/ None

3. Test animals:

Species: Rat
Strain: Wistar

Source:	[redacted]
Age:	No data
Sex:	Males and females
Weight at dosing:	Males: 190 – 200 g; Females: 150 – 160 g
Acclimation period:	One week
Diet/Food:	Standard Altromin rodent chow (except during the exposure period)
Water:	Fresh water, *ad libitum* (except during the exposure and the 4-hours post-exposure period)
Housing:	Groups of five animals in steel-Macrolon III cages on daily changed woodchips
Environmental conditions:	Temperature: 20 ± 2 °C Humidity: 45 – 70 % Air changes: 10/ hour 12 hours light/dark cycle

B. STUDY DESIGN AND METHODS

Test atmosphere generation:

The various aquaeous concentrations of glyphosate were maintained in homogenous state with a magnetic stirrer and released to a TUR-USI 50 therapeutic ultrasound generator at a rate providing complete vaporization.

Exposure chamber conditions:

The amounts of aerosol were measured at hourly intervals. The aerosol was introduced into the respiratory zone of the animals via the glass cone of the inhalation chamber. At hourly intervals 5 minute breaks were inserted meanwhile rats were observed for clinical signs and gravimetric measurements of the product were performed.

Particle size distribution:

No particle size distribution measurement was performed.

Animal assignment and treatment:

Three groups of five rats per sex received the test material at a dose level (nominal) of 0, 1138 and 2876 mg/m^3 via inhalation for 4 hours. The measured concentrations were 0, 653 and 1538 mg/m^3. During exposure symptoms could be observed only on a head region. Clinical observations were made immediately after dosing, at one and four hours postdose and once daily for 14 days. Individual body weights were recorded before treatment and subsequently at weekly intervals. At termination (day 14), all surviving rats were sacrificed and necropsied. Gross changes in the thoracic and abdominal organs as well as organ weights were recorded for brain, heart, thymus, stomach, spleen, lung, liver, kidneys, adrenal glands and testes and epididymis. Parallel with necropsies of rats organ fragments from lung, trachea, liver and kidney were sampled from all 20 rats of the control and high dose groups for histology. The histological changes were classified as slight, moderate and severe lesions. Significance of lesions attributable to product was controlled with the Fisher's exact test.

II. RESULTS

A. MORTALITY

No deaths occurred.

B. CLINICAL OBSERVATIONS

No clinical signs were observed neither during the exposure nor after the exposure up to the end of the observation period.

C. BODY WEIGHT

No statistically significant differences were noted in body weights. All animals gained the expected body weight.

D. ORGAN WEIGHT

The absolute and relative organ weights were not affected significantly by the test substance.

E. NECROPSY AND HISTOPATHOLOGY

No pathological findings were noted at necropsy.

No statistically significant histopathological lesions were observed. Males of the low dose group revealed a higher weight of the right testes.

III. CONCLUSIONS

Exposure to a mean concentration up to a nominal concentration of 2.876 mg/L did not result in any mortality. Thus, the acute inhalation LC_{50} of glyphosate technical after a 4-hour exposure to male and female rats, observed over a period of 14 days was calculated to be greater than 2.876 mg/L.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was performed similar to OECD 403 guideline. No information on the mode of exposure are reported. The tested concentrations are below the recommended limit concentration and no MMAD is provided. Based on these deviations, the study is considered as supportive. Based on the study results, the acute inhalation LC_{50} is > 2.876 mg/L air.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.3/016
Report author	[redacted]
Report year	1994
Report title	Glyphosate premix: Acute inhalation toxicity study four-hour exposure (nose only) in the rat
Report No	545/39
Document No	Not reported
Guidelines followed in study	OECD 403 (1981), Method B2
Deviations from current test guideline (OECD 403, 2009)	Limit test performed on 5 animals per sex. Body weight measured at Day 0 and once a week afterwards. These deviations did not affect the outcome of the study.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The test substance, glyphosate Premix containing 62.2 % as glyphosate isopropylamine salt (46.1 % as glyphosate), was evaluated for its acute inhalation toxicity potential, as a 70 % formulation with distilled water, by exposing a single group of ten Sprague-Dawley strain rats (five males and five females) to an aerosol atmosphere of 4.24 mg/L for four hours using a nose only exposure system. No mortality occurred during the study. Common abnormalities noted on the day of exposure were wet fur, hunched posture and piloerection. No similar abnormalities were observed at later time points until the end of the observation period. No mortality was observed. There was no effect on body weight gain. Six animals showed a dark area or multiple dark foci on the lungs at necropsy but no other abnormalities were detected.

The acute inhalation LC50 was determined to be > 4.24 mg/L air.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate Premix
Description:	Pale yellow liquid
Lot/Batch #:	290-JaK-146-4
Purity:	62.2 % as glyphosate isopropylamine salt; 46.1 % as glyphosate
Stability of test compound:	Stable at room temperature

2. Vehicle and/or positive control: Vehicle: distilled water (the test material was a 70 % formulation in distilled water)

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley

Source:	[redacted]
Age:	No data (youg adult)
Sex:	Males and females
Weight at dosing:	Male: 200 – 213g; Female: 190 – 203g
Acclimation period:	At least 5 days
Diet/Food:	Rat and Mouse Expanded Diet No. 1 (Special Diets Services Limited, Witham, Essex, UK), *ad libitum* (except during exposure)
Water:	drinking water, *ad libitum* (except during exposure)
Housing:	In groups of 5 animals per cage in solid floor, polypropylene cages, with sawdust bedding
Environmental conditions:	Temperature: 19 – 23 °C Humidity: 58 – 64 % Air changes: 15/hour 12 hours light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 1994-03-29 to 1994-05-10

Test atmosphere generation:

The test atmosphere was generated using a glass concentric jet nebuliser (Radleys, Saffron Walden, Essex).

Exposure chamber conditions:

The cylindrical exposure chamber had a volume of approximately 30 L. The concentration within the exposure chamber was controlled by adjusting the rate of the infusion pump and the air flow rate through the chamber. The extract from the exposure chamber passed through a scrubber trap and was connected with a high efficiency filter to a metered exhaust system. Prior to the start of the study test material atmospheres were generated within the exposure chamber. Chamber concentrations were measured at regular intervals during the exposure periods. A glass fibre filter was placed in a filter holder and temporarily sealed in a port in the exposure chamber in the animals breathing zone. Chamber air was drawn through the filter at a measured rate using a vacuum pump for a suitable time period.
Each filter was weighed before sampling then dried and weighed again in order to calculate by difference the weight of collected material. The difference in the two weights divided by the volume of atmosphere sampled was representative of the chamber concentration. Based on the results of the preliminary work these figures were adjusted for moisture content (29.5 %).

Particle size distribution:

The particle size distribution of the dispersed material inside the exposure chamber was estimated three times during the exposure period using a Cascade Impactor. The results were as follows:

Table 5.2.3-31: Glyphosate premix: Acute inhalation toxicity study four-hour exposure (nose only) in the rat ([redacted] 1994): Details of test atmosphere

Mean achieved actual concentration	MMAD	GSD	Respirable amount with particle size ≤1 µm
(mg/L air)	(µm)		(%)
4.24 ± 0.49	1.1	0.57	45.3

MMAD = mean mass median aerodynamic diameter
GSD = geometric standard deviation

The generated dust had a mass median aerodynamic diameter (MMAD) of 1.1 µm. The Geometric Standard Deviation (GSD) of the MMAD was calculated as 0.57. Every effort was made to vary the combination of airflow settings with test material input rate to achieve maximum concentrations and optimum particle size distribution.

Animal assignment and treatment:

One group of 5 male and 5 female rats was exposed to an atmosphere containing 4.24 mg/L of the test material, using a nose only exposure system, for a period of 4 h. All animals were observed for clinical signs at hourly intervals throughout the exposure period, on removal from the restraining tubes at the end of the exposure, one day after termination of the exposure and thereafter once daily for 14 days. Individual body weights were recorded on the day of exposure, days 7 and 14. All animals were subjected to full external and internal examination, and any macroscopic abnormalities were recorded. The respiratory tract was subjected to a detailed macroscopic examination for signs of irritancy or local toxicity. The lungs of animals showing abnormalities at necropsy were retained and preserved in 10 % buffered formalin.

II. RESULTS

A. MORTALITY

No deaths occurred.

B. CLINICAL OBSERVATIONS

During exposure wet fur was observed as a common alteration in any animal after exposure. On removal from the chamber hunched posture and pilo-erection were additionally noted in all animals. Wet fur was no longer evident one hour after completion of exposure whereas hunched posture and pilo-eretion was still abserved one hour after chamber removal. On day one following exposure and for the rest of the study no abnormalities were detected.

The clinical signs are summarized in table below.

Table 5.2.3-32: Glyphosate premix: Acute inhalation toxicity study four-hour exposure (nose only) in the rat ([redacted] 1994): Clinical observation data

Clinical sign	Sex	No. of animals exposed	Hours During exposure			On removal from chamber (4 hours)	On-hour Post-exposure	Post-exposure Day				
			1	2	3			1	2	3	4	5-14
Wet fur	Male	5	3	4	5	5	0	0	0	0	0	0
	Female	5	3	5	5	5	0	0	0	0	0	0
Hunched posture	Male	5	0	0	0	5	5	0	0	0	0	0
	Female	5	0	0	0	5	5	0	0	0	0	0
Pilo-erection	Male	5	0	0	0	5	5	0	0	0	0	0
	Female	5	0	0	0	5	5	0	0	0	0	0

C. BODY WEIGHT

No adverse effects on body weight gain were noted.

D. NECROPSY

Six animals showed a dark area or multiple dark foci on the lungs at necropsy but no other abnormalities were detected. The necropsy findings are summarized in table below.

Table 5.2.3-33: Glyphosate technical: Glyphosate premix: Acute inhalation toxicity study four-hour exposure (nose only) in the rat ([redacted]., 1994): Necropsy findings

Macroscopic observations	Sex	No. of animals exposed	No. of animals with present finding
Lungs: dark area	Male	5	1
	Female	5	1
Lungs: multiple dark foci	Male	5	0
	Female	5	4

III. CONCLUSIONS

Nose-only exposure to a mean concentration of 4.24 mg/L with a MMAD of 1.1 µm did not result in any mortality. Thus, the acute inhalation LC_{50} of the test substance glyphosate Premix containing 62.2 % as glyphosate isopropylamine salt (46.1 % as glyphosate) after a 4-hour exposure to male and female rats, observed over a period of 14 days was calculated to be greater than 4.24 mg/L.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was performed in accordance to OECD 403 guideline and GLP. The MMAD and the applied concentration were in the range of the recommended values of the current OECD guideline. Otherwise, only minor deviations to the current valide OECD guideline are present. Therefore the study is acceptable. Based on the study results, the acute inhalation LC_{50} is >4.24 mg/L air after an exposure period of 4 hours.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.3/017
Report author	[redacted]
Report year	1991
Report title	Acute inhalation toxicity study with glyphosate technical in Wistar rats
Report No	ES.877.AIN
Document No	Not reported
Guidelines followed in study	OECD 403 (1987)
Deviations from current test guideline (OECD 403, 2009)	MMAD not calculated. Exposure concentration below recommended limit dose (0.64 mg/L (maximum attainable concentration)). Limit test performed on 5 animals per sex instead of 3. Body weight measured at Day 0 and once a week afterwards.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes

Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

The test substance, glyphosate technical, was evaluated for its acute inhalation toxicity potential, by exposing groups of ten Wistar strain rats (five males and five females) to an aerosol atmosphere of 0 and 0.644 mg/L for 4 hours by whole body exposure. The test substance was dissolved in 10 % v/v of diethyleamine in distilled water and atomised to generate an aerosol. All animals were observed for clinical signs during the exposure for 4 hours (day 1) and once daily during post exposure (days 2-14). All rats were weighed before dosing and on days 7 and 14 post exposure. At sacrifice, all animals were subjected to gross necropsy.
No mortality occurred during the study. During exposure, nasal irritation was observed in many rats. All rats were normal by 24-hours post exposure. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no pathological findings.

The acute inhalation LC_{50} was calculated to be > 0.644 mg/L air.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate Technical
Description:	White odorless crystals
Lot/Batch #:	60 (code: FSG 03090 h/05 March 90)
Purity:	96.8 %
Stability of test compound:	July 1992

2. Vehicle and/or positive control: Vehicle: 10 % v/v of diethylamine in distilled water

3. Test animals:

Species:	Rat
Strain:	Wistar
Source:	[redacted]
Age:	12 weeks
Sex:	Males and females
Weight at dosing:	Males: 140 – 280g; Females: 136 – 240g
Acclimation period:	At least one week
Diet/Food:	Standard “Gold mohur” brand pelleted rat feed (M/s Lipton India Ltd., Bangalore, India), *ad libitum* (except during exposure)
Water:	Deep borewell water passed through activated charcoal filter and exposed to UV rays, *ad libitum* (except during exposure)
Housing:	In groups of 5 animals per cage in standard polypropylene rat cages (size: L 430 x W 270 x H 150 mm) during preexposure. Individual polypropylene cages (size: L 290 x W 220 x H 140 mm) with stainless steel top grill and steam sterilized clean paddy husk bedding during post exposure.

Environmental conditions: Temperature: 23 ± 2 °C
Humidity: 67 ± 6 %
Air changes: 10 – 15/hour
12 hours light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: No data

Test atmosphere generation:

The test substance was dissolved in 10 % v/v of diethyleamine in distilled water to receive a test substance concentration of 15 % (w/v). The solution was atomised at 1.2 kg/cm^2 pressure and an injection rate of 0.4 ml/min to generate the aerosol.

Exposure chamber conditions:
A whole-body exposure chambers consisting of rectangular stainless steel/glass chamber pyramidal at the top and the bottom was used. The exposure chamber had a volume of 0.5 m^3. Two atomizers were used per chamber with an injection rate of 0.4 mL/min. The output of the two atomizers was delivered to one chamber to attain high concentration of test material in the chamber. The chamber air was sampled once per hour at 15 L/min and the concentration in the air was determined by the colorimetric method of Glass (Glass, R.L. 1981).
No further details on exposure chamber conditions are available.

Particle size distribution:
No data on MMAD and GSD is available. Particle size of mist generated by spraying as measured by microscopic sedimentation analyser is shown in the table below.

Table 5.2.3-34: Acute inhalation toxicity study in Wistar rats ([redacted] 1991): Details of test atmosphere

Atomizer	Concentration of formulation	Median particle size
	(%)	(µm)
1	50	1.42 ± 0.35
	100	1.22 ± 0.18
2	50	0.95 ± 0.08
3	50	1.34 ± 0.21
4	50	1.34 ± 0.30

No further information available (assignment of atomiser to exposure group not provided)

Animal assignment and treatment:
Two groups of 5 male and 5 female rats was exposed for 4 hours to an atmosphere containing 0 and 0.644 mg/L of the test material (maximum attainable concentration), using a whole body exposure system. All animals were observed for clinical signs during the exposure for 4 hours (day 1) and once daily during post exposure (days 2-14). All rats were weighed before dosing and on days 7 and 14 post exposure. At sacrifice, all animals were subjected to gross necropsy.

II. RESULTS

A. MORTALITY

No deaths occurred.

B. CLINICAL OBSERVATIONS

Nasal irritation was observed in few rats of the control group and most rats of the test group during exposure. All rats were normal by 24-hours post exposure.

C. BODY WEIGHT

No adverse effects on body weight gain were noted except for two control rats which had lost weight on day 14.

D. NECROPSY

No gross necropsy observations were noted in any of the animals.

III. CONCLUSIONS

The acute inhalation LC_{50} of glyphosate technical after a 4-hour exposure to male and female rats, observed over a period of 14 days was calculated to be greater than 0.644 mg/L.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was not performed in accordance with the current OECD 403 guideline (2009). The tested concentration is far below the recommended limit concentration and no MMAD is provided. Based on these deviations, the study is considered as supportive. Based on the study results, the acute inhalation LC_{50} is > 0.644 mg/L air after an exposure period of 4 hours.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.3/018
Report author	[redacted]
Report year	1989
Report title	Glyphosate technical: Acute Inhalation Toxicity Study In Rats (Limit Test)
Report No	5993
Document No	Not reported
Guidelines followed in study	OECD 403 (1981), EPA Guidelines, subdivision F, 81-3
Deviations from current test guideline (OECD 403, 2009)	No MMAD calculated (mean measured particle size: 22.5 µm). Limit test performed on 5 animals per sex. Analytical purity of test material not provided.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The test substance, glyphosate technical, was evaluated for its acute inhalation toxicity potential in rats when exposed by snout-only exposure for a single continuous 4-hour period to an actual concentration of 4.98 ± 0.12 mg/L with a mean measured particle size of 22.5 µm.
No mortality occurred during the study. No clinical signs were observed. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no pathological findings.

The acute inhalation LC_{50} was calculated to be >4.98 mg/L air.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate Technical
Description:	Fine white powder
Lot/Batch #:	206-JAK-25-1
Purity:	No data (certificate of analysis not available) (Purity: 98.6 % according to CA 5.2.1/024)
Stability of test compound:	No data

2. Vehicle and/or positive control: none

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	No data
Sex:	Males and females
Weight at dosing:	118 – 147 g
Acclimation period:	No data
Diet/Food:	Rat and Mouse (Modified) No. 1 Diet SQC Expanded (Special Diets Services Limited, Stepfield, Witham, Essex), *ad libitum* (except during exposure)
Water:	tap water, *ad libitum* (except during exposure)
Housing:	In groups of 1-2 animals per cage in suspended stainless steel mesh cages on absorbent paper.
Environmental conditions:	Temperature: 20 – 25 °C Humidity: 50-66 % Air changes: no data 12 hours light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 1989-08-10 to 1989-08-25

Test atmosphere generation:
The test atmosphere was generated using an Aerostyle dust generator.

Exposure chamber conditions:
The cylindrical exposure chamber had a volume of approximately 41.5 L. The exposure system was truly dynamic, incorporating a single pass of the freshly generated material. The particles dispersed throughout the chamber and exited through the base to a filtered vacuum line. Chamber air flow rates were monitored continuously and the values recorded at 30 min intervals. Air flow to the chamber was maintained at 30 L/min and the extract flow at 32 L/min. Chamber concentrations were measured at regular intervals during the exposure periods. The gravimetric method used employed pressed glass fibre filters (Whatman GF/B) placed in a filter holder. The conical input side of the holder was positioned and temporarily sealed in a port in the exposure chamber at the animals' breathing zone. Chamber air was drawn through the filter at a measured rate of 1.0 L/min using a vacuum pump. The air flow during each sample was controlled by a critical orifice and timed for a suitable period.

Each filter was weighed before and after sampling in order to calculate by difference the weight of collected material. The chamber concentration was estimated by further calculation using the sample air volume.

Particle size distribution:

The particle size distribution of the dispersed material inside the exposure chamber was estimated twice during the exposure period using a Marple (Model 296) Cascade Impactor (Anderson Samplers Inc., Atlanta, Georgia, USA). The results were as follows:

Table 5.2.3-35: Glyphosate Technical: Acute Inhalation Toxicity Study In Rats (Limit Test) ([redacted] 1989): Details of test atmosphere

Actual concentration (gravimetric method)	Mass mean diameter	GSD	Respirable amount particle size ≤ 3.5 µm
(mg/L air)	(µm)		(%)
4.98 ± 0.12	22.5	4.6	10

GSD = geometric standard deviation

The generated dust had a mass mean diameter of 22.5 µm. The Geometric Standard Deviation (GSD) of the mass mean diameter was calculated as 4.6. The percentage of particles with a size < 3.5 µm was 10 % by weight.

Animal assignment and treatment:

One group of 5 male and 5 female rats each was exposed to an atmosphere containing 4.98 mg/L of the test material, via the inhalation route, by snout-only exposure for a single continuous 4 h period. All rats were observed for clinical signs at frequent intervals throughout the exposure period, for the first 1-2 h post dosing and thereafter at least once daily during the subsequent 14 day observation period. All rats were weighed immediately before dosing and on Days 2, 3, 4, 7, 10 and 14 post exposure. Each rat was examined externally prior to opening the abdominal and thoracic cavities. Any gross lesions observed were recorded in descriptive terms, including location(s), size (mm), colour and number. The respiratory tract was subjected to a detailed macroscopic examination for signs of irritancy or local toxicity. All organs were examined in situ. The lungs of each animal were removed and weighed to allow calculation of lung:body weight ratio.

II. RESULTS

A. MORTALITY

No deaths occurred.

B. CLINICAL OBSERVATIONS

All animals were slightly subdued after dosing but showed normal behaviour on Day 1 and throughout the remainder of the observation period.

C. BODY WEIGHT

No adverse effects on body weight gain were noted.

D. NECROPSY

There were no findings attributable to treatment with glyphosate technical noted during macropathological examination of the animals.
The lung to body weight ratios for all animals were considered to be within normal limits.

III. CONCLUSIONS

The acute inhalation LC_{50} of glyphosate technical after a 4-hour exposure to male and female rats, observed over a period of 14 days was calculated to be greater than 4.98 mg/L.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was performed similar to OECD 403 guideline. No MMAD was calculated, the mean measured particle size was 22.5 µm. Further, no analytical purity of the test material is provided. Based on these deviations, the study is considered as supportive. Based on the study results, the acute inhalation LC_{50} is >4.98 mg/L air after an exposure period of 4 hours.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.3/019
Report author	[redacted]
Report year	1989
Report title	4-hour acute inhalation toxicity study with glyphosate technical in rats
Report No	238105
Document No	Not available
Guidelines followed in study	Not available
GLP	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Short description of study design and observations:	Groups of five Wistar rats per sex were exposed to nebulised test substance (glyphosate salt, IPA with a purity of 62 %) for 4 hours via a nose-only exposure system. Animals were exposed to doses of 4.1, 4.42 and 6.49 mg/L air.
Short description of results:	No mortality occurred during the study. During exposure, nose bleeding and ruffled fur were observed. The acute inhalation LC_{50} was calculated to be >6.49 mg/L.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	Study report not available. Monograph (2000): The study is considered acceptable.

Reasons why the study report is not available for submission	The notifier has not access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance" (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL
Category study in AIR 5 dossier (L docs)	Category 4a

1. Information on the study

Data point:	CA 5.2.3/020
Report author	[redacted]
Report year	1988
Report title	Acute inhalation study of MON-8750 technical
Report No	[redacted]-87-228
Document No	Not reported
Guidelines followed in study	Not reported
Deviations from current test guideline (OECD 403, 2009)	Limit test performed on 5 animals per sex.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The test substance, glyphosate technical (MON 8750 Technical), was evaluated for its acute inhalation toxicity potential, by exposing a single group of ten Sprague-Dawley strain rats (five males and five females) to an aerosol atmosphere of 1.9 mg/L for four hours by whole body exposure. Exposure was followed by a 14-day observation period and subsequent necropsy. No deaths occurred as a result of the exposure, which was performed at the highest attainable concentration. due to the placement of the animals within the chamber, observations during exposure were difficult to make; however, of the animals visible, only hypoactivity was noted. Animals appeared normal immediately after exposure. The only notable observation during the post-exposure period was red/brown perinasal encrustation noted on post-exposure Days 1 and 2. Mean body weight of the animals increased throughout the study period. There were no gross necropsy observations noted at the terminal sacrifice.

The acute inhalation LC_{50} was determined to be > 1.9 mg/L air.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	MON 8750 Technical
Description:	White powder
Lot/Batch #:	XLH-270
Purity:	85.52 %
Stability of test compound:	Not reported

2. Vehicle and/or positive control:
None

3. Test animals:

Species:	Rat	
Strain:	Sprague-Dawley	
Source:	[redacted]	
Age:	9 weeks	
Sex:	Males and females	
Weight at dosing:	Male: approx. 322g; Female: approx. 214g	
Acclimation period:	20 days (arrival: 1987-11-03; first exposure: 1987-11-23)	
Diet/Food:	Purina Mills RODENT CHOW No. 5002 (Purina Mills Inc., St. Louis, MO), *ad libitum* (except during exposure)	
Water:	Sodium zeolite-conditioned St. Louis public water supply, *ad libitum* (except during exposure)	
Housing:	Individual suspended stainless steel mesh cages, over paper bedding	
Environmental conditions:	Temperature:	68 – 76 °F (approx. 20 - 24 °C)
	Humidity:	35 – 60 %
	Air changes:	No data
	12 hours' light/dark cycle	

B. STUDY DESIGN AND METHODS

In life dates: 1987-11-23 to 1987-12-7

Test atmosphere generation:

The test atmosphere was generated using a fluidized-bed dust generator.

Exposure chamber conditions:

Animals were exposed in a stainless steel chamber with a pyramidal top and bottom. The concentration within the exposure chamber was controlled by regulating the air flow rate through the dust generator. The chamber air flow, temperature and relative humidity was monitored continuously and recorded approximately every 30 minutes. Chamber concentrations were measured at regular intervals during the exposure periods. Approximately 10 L of test atmosphere was drawn at a know rate a glass fibre filter (25mm Gelman type A/E). Filter was weighed prior to and following the sampling period to determine the net weight of dust collected after drawing a known volume of air from the chamber. The nominal concentration was calculated once for each exposure by determining the total amount of test material delivered to the chamber and dividing this amount by the total air volume passing through the chamber.

Particle size distribution:

The particle size distribution of the dispersed material inside the exposure chamber was estimated once during the exposure period using a Andersen Cascade Impactor. The results were as follows:

Table 5.2.3-36: Acute inhalation study of MON-8750 Technical ([redacted] 1994): Details of test atmosphere

Mean analytical concentration (gravimetric)	MMAD	GSD	Respirable amount particle size ≤10 µm
(mg/L air)	(µm)		(%)
1.9	4.2	1.8	92.5

MMAD = mean mass median aerodynamic diameter
GSD = geometric standard deviation

The generated dust had a mass median aerodynamic diameter (MMAD) of 4.2 µm. The Geometric Standard Deviation (GSD) of the MMAD was calculated as 1.8.

Animal assignment and treatment:

One group of 5 male and 5 female rats were exposed to an atmosphere containing 1.9 mg/L of the test material for 4 hours by whole body exposure. All animals were observed for clinical signs at hourly intervals throughout the exposure period, however the placement of cages allowed only limited observation of animals, immediately following exposure and once daily (normal work days only) during 14-day observation period following exposure. The animals were observed twice daily for mortality and moribundity. Individual body weights were recorded on the day of exposure, days 2, 7 and 14. All animals were subjected to a full external and internal examination, and any macroscopic abnormalities were recorded.

II. RESULTS

A. MORTALITY

No deaths occurred.

B. CLINICAL OBSERVATIONS

During exposure hypoactivity was observed. No clinical signs were detected immediately after exposure. Red/Brown perinasal encrustation was observed in two males on Days 1-2 during the post-exposure period.

For the rest of the study, Days 3-14, all animals appeared normal. No abnormalities were detected in females.

The clinical signs are summarized in table below.

Table 5.2.3-37: Acute inhalation study of MON-8750 Technical ([redacted] 1994): Clinical observation data

Clinical sign	Sex	No. of animals exposed	Post-exposure Day													
			1	2	3	4	5	6	7	8	9	10	11	12	13	14
Red/Brown perinasal encrustation	Male	5	2	2	0	0	0	0	0	0	0	0	0	0	0	0
	Female	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0

C. BODY WEIGHT

No adverse effects on body weight gain were noted.

D. NECROPSY

No gross necropsy observations were noted in any of the animals.

III. CONCLUSIONS

The acute inhalation LC_{50} of glyphosate technical after a 4-hour exposure to male and female rats, observed over a period of 14 days was calculated to be greater than 1.9 mg/L.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was performed in accordance to OECD 403 guideline and GLP. The MMAD and the applied concentration were in the range of the recommended values of the current OECD guideline. Otherwise, only minor deviations to the current valide OECD guideline are present. Therefore the study is acceptable. Based on the study results, the acute inhalation LC_{50} is >1.9 mg/L air after an exposure period of 4 hours.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.3/021
Report author	[redacted]
Report year	1987
Report title	Acute Toxicity of Rodeo® Herbicide Administered by Inhalation to Male and Female Sprague-Dawley Rats
Report No	[redacted]-6582
Document No	Not reported
Guidelines followed in study	None
Deviations from current test guideline (OECD 403, 2009)	Exposure concentration slightly below recommended limit dose (1.3 mg/L (maximum attainable concentration of the herbizide containing approx. 54 % isopropylamine salt of glyphosate)). Limit test performed on 5 animals per sex. Body weight measured at Day 0, 2, 7 and 14 These deviations did not affect the outcome of the study.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The test substance, Rodeo® Herbicide,containing 53.8 % Isopropylamine salt of glyphosate was evaluated for its acute inhalation toxicity potential, by exposing a single group of ten Sprague-Dawley strain rats (five males and five females) to an aerosol/vapour atmosphere of 1.3 mg/L (maximum attainable concentration) for four hours by whole body exposure. Exposure was followed by a 14-day observation period and subsequent necropsy. One female rat died. Focal and/or generalized loss of hair developed in some animals during the observation period. There was a decrease in group mean body weight on post-exposure Day 2 relative to pre-exposure mean weight. On Days 7 and 14 group mean body weight was increased. Gross necropsy examination of animals revealed no alterations that were considered related to exposure.

The acute inhalation LC_{50} was calculated to be > 1.3 mg/L air.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Rodeo® Herbicide
Description:	Amber liquid
Lot/Batch #:	LHRO-12010 X
Purity:	Stated composition: 53.8 % Isopropylamine salt of glyphosate, 46.2 % Inert
Stability of test compound:	Expiry date: December 1987

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley (Crl: CD®(SD)BR)
Source:	[redacted]
Age:	8 – 10 weeks
Sex:	Males and females
Weight at dosing:	Male: 271 – 286 g; female: 235 – 242 g
Acclimation period:	At least 10 days
Diet/Food:	Purina Certified Rodent Chow® (5002), *ad libitum* (except during exposure)
Water:	Sodium zeolite-conditioned St. Louis public water supply, *ad libitum* (except during exposure)
Housing:	Individually in suspended stainless steel mesh cages
Environmental conditions:	Temperature: 68 – 76 °F (22 ± 2 °C) Humidity: 35 – 60 % Air changes: Not specified 12-hour light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 1986-07-29 to 1986-08-12

Test atmosphere generation:

The test atmosphere was generated by using a capillary restrictor from a tank to a Laskin-type nebuliser located in the top turret of the exposure chamber.

Exposure chamber conditions:

The exposure chamber was a 300 L New Yorker University-style stainless steel chamber, with a pyramidal top and bottom and a glass window in the door.

The concentration of test material in the chamber was controlled by regulating the pressure in the tank headspace and consequently, the flowrate of the test material into the nebuliser. Chamber airflow, temperature, and relative humidity were monitored continuously and recorded every 30 minutes. Airflow through the chamber ranged from 58.3 to 69.2 L/min during the exposure. The mean chamber temperature was 24 °C and the mean relative humidity was 66 %. Oxygen level was determined once during the exposure using a Draeger tube and was determined to be 19 %.

Test material concentrations in the inhalation chamber were routinely obtained four different times during the exposure at approximate one-hour intervals. The analytical concentration of test material was determined by liquid chromatography. The mean analytical concentration for the exposure was 1.3 mg/L in air that is considered to be the maximum attainable concentration. The nominal concentration of 47 mg/L was calculated by determining the amount of test material delivered to the chamber and dividing this amount by the air volume passing through the chamber. The high nominal/analytical ratio appeared to be due to loss from the exposure atmosphere of the test material aerosol because of impaction on the walls of the chamber, cages or animals.

Particle size distribution:

The particle size distribution of the dispersed material inside the exposure chamber was estimated once during the exposure period using an Andersen Cascade Impactor. The results were as follows:

Table 5.2.3-38: Acute Toxicity of Rodeo® Herbicide Administered by Inhalation to Male and Female Sprague-Dawley Rats ([redacted] 1987): Details of test atmosphere

Mean analytical concentration (gravimetric)	MMAD	GSD	Respirable amount particle size ≤ 10 µm
(mg/L air)	(µm)	(µm)	(%)
1.3	3.1	1.9	97.0

MMAD = mean mass median aerodynamic diameter
GSD = geometric standard deviation

Animal assignment and treatment:

One group of 5 male and 5 female rats were exposed to an aerosol/vapour atmosphere containing 1.3 mg/L of the test material for 4 hours by whole body exposure. All animals were observed during the exposure but placement of the cages allowed only limited observation. Animals were thoroughly examined following exposure and gross signs of toxicity were recorded. During the 14-day observation period the animals were scheduled for examination twice daily for mortality and once daily for gross signs of toxicity. Body weights were obtained prior to exposure (Day 0) and on post-exposure Days 2, 7 and 14. A standard macroscopic examination of the external appearance and of the tissues of the thoracic, abdominal, and cranial cavities was conducted on animals found dead and those sacrificed at the end of the study.

II. RESULTS

A. MORTALITY

Exposure to 1.3 mg/L resulted in mortality of one female on post-exposure Day 3. No further unscheduled death was observed until the end of the observation period.

B. CLINICAL OBSERVATIONS

Observations recorded following the exposure are presented in the table below.

Table 5.2.3-39: Acute Toxicity of Rodeo® Herbicide Administered by Inhalation to Male and Female Sprague-Dawley Rats [redacted] 1987): Clinical observation data

Exposure Level (mg/L)	Observation	Number Observed		Day observed	
		Male	Female	First	Last
1.3	Labored respiration	0	1	2	2
	Rapid respiration	0	1	2	2
	Yellow/brown nasal discharge	4	5	0	7
	Bloodlike oral discharge	0	1	2	2
	Red ocular discharge	2	1	0	1
	Periocular encrustation	1	1	2	3
	Auricular encrustation	1	0	9	14
	Focal loss of hair	2	2	3	14
	Generalized loss of hair	2	0	4	14
	Red/brown perinasal encrustation	2	1	1	1
	Diarrhea	1	0	2	7
	Emaciated	1	1	2	7

Yellow/brown nasal discharge was observed in most of the exposed animals (8/10) immediately after exposure (Day 0). This sign did not persist - on the day after exposure (Day 1) only 2/10 animals exhibited this observation and on Day 8 this sign was not seen in any animal. Focal and/or generalized loss of hair developed in some animals (5/10) during the 14-day observation period.

C. BODY WEIGHT

On post-exposure Day 2 both males and females revealed decreases in mean body weights, however, mean body weights were increased over initial weights on Days 7 and 14 (see table below).

Table 5.2.3-40: Acute Toxicity of Rodeo® Herbicide Administered by Inhalation to Male and Female Sprague-Dawley Rats ([redacted] 1987): Body weight

Day		0	2	7	14	0	2	7	14
Sex		Males				Females			
Exposure level (mg/L)	Animal No.	Body weight [g]							
1.3	1	286	288	321	362	241	237	240	247
	2	278	276	298	333	235	212	-	-
	3	284	257	264	312	242	234	246	251
	4	282	277	287	320	241	235	255	268
	5	271	270	298	326	236	229	235	245
	Mean	**280**	**274**	**294**	**331**	**239**	**229**	**244**	**253**
	± SD	**± 6**	**± 11**	**± 21**	**± 19**	**± 3**	**± 10**	**± 9**	**± 10**

- = animal dead

D. NECROPSY

Gross necropsy examination revealed alterations in the kidneys, lung, skin, thymus, and uterus (see table below). None of these alterations were considered related to the exposure and are considered findings that are likely due to post mortem changes or are observations frequently seen in animals of this species and strain processed similarly.

Table 5.2.3-41: Acute Toxicity of Rodeo® Herbicide Administered by Inhalation to Male and Female Sprague-Dawley Rats [redacted] 1987): Necropsy data

Exposure level [mg/L]	**1.3**	
Sex	**Males**	**Females**
Animals examined	5	4*
Kidney		
- Abnormal color, brown/yellow/tan	1	1
- Focus, brown/yellow/tan	1	
- Focus, red/purple/black	2	
Lung		
- Focus	3	3
Skin		
- Alopecia	2	
Thymus		
- Multiple, purple-red foci	5	3
Uterus	0	
- Hydrometra		1

* No gross necropsy alterations were observed in the dead female.

III. CONCLUSIONS

The acute inhalation LC_{50} of the test substance, Rodeo® Herbicide, containing 53.8 % Isopropylamine salt of glyphosate after a 4-hour exposure to male and female rats, observed over a period of 14 days was calculated to be greater than 1.3 mg/L.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was performed similar to OECD 403 guideline and according to GLP. The maximum attainable concentration of 1.3 mg/L air contained 53.8 % isopropylamine salt of glyphosate. The MMAD and GSD was in the range of the recommended values of the current OECD guideline. Otherwise, only minor deviations to the current valide OECD guideline are present. Therefore the study is acceptable as supportive. Based on the study results, the acute inhalation LC_{50} is >1.3 mg/L air after an exposure period of 4 hours.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.3/022
Report author	[redacted]
Report year	1983
Report title	Report on acute inhalation toxicity in rats (4 hours) of glyphosate (technical)
Report No	Not given
Document No	Not reported
Guidelines followed in study	None
Deviations from current test guideline (OECD 403, 2009)	Yes, the following information were not given: Purity and stability of test substance, strain of test animals, acclimation period, vehicle. Age of animals was in the range of 6 - 7 weeks instead of 8 - 12 weeks. Body weights of animals after exposure were not determined. Individual animal data not reported. These deviations did not affect the study outcome.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

The test substance, glyphosate technical (Glycel 4l SL), was evaluated for its acute inhalation toxicity potential, by exposing groups of ten rats (five males and five females) to a nominal aerosol atmosphere of 0, 1.4, 2.3 and 4.5 mg/L for four hours. Nose- and mouth-inhalation route was used. All animals were observed for clinical signs before and at hourly intervals during the exposure for 4 hours (day 1) and once daily during post exposure (days 2-14). At sacrifice, all animals were subjected to gross necropsy.
No mortality occurred during the study. No toxic symptoms and no remarkable pathological changes were observed in any of the animals exposed to the test material.

The acute inhalation LC_{50} was determined to be > 4.5 mg/L air.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glycel 4l SL
Description:	Not specified
Lot/Batch #:	Not specified
Purity:	Not specified
Stability of test compound:	Not specified

2. Vehicle and/or positive control: No information provided

3. Test animals:

Species: Rat

Strain:	Not specified
Source:	Breeding colony, not further specified
Age:	6 – 7 weeks
Sex:	Males and females
Weight at dosing:	90 – 100 g
Acclimation period:	Not specified
Diet/Food:	Gold Mohor rat food (Hindustan Lever Limited), *ad libitum* (except during exposure)
Water:	*ad libitum* (except during exposure)
Housing:	In groups of 5 animals per cage in polypropylene cages
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 25 – 75 % Air changes: 12/hour 12 hours light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: No data

Test atmosphere generation:

The test material was nebulised with compressed air at 10 psi and at the rate of 10 L/min.
No further details on test atmosphere generation are available.

Exposure chamber conditions:

The chamber was exhausted by the use of vacuum pump evaluating 20 L/min. The exhausted air was led passively to the outside of the building and vented. The size of the chamber was 78 L.

The temperature (19 – 25 °C) and humidity (25 – 75 %) inside the exposure chamber was measured continuously and recorded at hourly intervals throughout each exposure period. The oxygen concentration inside the chambers was monitored continuously using oxygen analyser and recorded at hourly intervals. The mean oxygen concentration recorded for all groups was not less than 21 %. The mean diluent air flow rates were similar for all groups.

The absolute exposure chamber atmosphere concentration was measured hourly by withdrawing air from the chamber at the rate of 4.86 L/min on a Whatman 4l filter paper in a filter assembly. The difference in weights of the paper before and after the exposure gave the weight of the product. The nominal concentration was the weight of test material used divided by the volume of diluent air used. The nominal concentrations were 4.6 mg/L (low dose), 9.1 mg/L (intermediate dose) and 16.7 mg/L (high dose). The overall mean measured concentrations were 1.4 mg/L, 2.3 mg/L and 4.5 mg/L for low, intermediate and high dose, respectively.

No further details on exposure chamber conditions are available.

Particle size distribution:

The particle size of the generated atmosphere of the test material was determined hourly using the method and operations of an Anderson Sampler Mark II. The mass median aerodynamic diameters (MMAD) mean values were 3.8 µm (low dose), 4.0 µm (intermediate dose) and 3.9 µm (high dose).

Animal assignment and treatment:

Four groups of 5 male and female rats were exposed for 4 hours to an atmosphere containing nominal concentrations of 0, 1.4, 2.3 and 4.5 mg/L (maximum attainable concentration) of the test material. Nose- and mouth-inhalation route was used. All animals were observed for clinical signs before, at hourly intervals during the exposure for 4 hours (day 1) and once daily during post exposure (days 2-14). At sacrifice, all animals were subjected to gross necropsy.

II. RESULTS

A. MORTALITY
No deaths occurred.

B. CLINICAL OBSERVATIONS
No toxic symptoms were seen in any of the animals.

C. BODY WEIGHT
Body weights were not recorded.

D. NECROPSY
No remarkable pathological changes were seen in any of the animals.

III. CONCLUSIONS

The acute inhalation LC_{50} of glyphosate technical (Glycel 41 SL) after a 4-hour exposure to male and female rats, observed over a period of 14 days was calculated to be greater than 4.5 mg/L.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was not performed according to any guideline. No information on GLP status is reported. Due to limited reporting, and minor deviations, the study is considered as supportive. Based on the study results, the acute inhalation LC_{50} is > 4.5 mg/L air after an exposure period of 4 hours.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

CA 5.2.4 Skin irritation

A variety of skin irritating tests are available for glyphosate tested as acid or salts.
According to the first EU evaluation (Monograph, 2002) and the respective data, glyphosate acid and its salts were considered not to be irritating to intact skin and only slightly irritating to abraded skin. For the re-evaluation in 2015, 13 additional studies with glyphosate acid on rabbits were submitted. Three of them revealed mildly or slightly irritating effects; however, based on the data, the criteria of Regulation (EC) 1272/2008 (CLP), for classification for skin irritation are not met.
For the current re-evaluation (2020) two additional studies were submitted, one conducted with glyphosate by [redacted], 1983 and the other with the isopropylamine (IPA) salt of glyphosate by [redacted], 1981. The glyphosate salt caused slight erythema on the intact skin in one of six animals 24-hours after patch removal (score 1), without effects at other time points and in other animals. No irritating effects were seen in the study conducted with glyphosate. In both studies the test substance was concluded not to have an irritating potential to the skin for rabbits.
Based on these data, the previous evaluation was confirmed; glyphosate is considered to be not skin irritating and no classification for skin irritation is warranted.

Table 5.2.4-1: Studies on skin irritation with glyphosate

Annex Point	Study	Study type	Substance(s)	Reference list- related category§	Result
CA 5.2.4/001	[redacted] 2011	Skin irritation *in vivo*: New Zealand White rabbits, ♂	Glyphosate technical (Batch: 569753 (BX20070911), Purity: 96.3 % w/w)	Valid, Category 2a	Not irritating
CA 5.2.4/002	[redacted] 2010	Skin irritation *in vivo*: Himalayan rabbits, ♂	Glyphosate TC (Batch: 20090506, Purity: 97.3 %)	Valid#, Category 2a	Not irritating
CA 5.2.4/003	[redacted], 2009	Skin irritation *in vivo*: Himalayan rabbits, ♂	Glyphosate TC (Batch: 2009051501, Purity: 96.4 %)	Valid#, Category 2a	Not irritating
CA 5.2.4/004	[redacted] 2009	Skin irritation test *in vivo*: Himalayan rabbits, ♂	Glyphosate TC (Batch: 20080801, Purity: 98.8 %)	Valid#, Category 2a	Not irritating
CA 5.2.4/005	[redacted] 2009	Skin irritation *in vivo*: New Zealand White rabbits, ♂ / ♀	Glyphosate Tech Grade Mixed 5-Batch (Batch: 080704-1 thru 5, Purity: 96.4 %)	Valid, Category 2a	Not irritating
CA 5.2.4/006	[redacted] 2008	Skin irritation *in vivo*: New Zealand White rabbits, ♀	Glyphosate technical (Batch: 20070606, Purity: 98.05 %)	Valid, Category 2a	Not irritating
CA 5.2.4/007	[redacted], 2007	Skin irritation *in vivo*: New Zealand White rabbits, ♂	Glyphosate Technical Material (Batch: 0507, Purity: 96.1 % w/w glyphosate acid).	Valid, Category 2a	Not irritating
CA 5.2.4/008	[redacted], 2007	Skin irritation *in vivo*: New Zealand White rabbits, ♂ / ♀	Glyphosate technical (NUP 05068, Batch: 200609062, Purity: 95.1 %)	Valid, Category 2a	Not irritating
CA 5.2.4/009	[redacted], 2005	Skin irritation *in vivo*: New Zealand White rabbits, ♂	Glyphosate acid technical (Batch: 040205, Purity: 97.23 %)	Valid, Category 2a	Not irritating
CA 5.2.4/010	[redacted], 1996	Skin irritation *in vivo*: New Zealand White rabbits, ♀	Glyphosate acid (Batch: P24, Purity: 95.6 %)	Valid, Category 2a	Not irritating
CA 5.2.4/011	[redacted], 1995	Skin irritation *in vivo*: New Zealand White Kbl:SPF rabbits, ♀	Glyphosate technical (HR-001, Batch: T-941209, Purity: 97.56 %)	Valid, Category 2a	Not irritating
CA 5.2.4/012	[redacted], 1994	Skin irritation *in vivo*: New Zealand White rabbits, ♂ / ♀	Glyphosate premix (Batch: 290-JaK-146-4; Purity: 46.1 % glyphosate).	Valid, Category 3a	Not irritating
CA 5.2.4/013	[redacted], 1994	Skin irritation *in vivo*: rabbits, ♂ / ♀	Glyphosate salt, purity 360 g/L	Invalid, Category 4b	Not irritating
CA 5.2.4/014	[redacted] 1994	Skin irritation *in vivo*: New Zealand White rabbits	Glyphosate (Batch: 36300892; Purity: 99.6 %)	Valid, Category 3a	Not irritating

Table 5.2.4-1: Studies on skin irritation with glyphosate

CA 5.2.4/015	[redacted], 1991	Skin irritation *in vivo*: New Zealand White rabbits, ♂ / ♀	Glyphosate technical (Batch: 60; Purity: 96.8 %)	Valid, Category 3a	Not irritating
CA 5.2.4/016	[redacted] 1991	Skin irritation *in vivo*: New Zealand White rabbits, ♂	Glyphosate acid (Purity: 98 %)	Invalid, Category 4b	Not irritating
CA 5.2.4/017	[redacted], 1990	Skin irritation *in vivo*: New Zealand White rabbits, ♂	Glyphosate technical (Batch: 0190 A; Purity: 98.1 %)	Valid, Category 3a	Not irritating
CA 5.2.4/018	[redacted] 1989	Skin irritation *in vivo*: New Zealand White rabbits, ♂ / ♀	Glyphosate technical (Batch: 206-Jak-25-1; Purity: 98.6 %)	Valid, Category 3a	Not irritating
CA 5.2.4/019	[redacted] 1989	Skin irritation *in vivo*: rabbits, ♂ / ♀	Glyphosate salt (Purity: 62 %)	Invalid, Category 4b	Not irritating
CA 5.2.4/020	[redacted] 1988	Skin irritation *in vivo*: New Zealand White rabbits, ♂ / ♀	Glyphosate (Batch: XLI-55; Purity: 97.76 %)	Valid, Category 2a	Not irritating
CA 5.2.4/021	[redacted], 1987	Skin irritation *in vivo*: New Zealand White rabbits, ♂	MON 8750, FD-86-431 (Batch XLG-225, Purity: 90.8 %)	Valid, Category 3a	Not irritating
CA 5.2.4/022	[redacted] 1983	Skin irritation *in vivo*: NWS rabbits, ♂	Glyphosate technical (Purity: 95 %)	Valid, Category 3a	Not irritating
CA 5.2.4/023	[redacted] 1981	Skin irritation *in vivo*: New Zealand White Isf: (NZW) rabbits, ♂ / ♀	MON 0139 (Batch: SSRT-11012, Purity: 65 %)	Valid, Category 3a	Not irritating
CA 5.2.4/024	[redacted] 1979	Skin irritation *in vivo*: New Zealand White rabbits, ♂ / ♀	Glyphosate technical (Batch: XLI-180, Purity: 99 %)	Valid, Category 2a	Not irritating

[#] performed at [redacted]

§: The category describes the acceptability/reliability of the study within the AIR 5 submission. This category does not describe hazard classification. (for details please refer to the Doc ID: 110054-B-GRG_Jun_2020)

1. Information on the study

Data point:	CA 5.2.4/001
Report author	[redacted]
Report year	2011
Report title	Glyphosate technical - Primary skin irritation study in rabbits
Report No	10/218-006N
Document No	Not reported
Guidelines followed in study	OECD 404 (2002), US EPA OPPTS 870.2500 (1998), EC No 44/2008, B.4 (2008)
Deviations from current test guideline (OECD 404, 2015)	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

In a primary dermal irritation study, three male, young adult, New Zealand White rabbits were each given a dermal application of 0.5 g of undiluted glyphosate technical (Batch: 569753 (BX20070911), Purity: 96.3 % w/w). The test substance was applied to an area of the intact shaved flank (2.5 cm × 2.5 cm), under a semi-occlusive dressing. The patch was hold in place with a surrounding adhesive hypoallergenic plaster. After 4 hours, the dressing was removed, and the skin was flushed with lukewarm tap water to clean the application site. Initially, a single animal was treated. As neither a corrosive effect nor a severe irritant effect was observed after the 1-hour exposure, the test was completed using the two remaining animals.

The skin reaction was assessed according to the numerical scoring system listed in the Commission Directive 2004/73/EC, April 29, 2004 which was based on the Draize scoring system. The scoring of skin reactions was performed 1, 24, 48 and 72-hours after removal of the dressing. 1 and 24-hours after patch removal, very slight erythema (score 1) was observed in one animal. No signs of irritation were observed in the other treated animals throughout the study. The individual mean score for the 24, 48 and 72-hour readings were 0.33 for one animal and 0.0 for the remaining animals for erythema and 0.0 for oedema for all animals, respectively.

Based on the study, glyphosate technical does not show a skin irritating potential under the test conditions chosen.

Materials and methods

A: Materials

1. Test material:

Identification:	Glyphosate technical
Description:	Dry white powder
Lot/Batch number:	569753 (BX20070911)
Purity:	96.3 % w/w
Stability of test compound:	Stable under storage conditions (room temperature range < 30 °C), Expiry date: 2011-08-31

2. Vehicle and/or positive control:	Not specified
3. Test animals:	
Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Approximately 12 weeks
Sex:	Male
Weight at dosing:	2.995 – 3.095 kg
Acclimatisation period:	5 days
Diet:	Purina Base – Lap gr. diet (AgribrandsEurope Hungary PLC, H-5300 Karcag, Madarasi út, Hungary), *ad libitum*
Water:	Municipal tap water *ad libitum*
Housing:	Individually in metal cages
Environmental conditions:	Temperature: 17 – 20 °C Humidity: 30 – 70 % Air changes: 15 – 20 / hour Photocycle: 12 hours light / dark
4. Test conditions:	
Patch site preparation technique:	The back and flanks were clipped (area of approximately 100 cm^2) with an electric clipper approximately 24-hours before treatment.
Patching technique:	Dermal application onto shaved, intact dorsal skin (area 2.5 cm × 2.5 cm). The patch was kept in contact with the skin by a patch with surrounding adhesive hypoallergenic plaster.
Chemical preparation:	Glyphosate Technical was applied undiluted
Chemical application:	0.5 g / animal
Chemical removal:	Skin was flushed with lukewarm tap water

B: STUDY DESIGN AND METHODS:

In-life dates: 2010-11-02 to 2010-11-05

Animal assignment and treatment:

In a primary dermal irritation study, three male, young adult, New Zealand White rabbits were each given a dermal application of 0.5 g of undiluted glyphosate technical (96.3 % w/w glyphosate technical).

Approximately 24-hours prior to the test the hair was clipped from the back and flanks of the animals with an electric clipper, exposing an area approximately 10 cm × 10 cm. Animals with overt signs of skin injury or marked irritation which may have interfered with the interpretation of the results were not used in the test.

On the day of treatment, 0.5 g of glyphosate technical was placed on a surgical gauze pad (approximately 2.5 cm × 2.5 cm). This gauze pad was applied to the intact skin of the clipped area and was kept in contact with the skin by a patch with a surrounding adhesive hypoallergenic plaster. The entire trunk of the animals was then wrapped with plastic wrap held in place with an elastic stocking. The dressing was left in place for 4 hours, after which it was removed, and the skin was flushed with lukewarm tap water to clean the application site so that any reactions (erythema) were clearly visible.

As it was suspected that the test item might produce irritancy, a single animal was treated first. As no

corrosive effect was observed after the 4-hour exposure, the test was completed using the two remaining animals. The animals were checked daily for signs of systemic toxicity and mortality. Body weights were recorded on the day of application and at termination of observations.

The skin reaction was assessed according to the numerical scoring system listed in the Commission Directive 2004/73/EC, April 29, 2004, which was based on the Draize scoring system (see table below), approximately 1, 24, 48 and 72-hours after the removal of the dressing, gauze patch and test item. The mean score was calculated across three scoring times (24, 48 and 72-hours after patch removal) for each animal for erythema/eschar grades and for oedema grades, separately.

Skin reaction grading according to Draize criteria used by [redacted] (2011)

Skin Reaction	Grading
Erythema and eschar formation	
No erythema	0
Very slight erythema	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef redness) to eschar formation (injuries in depth preventing erythema) reading	4
Oedema formation	
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well-defined by definite raising)	2
Moderate oedema (edges raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre and extending beyond area of exposure)	4

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed in the animals during the study.

C. BODY WEIGHT

The body weights of the rabbits were considered to be within the normal range of variability.

D. NECROPSY

No necropsy was performed.

E. SKIN OBSERVATIONS

The test substance did not elicit any skin reactions at the application site of any animal at any of the observation times (all scores 0). 1 and 24-hours after patch removal, very slight erythema (score 1) was observed in one animal. No signs of irritation were observed in the other treated animals throughout the study.

The individual mean score for the 24, 48 and 72-hour readings were 0.33 for one animal and 0.0 for the remaining animals for erythema and 0.0 for oedema for all animals, respectively. No staining of the treated skin or other alterations or corrosive effects was observed.

As no signs of irritation were observed 72-hours after patch removal, the study was terminated after the 72-hour observation.

Table 5.2.4-1 Glyphosate technical - Primary Skin Irritation Study in Rabbits [redacted] 2011): Skin irritation scores (individual values)

Evaluation interval	Animal No.	Erythema	Oedema
1 hour	00606 (♂)	0	0
	00622 (♂)	1	0
	00620 (♂)	0	0
24-hours	00606 (♂)	0	0
	00622 (♂)	1	0
	00620 (♂)	0	0
48 hours	00606 (♂)	0	0
	00622 (♂)	0	0
	00620 (♂)	0	0
72-hours	00606 (♂)	0	0
	00622 (♂)	0	0
	00620 (♂)	0	0
Individual 24 – 72 h means	**00606 (♂)**	**0.0**	**0.0**
	00622 (♂)	**0.33**	**0.0**
	00620 (♂)	**0.0**	**0.0**

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study is in concordance with the current OECD TG 404 (2015). Therefore, the study is considered acceptable and the outcome can be reported as valid.

A single semi-occlusive application of 0.5 g glyphosate technical to intact rabbit skin for 4 hours elicited a very slight erythema (score 1) in one animal after 1 and 24-hours. No dermal observation was recorded in this animal thereafter. No skin reactions at the application site were observed in the other two treated animals at any observation time. The individual mean score for the 24, 48 and 72-hour readings were 0.33 for one animal and 0.0 for the remaining animals for erythema and 0.0 for oedema for all animals, respectively.

Thus, glyphosate is not considered a skin irritant.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.4/002
Report author	[redacted]
Report year	2010
Report title	Acute Dermal Irritation/Corrosion Test (Patch Test) of Glyphosate TC in Rabbits
Report No	24605
Document No	Not reported
Guidelines followed in study	OECD 404 (2002), EC method B.4. (2004/73/EC), US EPA OPPTS 870.2500 (1998)
Deviations from current test guideline (OECD 404, 2015)	Himalayan rabbits were used instead of New Zealand White rabbits. This deviation is not considered to affect the study outcome.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid[#]
Category study in AIR 5 dossier (L docs)	Category 2a

#: Following the feedback from the Assessment Group Glyphosate in March 2020, the Notifier is aware of concerns regarding work conducted at this specific contract laboratory [redacted]. For the sake of completeness, this study is included in the dossier submission since it was part of the data package of previous EU evaluation processes.

2. Full summary

The test substance, glyphosate TC (Batch: 20090506, Purity: 97.3 %), was evaluated for its potential to cause irritant / corrosive effects. In a primary dermal irritation study, three young male Himalayan rabbits were dermally exposed to glyphosate TC. The clipped intact dorsal skin of the trunk was exposed to 0.5 g of the solid test item (area: approximately 6 cm^2), moistened with 0.5 or 1 mL *aqua ad iniectabilia*, for 4 hours under semi-occlusive conditions. The patch was held in contact with the skin with a non-irritating tape. Skin irritation was scored using the Draize scheme 1, 24, 48 and 72-hours after removal of the test substance.

None of three male rabbits exposed for 4 hours to 0.5 g glyphosate TC per animal (semi-occlusive conditions) showed any test item-related changes. The mean for the 24, 48 and 72-hour readings for each animal was 0.0 for erythema and 0.0 for oedema.

Based on the study, glyphosate TC does not show a skin irritating potential under the test conditions chosen.

Materials and methods

A: Materials

1. Test material:

Identification:	Glyphosate TC
Description:	White solid powder
Lot/Batch #:	20090506
Purity:	97.3 %
Water solubility:	Yes
Stability of test compound:	Expiry date: 2011-05

2. Vehicle and/or positive control: Purified water for injection

3. Test animals:

Species:	Rabbit
Strain:	Himalayan
Source:	[redacted]
Age:	Approximately 6 – 7 months
Sex:	Male
Weight at dosing:	2.4 – 2.9 kg
Acclimation period:	At least 20 days
Diet/Food:	Commercial diet, ssniff® K-H V2333 (ssniff Spezialdiäten GmbH, 59494 Soest, Germany), *ad libitum*, except during the exposure period
Water:	Tap water, *ad libitum,* except during the exposure period
Housing:	Individually, in cages measuring 380 mm × 425 mm × 600 mm (manufacturer: Dipl. Ing. W. EHRET GmbH, 16352 Schönwalde, Germany) before and after the exposure period. During the exposure period the animals were kept singly in restrainers which allowed free movement of the head but prevented a complete body turn.
Environmental conditions:	Temperature: 20 ± 3 °C Humidity: 30 – 70 % Air changes: Not reported Photocycle: 12-hour light / dark cycle

4. Test conditions:

Patch site preparation technique:	The dorsal area of the trunk was closely clipped, 24-hours before application.
Patching technique:	Dermal application onto shaved, intact dorsal skin (6 cm^2). The patch was held in position with non-irritating tape for exposure period duration.
Chemical preparation:	1000 or 2000 mg was mixed with 0.5 or 1 mL *aqua ad iniectabilia* [water for injection] respectively and 750 mg were applied per animal (≈ 0.5 g test item/animal).
Chemical application:	0.5 g glyphosate TC / animal
Chemical removal:	No

B: Study design and methods

In life dates: 2009-10-26 to 2009-11-06

Animal assignment and treatment:

Approximately 24-hours before the test, the fur was removed by closely clipping the dorsal area of the trunk of the animals. Care was taken to avoid abrading the skin. Only animals with healthy intact skin were used.

A dose of 0.5 g of the test item was applied to the test site (area: approximately 6 cm²) and then covered with a gauze patch. The patch was held in contact with the skin with non-irritating tape for the duration of

the exposure period. The surrounding untreated skin served as a control. Exposure time was 4 hours. During the exposure the animals were kept in comfortable restrainers. At the end of the exposure time no residual test item had to be removed.

As it was expected that the test item would not produce any severe irritancy or corrosion, the test was started using at first only one animal, receiving a single patch for an exposure period of 4 hours. As neither a corrosive effect nor a severe irritant effect was observed after a 4 hour exposure period, the test was completed using two additional animals, each with one patch only, for an exposure period of 4 hours.

Skin reactions were assessed approximately 1, 24, 48 and 72-hours after removal of the patch according to the Draize scheme (see table below).

Skin reaction grading according to Draize criteria used by [redacted] (2010)

Skin Reaction	**Grading**
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef redness) to eschar formation preventing grading of erythema	4
Oedema formation	
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre and extending beyond area of exposure)	4

Results

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed during the study.

C. BODY WEIGHT

All rabbits showed the expected body weight gain.

D. NECROPSY

No necropsy was performed.

E. SKIN OBSERVATION

The reactions of the intact skin were evaluated at 60 minutes and then at 24, 48 and 72-hours after patch removal. None of the three male rabbits showed any significant test item-related lesions at these examination time points.

Table 5.2.4-2 Acute Dermal Irritation/Corrosion Test (Patch Test) of Glyphosate TC in Rabbits [redacted] 2010): Skin irritation scores (individual values)

Evaluation interval	Animal No.	Erythema	Oedema
1 hour	1 (♂)	0	0
	2 (♂)	0	0
	3 (♂)	0	0
24-hours	1 (♂)	0	0
	2 (♂)	0	0
	3 (♂)	0	0
48 hours	1 (♂)	0	0
	2 (♂)	0	0
	3 (♂)	0	0
72-hours	1 (♂)	0	0
	2 (♂)	0	0
	3 (♂)	0	0
Individual 24 – 72 h means	**1 (♂)**	**0.0**	**0.0**
	2 (♂)	**0.0**	**0.0**
	3 (♂)	**0.0**	**0.0**

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD TG 404 (2015). Therefore, the study is considered acceptable and the outcome can be reported as valid.
A single semi-occlusive application of glyphosate to intact rabbit skin for four hours elicited no skin reactions at the application site of any animal at any observation time. The individual mean score over 24, 48 and 72-hours was 0.0 for erythema and 0.0 for oedema for all animals.
Thus, glyphosate is not considered a skin irritant.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.4/003
Report author	[redacted]
Report year	2009
Report title	Acute Dermal Irritation/Corrosion Test (Patch Test) of Glyphosate TC in Rabbits
Report No	24877
Document No	Not reported
Guidelines followed in study	OECD 404 (2002), Commission Directive 2004 B.4 (2004/73/EC), US EPA OPPTS 870.2500 (1998)
Deviations from current test guideline (OECD 404, 2015)	Himalayan rabbits were used instead of New Zealand White rabbits. This deviation is not considered to affect the study outcome.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid#

Category study in AIR 5 dossier (L docs)	Category 2a

#: Following the feedback from the Assessment Group Glyphosate in March 2020, the Notifier is aware of concerns regarding work conducted at this specific contract laboratory ([redacted]). For the sake of completeness, this study is included in the dossier submission since it was part of the data package of previous EU evaluation processes.

2. Full summary

In a primary dermal irritation study, three young male adult Himalayan rabbits were dermally exposed to glyphosate TC (Batch: 2009051501, Purity: 96.4 %). The clipped, intact skin was exposed to 0.5 g of the solid test item, moistened with purified water, for 4 hours under semi-occlusive conditions. The rabbits were observed for 72-hours. Skin irritation was scored using the Draize scheme 1, 24, 48 and 72-hours after removal of the test substance.

No skin reactions were observed at the application site of any animal at any observation time point. The mean for the 24, 48, and 72-hour readings for each animal were 0.0 for erythema and 0.0 for oedema.

Based on the study, glyphosate TC does not show a skin irritating potential under the test conditions chosen.

Materials and methods

A: Materials

1. Test material:

Identification:	Glyphosate TC
Description:	White solid powder
Lot/Batch #:	2009051501
Purity:	96.4 %
Stability of test compound:	At room temperature in the dark stable Expiry date: 2011-05-15

2. Vehicle and/or positive control: Purified water for injection

3. Test animals:

Species:	Rabbit
Strain:	Himalayan
Source:	[redacted]
Age:	Approximately 7.5 – 8.5 months
Sex:	Male
Weight at dosing:	2.6 – 3.2 kg
Acclimation period:	At least 20 days
Diet/Food:	Commercial diet, ssniff K-H V2333 (ssniff Spezialdiäten GmbH, 59494 Soest, Germany), *ad libitum* except during the exposure period
Water:	Tap water, *ad libitum* except during the exposure period
Housing:	Individual housing in cages (manufacturer: Dipl. Ing. W. EHRET GmbH, 16352 Schönwalde, Germany) before and after the exposure period. During the exposure period the animals were kept singly in restrainers which allowed free movement of the head but prevented a complete body turn.

Environmental conditions:	Temperature:	20 ± 3 °C
	Humidity:	30 – 70 %
	Air changes:	Not specified
	Photoperiod:	12 hours light / dark cycle

4. Test conditions:

Patch site preparation technique:	Closely clipping the dorsal area of the trunk, 24-hours before application.
Patching technique:	Dermal application onto shaved, intact dorsal skin. The patch was held with non-irritating tape for exposure period duration.
Chemical preparation:	1000 or 2000 mg was mixed with 0.5 or 1 mL *aqua ad iniectabilia* [water for injection], respectively and 750 mg were applied per animal (≈ 0.5 g test material/animal).
Chemical application:	0.5 g glyphosate TC / animal
Chemical removal:	No

B: Study design and methods

In life dates: 2009-10-15 to 2009-10-23

Animal assignment and treatment:

The test was conducted using three young male adult Himalayan rabbits. The test was performed in a sequential manner, first using one animal. Since no signs of corrosion were observed in the first animal the test was completed using the remaining two rabbits. An amount of 0.5 g of the solid test substance was moistened with purified water and applied to the intact skin of the rabbits on an approx. 6 cm^2 gauze patch. The patch was covered with a semi-occlusive dressing. After 4 hours of exposure the dressing was removed. No residual test item had to be removed.

Skin reactions were assessed approximately 1, 24, 48 and 72-hours after removal of the patch according to the scoring system of the Draize scheme (see table below).

Skin reaction grading according to Draize criteria used by [redacted] (2009)

Skin Reaction	Grading
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef redness) to eschar formation preventing grading of erythema	4
Oedema formation	
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approx. 1 millimetre)	3
Severe oedema (raised more than 1 millimetre and extending beyond area of exposure)	4

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed during the study.

C. BODY WEIGHT

All rabbits showed the expected body weight gain.

D. NECROPSY

No necropsy was performed.

E. SKIN OBSERVATIONS

No skin reactions were observed at the application site of any animal at any observation time point (all scores were 0). The overall mean for the 24, 48 and 72-hours readings were 0.0 for erythema and 0.0 for oedema. The test substance produced no staining on the treated skin. In addition, neither alterations of the treated skin, nor corrosive effects were observed.

Table 5.2.4-3 Acute Dermal Irritation/Corrosion Test (Patch Test) of Glyphosate TC in Rabbits ([redacted] 2009): Skin irritation scores (individual values)

Evaluation interval	Animal No.	Erythema	Oedema
1 hour	1 (♂)	0	0
	2 (♂)	0	0
	3 (♂)	0	0
24-hours	1 (♂)	0	0
	2 (♂)	0	0
	3 (♂)	0	0
48 hours	1 (♂)	0	0
	2 (♂)	0	0
	3 (♂)	0	0
72-hours	1 (♂)	0	0
	2 (♂)	0	0
	3 (♂)	0	0
Individual 24 – 72 h means	**1 (♂)**	**0.0**	**0.0**
	2 (♂)	**0.0**	**0.0**
	3 (♂)	**0.0**	**0.0**

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD TG 404 (2015). Therefore, the study is considered acceptable and the outcome can be reported as valid.
A single semi-occlusive application of glyphosate to intact rabbit skin for four hours elicited no skin reactions at the application site of any animal at any observation time. The individual mean score over 24, 48 and 72-hours was 0.0 for erythema and 0.0 for oedema for all animals.
Thus, glyphosate is not considered a skin irritant.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.4/004
Report author	[redacted]
Report year	2009
Report title	Acute Dermal Irritation/Corrosion Test (Patch Test) of Glyphosate TC in Rabbits
Report No	23913
Document No	Not reported
Guidelines followed in study	OECD 404 (2002), EC method B.4. (2004/73/EC), US EPA OPPTS 870.2500 (1998)
Deviations from current test guideline (OECD 404, 2015)	Himalayan rabbits were used instead of albino New Zealand rabbits. This deviation is not considered to affect the study outcome.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid[#]
Category study in AIR 5 dossier (L docs)	Category 2a

#: Following the feedback from the Assessment Group Glyphosate in March 2020, the Notifier is aware of concerns regarding work conducted at this specific contract laboratory [redacted]. For the sake of completeness, this study is included in the dossier submission since it was part of the data package of previous EU evaluation processes.

2. Full summary

The test substance, glyphosate TC (Batch: 20080801, Purity: 98.8 %), was evaluated for its potential to cause irritant / corrosive effects. In a primary dermal irritation study, three young male Himalayan rabbits were dermally exposed to glyphosate TC. The clipped intact dorsal skin of the trunk was exposed to 0.5 g of the solid test material (area: approximately 6 cm^2), moistened with 0.5 or 1 mL *aqua ad iniectabilia*, for 4 hours under semi-occlusive conditions. The patch was held in contact with the skin with a non-irritating tape. Skin irritation was scored using the Draize scheme 1, 24, 48 and 72-hours after removal of the test substance.

None of three male rabbits exposed for 4 hours to 0.5 g glyphosate TC per animal (semi-occlusive conditions) showed any test item-related changes. The mean for the 24, 48 and 72-hour readings for each animal were 0.0 for erythema and 0.0 for oedema.

Based on the study, glyphosate TC does not show a skin irritating potential under the test conditions chosen.

Materials and methods

A: Materials

1. Test material:

Identification: Glyphosate TC
Description: White solid powder
Lot/Batch #: 20080801
Purity: 98.8 %
Water solubility: Yes

Stability of test compound:	Expiry date: 2010-08-01
2. Vehicle and/or positive control:	Purified water for injection
3. Test animals:	
Species:	Rabbit
Strain:	Himalayan
Source:	[redacted]
Age:	Approximately 4 – 5 months
Sex:	Male
Weight at dosing:	3.8 – 4.4 kg
Acclimation period:	At least 20 days
Diet/Food:	Commercial diet, ssniff® K-H V2333 (ssniff Spezialdiäten GmbH, 59494 Soest, Germany), *ad libitum* except during the exposure period
Water:	Tap water, *ad libitum* except during the exposure period
Housing:	Individually in cages measuring 380 mm × 425 mm × 600 mm (manufacturer: Dipl. Ing. W. EHRET GmbH, 16352 Schönwalde, Germany) before and after the exposure period. During the exposure period the animals were kept singly in restrainers which allowed free movement of the head but prevented a complete body turn.
Environmental conditions:	Temperature: 20 ± 3 °C Humidity: 30 – 70 % Air changes: Not specified Photoperiod: 12-hour light / dark cycle
4. Test conditions:	
Patch site preparation technique:	The dorsal area of the trunk was closely clipped, 24-hours before application.
Patching technique:	Dermal application onto shaved, intact dorsal skin. The patch was held in position with non-irritating tape for exposure period duration.
Chemical preparation:	1000 or 2000 mg was mixed with 0.5 or 1 mL *aqua ad iniectabilia* [water for injection] respectively, and 750 mg were applied per animal (≈ 0.5 g test item / animal).
Chemical application:	0.5 g glyphosate TC / patch and animal
Chemical removal:	No

B: Study design and methods

In life dates: 2009-02-04 to 2009-02-13

Animal assignment and treatment:

Approximately 24-hours before the test, the fur was removed by closely clipping the dorsal area of the trunk of the animals. Care was taken to avoid abrading the skin. Only animals with healthy intact skin were used.

A dose of 0.5 g of the test item was applied to the test site (area: approximately 6 cm²) and then covered

with a gauze patch. The patch was held in contact with the skin with non-irritating tape for the duration of the exposure period. The surrounding untreated skin served as a control. Exposure time was 4 hours. During the exposure the animals were kept in comfortable restrainers. At the end of the exposure time no residual test item had to be removed.

As it was expected that the test item would not produce any severe irritancy or corrosion, the test was started using at first only one animal, receiving a single patch for an exposure period of 4 hours. As neither a corrosive effect nor a severe irritant effect was observed after a four-hour exposure period, the test was completed using two additional animals, each with one patch only, for an exposure period of 4 hours.

Skin reactions were assessed approximately 1, 24, 48 and 72-hours after removal of the patch according to the Draize scheme (see table below).

Skin reaction grading according to Draize criteria used by [redacted] (2009)

Skin Reaction	**Grading**
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef redness) to eschar formation preventing grading of erythema	4
Oedema formation	
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre and extending beyond area of exposure)	4

Results

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed during the study.

C. BODY WEIGHT

All rabbits showed the expected body weight gain.

D. NECROPSY

No necropsy was performed.

E. SKIN OBSERVATION

The reactions of the intact skin were evaluated at 60 minutes and then at 24, 48 and 72-hours after patch removal. None of the three male rabbits showed any significant test item-related lesions at these examination time points.

Table 5.2.4-4 Acute Dermal Irritation/Corrosion Test (Patch Test) of Glyphosate TC in Rabbits ([redacted] 2009): Skin irritation scores (individual values)

Evaluation interval	Animal No.	Erythema	Oedema
1 hour	1 (♂)	0	0
	2 (♂)	0	0
	3 (♂)	0	0
24-hours	1 (♂)	0	0
	2 (♂)	0	0
	3 (♂)	0	0
48 hours	1 (♂)	0	0
	2 (♂)	0	0
	3 (♂)	0	0
72-hours	1 (♂)	0	0
	2 (♂)	0	0
	3 (♂)	0	0
Individual 24 – 72 h means	**1 (♂)**	**0.0**	**0.0**
	2 (♂)	**0.0**	**0.0**
	3 (♂)	**0.0**	**0.0**

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD TG 404 (2015). Therefore, the study is considered acceptable and the outcome can be reported as valid.
A single semi-occlusive application of glyphosate to intact rabbit skin for four hours elicited no skin reactions at the application site of any animal at any observation time. The individual mean score over 24, 48 and 72-hours was 0.0 for erythema and 0.0 for oedema for all animals.
Thus, glyphosate is not considered a skin irritant.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.4/005
Report author	[redacted]
Report year	2009
Report title	Glyphosate – Acute Dermal Irritation Study in Rabbits
Report No	12173-08
Document No	Not reported
Guidelines followed in study	US EPA OPPTS 870.2500
Deviations from current test guideline (OECD 404, 2015)	Step-wise approach by initial testing in one animal was not performed and three animals were treated simultaneously. Rationale for *in vivo* testing and consideration of pre-existing data was not documented. Water solubility was not documented. Humidity was in the range of 43 – 92 % instead of 30 – 70 %. These deviations did not compromise the negative study outcome.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised	Yes

testing facilities	
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The test substance, glyphosate tech grade mixed 5-batch (Batch: 080704-1 thru 5, Purity: 96.4 %), was evaluated for its potential to cause irritant / corrosive effects. Therefore, a primary dermal irritation study was conducted on three New Zealand White rabbits (one male, two females).

The clipped intact dorsal skin of the trunk was exposed to 0.5 g of the solid test item (2.5 × 2.5 cm), moistened with 0.2 mL deionised water, for 4 hours under semi-occlusive conditions. The patch was held in contact with the skin with a strip of non-irritating adhesive tape. Skin irritation was scored using the Draize scheme 1, 24, 48 and 72-hours after removal of the test substance.

None of three rabbits exposed for 4 hours to 0.5 g glyphosate tech grade mixed 5-batch (semi-occlusive conditions) showed any test item-related changes. The mean individual score for the 24, 48 and 72-hour readings was 0.0 for erythema and 0.0 for oedema.

Based on the study, glyphosate tech grade mixed 5-batch does not show a skin irritating potential under the test conditions chosen.

Materials and methods

A: Materials

1. Test material:

Identification:	Glyphosate Tech Grade; Mixed 5-Batch
Description:	White powder
Lot/Batch #:	080704-1 thru 5
Purity:	96.40 % (per certificate of analysis dated 2008-10-17)
Stability of test compound:	A second certificate of analysis dated 2009-01-08 with a purity value of 96.71 % demonstrated that the test substance was stable for the duration of the test.

2. Vehicle and/or positive control: Deionised water

3. Test animals:

Species:	Albino rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Approx. 3 months
Sex:	Male and female (nulliparous and non-pregnant)
Weight at dosing:	Male: 2.000 kg; Females: 2.600 kg
Acclimation period:	5 days
Diet/Food:	PMI Feeds, Inc.™ Lab Rabbit Diet #5321, 8 oz. daily
Water:	Tap water, *ad libitum*
Housing:	Individual housing in suspended, wire bottom, stainless steel cages

Environmental conditions:	Temperature:	17 – 22 °C
	Humidity:	43 – 92 %
	Air changes:	10 – 12 / hour
	Photoperiod:	12-hour light / dark cycle

4. Test conditions:

Patch site preparation technique:	The dorsal area of the trunk was clipped, 24-hours before application to expose at least 8 × 8 cm.
Patching technique:	Dermal application onto shaved, intact dorsal skin (2.5 × 2.5 cm) and the patch was held in position with a strip of non-irritating adhesive tape for exposure period duration (semi-occlusive dressing).
Chemical preparation:	0.5 g glyphosate was moistened with 0.2 mL of deionised water
Chemical application:	0.5 g / animal
Chemical removal:	Skin was washed with room temperature tap water

B: Study design and methods

In life dates: 2008-11-11 to 2008-11-14

Animal assignment and treatment:

Each animal was prepared on the day prior to treatment by clipping the dorsal area of the trunk free of hair to expose an area at least 8 × 8 cm. Only those animals with exposure areas free of pre-existing skin irritation or defects were selected for testing. A single intact exposure site was selected as the test site while the contralateral intact site served as a control site.

On Day 0, 0.5 g of test substance moistened with 0.2 mL of deionised water was applied to each test site and covered with a 4 ply, 2.5 × 2.5 cm surgical gauze patch. Each patch was secured in place with a strip of non-irritating adhesive tape. The entire trunk of each animal was loosely wrapped with a semi-permeable dressing (orthopaedic stockinette) which was secured on both edges with strips of tape to retard evaporation of volatile substances and to prevent possible ingestion of the test substance. After 4 hours, the patches and wrappings were removed. The test sites were gently washed with room temperature tap water and a clean cloth to remove as much residual test substance as possible.

Skin reactions were assessed approximately 1, 24, 48 and 72-hours after removal of the patch according to the Draize scheme (see table below).

Skin reaction grading according to Draize criteria used by [redacted] (2009)

Skin Reaction	Grading
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef redness) to slight eschar formation (injuries in depth)	4
Oedema formation	
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approximately 1 mm)	3
Severe oedema (raised more than 1 mm and extending beyond area of exposure)	4

Results

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

No data were reported.

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance.

D. NECROPSY

No data on necropsy were reported.

E. SKIN OBSERVATIONS

No skin reactions were observed at the application site of any animal at any observation time point (all scores were 0). The overall mean for the 24, 48 and 72-hour readings were 0.0 for erythema and 0.0 for oedema.

Table 5.2.4-5 Glyphosate – Acute Dermal Irritation Study in Rabbits ([redacted] 2009): Skin irritation scores (individual values)

Evaluation interval	Animal No.	Erythema	Oedema
1 hour	3228 (♂)	0	0
	3227 (♀)	0	0
	3229 (♀)	0	0
24-hours	3228 (♂)	0	0
	3227 (♀)	0	0
	3229 (♀)	0	0
48 hours	3228 (♂)	0	0
	3227 (♀)	0	0
	3229 (♀)	0	0
72-hours	3228 (♂)	0	0
	3227 (♀)	0	0
	3229 (♀)	0	0
Individual 24 – 72 h means	**3228 (♂)**	**0.0**	**0.0**
	3227 (♀)	**0.0**	**0.0**
	3229 (♀)	**0.0**	**0.0**

3. Assessment and conclusion

Assessment and conclusion by applicant:
Compared to the current OECD TG 404 (2015), deviations such as no step-wise approach, no report of water solubility and humidity in the range of 43 – 92 % instead of 30 – 70 % are noted. Nevertheless, these deviations did not compromise the negative study outcome. Therefore, the study is considered acceptable and the outcome can be reported as valid.
A single semi-occlusive application of glyphosate to intact rabbit skin for four hours elicited no skin reactions at the application site of any animal at any observation time. The individual mean score over 24, 48 and 72-hours was 0.0 for erythema and 0.0 for oedema for all animals.
Thus, glyphosate is not considered a skin irritant.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.4/006
Report author	[redacted]
Report year	2008
Report title	Acute Dermal Irritation/Corrosion Study in Rabbits with Glyphosate Technical
Report No	[redacted]-3996.311.476.07
Document No	Not reported
Guidelines followed in study	OECD 404 (2002)
Deviations from current test guideline (OECD 404, 2015)	None.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The acute dermal irritation/corrosion potential of glyphosate technical (Batch: 20070606, Purity: 98.05 %) was evaluated in three New Zealand White rabbits (females). The test was initially conducted using one single rabbit. Because no dermal reaction was observed in the initial test, two additional animals were tested to confirm the response. A moistened gauze patch containing 0.5 g of the undiluted test item was applied to the clipped back (approx. 6 cm^2) of each animal. The patch was held in contact with the skin by an adhesive and a non-irritating tape. After the 4-hour semi-occlusive exposure period, the patches were removed, any residual test item was washed using physiological saline solution, and the animals were examined at approximately 1, 24, 48 and 72-hours to verify the erythema, eschars, and oedema formation, and for behavioural and clinical alterations. Adjacent untreated shaved areas of the skin were used as the negative control.

The test item applied on the skin of the rabbits did not cause any dermal irritation. The mean for the 24, 48 and 72-hour readings were 0.0 for erythema and 0.0 for oedema. No treatment-related behavioural or clinical alterations were noted during the observation period.

Based on the study, glyphosate technical does not show a skin irritating potential under the test conditions chosen.

Materials and methods

A: Materials

1. Test material:

Identification:	Glyphosate Technical
Description:	Solid; molecular weight 169.1 g/mol
Lot/Batch #:	20070606
Purity:	98.05 %
Water solubility:	Yes
Stability of test compound:	No data given in the report.

2. Vehicle and/or positive control: Not specified

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	17 weeks
Sex:	Female (nulliparous and non-pregnant)
Weight at dosing:	2.907 – 3.145 kg
Acclimation period:	5 to 6 days
Diet/Food:	Pelleted and autoclaved commercial diet for rabbits (Guabi, Mogiana Alimentos S.A. - Brazil), *ad libitum*
Water:	Filtered tap water, *ad libitum*
Housing:	The animals were housed individually in galvanized steel cages. Autoclaved wood shavings were placed in a tray below the cages to collect excrements.
Environmental conditions:	Temperature: 17 – 22 °C Humidity: 30 – 70 % Air changes: 10 – 15 / hour Photocycle: 12-hour light / dark cycle

4. Test conditions:

Patch site preparation technique:	The fur from the back was clipped using a small animal clipper (Oster model Golden A5, Electric Razor), 24-hours before application.
Patching technique:	Dermal application onto shaved, intact dorsal skin (approximately 6 cm^2) and the patch was held in the test site by an adhesive and non-irritating tape (Micropore®).
Chemical preparation:	Glyphosate technical was applied onto a moistened gauze dressing
Chemical application:	0.5 g / animal
Chemical removal:	Skin was washed with physiological saline.

B: Study design and methods

In life dates: 2008-05-20 to 2008-05-24

Animal assignment and treatment:

Each animal provisionally selected for the test was prepared by clipping the fur from the back approximately 24-hours prior to the application of the test item, using a small animal clipper (Oster model Golden A5, Electric Razor) with great care taken to avoid abrading the skin during the clipping procedure, so as not to alter its permeability. The clipped area was large enough to allow clear visualization of the test site. After being clipped, visual examination of the skin confirmed the skin was intact and healthy.

Five-tenths of a gram (0.5 g) of the test item was applied over the skin of each animal. The test item was first placed onto a moistened gauze dressing, which was applied over a small section of the test area (approximately 6 cm^2) in such a manner that there was good contact and uniform distribution of the test item on the skin. After application, the gauze was held in the test site by an adhesive and non-irritating tape. Removal and ingestion of the test item was prevented by placing a suitable adhesive tape (semi-occlusive dressing) around the trunk and test area. Adjacent untreated shaved areas of the skin were used as the negative control. After the 4-hour exposure period, the gauze patches were removed, any residual test item washed using physiological saline.

The treated areas examined for signs of irritation 1, 24, 48 and 72-hours after removal of the patch according to the table below. During the observation period animals were observed once daily for general health/morbidity and mortality.

The test was performed initially using one single animal for evaluation of any irritant / corrosive effect of the test item to the skin. Because no severe dermal reaction was observed in the initial test, two additional animals were tested to confirm the response.

Skin reaction grading according to Draize criteria used by [redacted] (2008)

Skin Reaction	Grading
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef redness) to slight eschar formation (injuries in depth)	4
Oedema formation	
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre and extending beyond area of exposure)	4

Results

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed during the study.

C. BODY WEIGHT

Body weight was unaffected by the administration of the test item.

D. NECROPSY

No data on necropsy were reported.

E. SKIN OBSERVATIONS

No signs of dermal irritation were observed at any of the time points in any of the animals tested.

Table 5.2.4-6 Acute Dermal Irritation/Corrosion Study in Rabbits with Glyphosate Technical [redacted] 2008): Skin irritation scores (individual values)

Evaluation interval	Animal No.	Erythema	Oedema
1 hour	97 (♀)	0	0
	98 (♀)	0	0
	99 (♀)	0	0
24-hours	97 (♀)	0	0
	98 (♀)	0	0
	99 (♀)	0	0
48 hours	97 (♀)	0	0
	98 (♀)	0	0
	99 (♀)	0	0
72-hours	97 (♀)	0	0
	98 (♀)	0	0
	99 (♀)	0	0
Individual 24 – 72 h means	**97 (♀)**	**0.0**	**0.0**
	98 (♀)	**0.0**	**0.0**
	99 (♀)	**0.0**	**0.0**

C. BODY WEIGHT

All animals presented gain in body weight during the observation period.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD TG 404 (2015). Therefore, the study is considered acceptable and the outcome can be reported as valid.
A single semi-occlusive application of glyphosate to intact rabbit skin for 4 hours elicited no skin reactions at the application site of any animal at any observation time. The individual mean score over 24, 48 and 72-hours was 0.0 for erythema and 0.0 for oedema for each animal.
Thus, glyphosate is not considered a skin irritant.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.4/007
Report author	[redacted]
Report year	2007
Report title	Glyphosate Technical Material: Primary Skin Irritation Study in Rabbits (4-Hour Semi-Occlusive Application)
Report No	B02777
Document No	Not reported
Guidelines followed in study	OECD 404 (2002), US EPA OPPTS 870.2500 (1998), 2004/73/EC B.4 (2004), 12 NohSan No. 8147 (2000)
Deviations from current test guideline (OECD 404, 2015)	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities[1,2]	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

In a primary dermal irritation study, young adult New Zealand White rabbits (one male and two females) were dermally exposed to glyphosate technical material (Batch: 0507, Purity: 96.1 % w/w glyphosate acid). The clipped, intact skin of the left flank was exposed to 0.5 g of the test material, moistened with about 0.5 mL water, for 4 hours under semi-occlusive conditions. The rabbits were observed for 72-hours. Skin irritation was scored using the Draize scheme 1, 24, 48 and 72-hours after removal of the test substance.

No skin reactions were observed at the application site of any animal at any observation time point. The overall mean for the 24, 48 and 72-hour readings were 0.0 for erythema and 0.0 for oedema. No clinical signs were observed.

Based on the study, glyphosate technical material does not show a skin irritating potential under the test conditions chosen.

Materials and methods

A: Materials

1. Test material:

Identification:	Glyphosate Technical Material
Description:	White powder
Lot/Batch number:	0507
Purity:	96.1 % w/w Glyphosate acid
Stability of test compound:	Stable under storage conditions of room temperature (range of 20 °C ± 5 °C), protected from light and humidity. Expiry date: 2008-08

2. Vehicle and/or positive control: Purified water

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White (SPF)

Source:	[redacted]
Age:	Male: 10 – 11 weeks; female: 15 – 16 weeks
Sex:	Male and female
Weight at dosing:	Male: 2.440 kg; female: 2.749 and 2.815 kg
Acclimation period:	5 – 6 days
Diet:	Pelleted standard Provimi Kliba 3418 rabbit maintenance diet (Provimi Kliba, CH-4303 Kaiseraugst, Switzerland), *ad libitum;* wood blocks (RCC Ltd, Fullinsdorf, Switzerland) and hay sticks 4642 (Provimi Kliba AG, CH-4303 Kaiseraugst, Switzerland) were provided for gnawing.
Water:	Community tap water, *ad libitum*
Housing:	Individually in stainless steel cages equipped with feed hoppers and drinking water bowls
Environmental conditions:	Temperature: 17 – 23 °C Humidity: 30 – 70 % Air changes: 10 – 15 / hour Photocycle: 12 hours light / dark

4. Test conditions:

Patch site preparation technique:	The left flank was clipped (area of approximately 100 cm^2) with an electric clipper 4 days before treatment.
Patching technique:	Dermal application onto shaved, intact dorsal skin (patch area 2.5 cm × 2.5 cm). The patch was covered with a semi-occlusive dressing which was wrapped around the abdomen and anchored with tape.
Chemical preparation:	Glyphosate technical material was moistened with approximately 0.5 mL purified water.
Chemical application:	0.5 g / animal
Chemical removal:	Skin was flushed with lukewarm tap water

B: Study design and methods

In-life dates: 2006-12-18 to 2006-12-22

Animal assignment and treatment:

Three young adult (one male and two female) New Zealand White rabbits were used in the study. As it was suspected that the test substance might produce irritancy, a single animal (one female) was treated first. As no corrosive effect was observed after the 4-hour exposure, the test was completed using the two remaining animals for an exposure period of 4 hours.

Four days before treatment, the left flank was clipped, exposing an area of approximately 100 cm^2 (10 cm × 10 cm). The skin of the animals was examined one day before treatment, and regrown fur of all animals was clipped again. Animals with overt signs of skin injury or marked irritation which may have interfered with the interpretation of the results were not used in the test.

On the day of treatment, 0.5 g of glyphosate technical material was placed on a surgical gauze patch (2.5 cm × 2.5 cm). This gauze patch was applied to the intact skin of the clipped area. The patch was covered with a semi-occlusive dressing which was wrapped around the abdomen and anchored with tape. The duration of treatment was 4 hours after which the dressing was removed, and the skin was flushed with lukewarm tap water to clean the application site so that any reactions (erythema) were clearly visible at that time.

Observations for viability, mortality and clinical signs were carried out daily from acclimatisation of the animals to the termination of the study. Body weights of individual animals were recorded at the start of the acclimatisation period, on the day of application, and at termination of the observation period. Skin reactions were assessed according to the scoring system listed in Commission Directive 2004/73/EC (see table below) approximately 1, 24, 48 and 72-hours after removal of the dressing, gauze patch and test substance.

Skin reaction grading according to Draize criteria used by [redacted] (2007)

Skin Reaction	Grading
Erythema and eschar formation	
No erythema	0
Very slight erythema	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef redness) or eschar formation (injuries in depth preventing erythema) reading	4
Oedema formation	
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well-defined by definite raising)	2
Moderate oedema (edges raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre and extending beyond area of exposure)	4

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed in the animals during the study.

C. BODY WEIGHT

The body weights of the rabbits were considered to be within the normal range of variability.

D. NECROPSY

No necropsy was performed.

E. SKIN OBSERVATIONS

The test substance did not elicit any skin reactions at the application site of any of the three animals at any of the observation times (all scores 0). The individual mean score for the 24, 48 and 72-hour readings were 0.0 for erythema and 0.0 for oedema for each of the animals. No staining of the treated skin was observed.

Table 5.2.4-7 Glyphosate Technical Material: Primary Skin Irritation Study in Rabbits (4-Hour Semi-Occlusive Application) ([redacted] 2007): Skin irritation scores (individual values)

Evaluation interval	Animal No.	Erythema	Oedema
1 hour	31 (♂)	0	0
	32 (♀)	0	0
	33 (♀)	0	0

Table 5.2.4-7 Glyphosate Technical Material: Primary Skin Irritation Study in Rabbits (4-Hour Semi-Occlusive Application) ([redacted] 2007): Skin irritation scores (individual values)

24-hours	31 (♂)	0	0
	32 (♀)	0	0
	33 (♀)	0	0
48 hours	31 (♂)	0	0
	32 (♀)	0	0
	33 (♀)	0	0
72-hours	31 (♂)	0	0
	32 (♀)	0	0
	33 (♀)	0	0
Individual 24 – 72 h means	**31 (♂)**	**0.0**	**0.0**
	32 (♀)	**0.0**	**0.0**
	33 (♀)	**0.0**	**0.0**

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD TG 404 (2015). Therefore, the study is considered acceptable and the outcome can be reported as valid.
A single semi-occlusive application of glyphosate to intact rabbit skin for 4 hours elicited no skin reactions at the application site of any animal at any observation time. The individual mean score over 24, 48 and 72-hours was 0.0 for erythema and 0.0 for oedema for all animals.
Thus, glyphosate is not considered a skin irritant.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.4/008
Report author	[redacted]
Report year	2007
Report title	Glyphosate Technical (NUP 05068): Primary Skin Irritation Study in Rabbits (4-Hour Semi-Occlusive Application)
Report No	B02294
Document No	Not reported
Guidelines followed in study	OECD 404 (2002), Commission Directive 2004/73/EC B.4 (2004), JMAFF 12 NohSan No. 8147 guideline 2-1-4 (2005)
Deviations from current test guideline (OECD 404, 2015)	Yes, the test patch used had a surface of 16 cm^2 instead of 6 cm^2. This deviation is not considered to affect the study outcome.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

In a primary dermal irritation study, young adult New Zealand albino rabbits (1 male, 2 females) were dermally exposed to glyphosate technical (NUP 05068, Batch: 200609062, Purity: 95.1 %). The clipped, intact skin of the left flank was exposed to 0.5 g of the solid test item, moistened with about 0.5 mL water, for 4 hours under semi-occlusive conditions. The rabbits were observed for 72-hours. Skin irritation was scored using the Draize scheme 1, 24, 48 and 72-hours after removal of the test substance.
No skin reactions were observed at the application site of any animal at any observation time point. The mean for the 24, 48 and 72-hour readings for each animal were 0.0 for erythema and 0.0 for oedema, respectively.

Based on the study, glyphosate technical does not show a skin irritating potential under the test conditions chosen.

Materials and methods

A: Materials

1. Test material:

Identification:	Glyphosate Technical (NUP 05068)
Description:	Solid
Lot/Batch #:	200609062
Purity:	95.1 %
Water solubility:	Yes
Stability of test compound:	Stable under storage conditions (20 ± 5 °C) Expiry date: 2008-09-14

2. Vehicle and/or positive control: Purified water

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White, SPF
Source:	[redacted]
Age:	13 weeks (male); 14 weeks (females)
Sex:	Male and female
Weight at dosing:	Male: 2.662 kg; female: 2.637 kg and 2.970 kg
Acclimation period:	At least four days
Diet/Food:	Pelleted standard Provimi Kliba 3418 rabbit maintenance diet (Provimi Kliba AG, CH-Kaiseraugust, Switzerland), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in stainless steel cages with feed hoppers and drinking water bowls. Wood blocks and hay sticks were provided for gnawing.
Environmental conditions:	Temperature: 17 – 23 °C Humidity: 30 – 70 % Air changes: 10 – 15 / hour Photocycle: 12 hours light / dark cycle

4. Test conditions:

Patch site preparation technique:	The left flank was clipped with an electric clipper (approximately 100 cm^2; 10 cm × 10 cm), 4 days before application
Patching technique:	Dermal application onto shaved, intact skin (4 cm × 4 cm). The patch was covered with a semi-occlusive dressing which was wrapped around the abdomen and anchored with tape.
Chemical preparation:	0.5 g glyphosate / animal was weighed and moistened with approximately 0.5 mL of purified water before application.
Chemical application:	0.5 g glyphosate technical
Chemical removal:	Skin was flushed with lukewarm tap water.

B: Study design and methods

In life dates: 2007-01-09 to 2007-01-15

Animal assignment and treatment:

The test was conducted using young adult New Zealand White rabbits (one male, two females). The test was performed in a sequential manner, first using one animal. Since no signs of corrosion were observed in the first animal the test was completed using the remaining two rabbits. An amount of 0.5 g of the solid test substance moistened with approximately 0.5 mL of purified water was applied to the intact skin of the clipped left flank of the rabbits on an approx. 16 cm^2 gauze patch. The patch was covered with a semi-occlusive dressing. After 4 hours of exposure the dressing was removed, and the skin was cleaned with lukewarm tap water.

Skin reactions were assessed according to the scoring system listed in Commission Directive 2004/73/EC approximately 1, 24, 48 and 72-hours after removal of the patch (see table below). The animals were observed for mortality and clinical signs daily. Body weights were determined at beginning of acclimatisation, on the day of application and at termination.

Skin reaction grading according to Draize criteria used by [redacted] (2007d)

Skin Reaction	**Grading**
Erythema and eschar formation	
No erythema	0
Very slight erythema	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef redness) to eschar formation (preventing grading of erythema) reading	4
Oedema formation	
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approx. 1 millimetre)	3
Severe oedema (raised more than 1 millimetre and extending beyond area of exposure)	4

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed during the study.

C. BODY WEIGHT

All rabbits showed the expected body weight gain.

D. NECROPSY

No necropsy was performed.

E. SKIN OBSERVATIONS

No skin reactions were observed at the application site of any animal at any observation time point (all scores were 0). The overall mean for the 24, 48 and 72-hour readings were 0.0 for erythema and 0.0 for oedema. The test substance produced no staining on the treated skin. In addition, neither alterations of the treated skin, nor corrosive effects were observed.

Table 5.2.4-8 Glyphosate Technical (NUP 05068): Primary Skin Irritation Study in Rabbits (4-Hour Semi-Occlusive Application) ([redacted] 2007): Skin irritation scores (individual values)

Evaluation interval	Animal No.	Erythema	Oedema
1 hour	7 (♂)	0	0
	8 (♀)	0	0
	9 (♀)	0	0
24-hours	7 (♂)	0	0
	8 (♀)	0	0
	9 (♀)	0	0
48 hours	7 (♂)	0	0
	8 (♀)	0	0
	9 (♀)	0	0
72-hours	7 (♂)	0	0
	8 (♀)	0	0
	9 (♀)	0	0
Individual 24 – 72 h means	7 (♂)	**0.0**	**0.0**
	8 (♀)	**0.0**	**0.0**
	9 (♀)	**0.0**	**0.0**

3. Assessment and conclusion

Assessment and conclusion by applicant:
Except the minor deviation of a test patch size of 16 cm² instead of 6 cm² the study is in concordance with the current OECD TG 404 (2015). This deviation did not compromise the acceptability of the study. Therefore, the study is considered acceptable and the outcome can be reported as valid.
A single semi-occlusive application of glyphosate to intact rabbit skin for four hours elicited no skin reactions at the application site of any animal at any observation time. The individual mean score over 24, 48 and 72-hours was 0.0 for erythema and 0.0 for oedema for all animals.
Thus, glyphosate is not considered a skin irritant.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.4/009
Report author	[redacted]
Report year	2005
Report title	Glyphosate Acid Technical – Primary Skin Irritation Study in Rabbits
Report No	15278
Document No	P326
Guidelines followed in study	US EPA OPPTS 870.2500 (1998), OECD 404 (2002), JMAFF 59 NohSan No. 4200 (1985)
Deviations from current test guideline (OECD 404, 2015)	Yes, step-wise approach by initial testing in one animal was not performed and three animals were treated simultaneously. Rationale for *in vivo* testing and consideration of pre-existing data was not documented. Individual animal weights at start and conclusion of the test not provided. No data about the relative humidity, air changes, specific age of animals, or animal body weight were given. These deviations are not considered to affect the study outcome.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

A primary skin irritation test was conducted with three young New Zealand albino rabbits (males) to determine the potential for glyphosate acid technical (Batch: 040205, Purity: 97.23 %) to produce irritation after a single topical application. Five-tenths of a gram (0.5 g) of the test substance was moistened with distilled water and applied to the clipped intact dorsal area and the trunk (6 cm^2) of three rabbits for 4 hours under semi-occlusive conditions. The patch was held in contact with the skin with a Micropore tape. After removal of the patch, the test sites were gently cleansed of any residual test substance. Skin reactions were scored according to the Draize method and recorded 1, 24, 48 and 72-hours after removal of the test material.

One hour after patch removal, one animal showed very slight erythema (score 1). Dermal irritation cleared from this animal by 24-hours. No skin irritation was noted for the other two animals.

Based on the study, glyphosate acid technical does not show a skin irritating potential under the test conditions chosen.

Materials and methods

A: Materials

1. Test material:

Identification:	Glyphosate Acid Technical (CAS No. 1071-83-6)
Description:	White crystalline powder
Lot/Batch #:	040205
Purity:	97.23 %
Water solubility:	12 g/L; insoluble in organic solvents
Stability of test compound:	No data given in the report

2. Vehicle and/or positive control: Distilled water

3. Test animals:

Species:	Rabbit
Strain:	New Zealand albino
Source:	[redacted]
Age:	Young adult (not further specified)
Sex:	Male
Weight at dosing:	Not specified
Acclimation period:	21 days
Diet/Food:	Pelleted Purina Rabbit Chow #5326
Water:	Filtered tap water, *ad libitum*
Housing:	Individual housing in suspended stainless steel cages with mesh floors which conform to the size recommendations in the at study timepoint valid *Guide for the Care and Use of Laboratory Animals DHEW (NIH)*. Litter paper was placed beneath the cage and was changed at least three times per week.
Environmental conditions:	Temperature: 18 – 22 °C Humidity: Not specified Air changes: Not specified Photoperiod: 12-hour light / dark cycle

4. Test conditions:

Patch site preparation technique:	Clipping the dorsal area and the trunk, 24-hours before application.
Patching technique:	Dermal application onto shaved, intact dorsal skin (6 cm^2) and the patch and entire trunk were wrapped with semi-occlusive 3-inch Micropore tape.
Chemical preparation:	Glyphosate Acid Technical was moistened with distilled water (70 % w/w mixture).
Chemical application:	0.5 g (0.71 g test mixture) / animal
Chemical removal:	Skin was cleaned

B: Study design and methods

In life dates: 2004-05-05 to 2004-05-08

Animal assignment and treatment:
On the day before application, a group of animals was prepared by clipping (Oster model #A5-small) the dorsal area and the trunk. On the day of dosing, but prior to application, the animals were examined for health and the skin checked for any abnormalities. Three healthy animals without pre-existing skin irritation were selected for test. Prior to application, the test substance was moistened with distilled water to achieve a dry paste by preparing a 70 % w/w mixture. Five-tenths of a gram (0.5 g) of the test substance (0.71 g of the test mixture) was placed on an l-inch x l-inch, 4-ply gauze pad and applied to one 6 cm^2 intact dose site on each animal. The pad and entire trunk of each animal were then wrapped with semi-occlusive 3-inch Micropore tape to avoid dislocation of the pad. Elizabethan collars were placed on each rabbit and they were returned to their designated cages.

After 4 hours of exposure to the test substance, the pads and collars were removed, and the test sites were gently cleansed of any residual test substance. Individual dose sites were scored according to the Draize scoring system at approximately 1, 24, 48 and 72-hours after patch removal. The animals were observed for signs of gross toxicity and behavioural changes at least once daily during the test period.

Skin reaction grading according to Draize criteria used by [redacted] (2005d)

Skin Reaction	Grading
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef redness) to slight eschar formation (injuries in depth)	4
Oedema formation	
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre and extending beyond area of exposure)	4

Results

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

No signs of gross toxicity, adverse effects, or abnormal behaviour were noted.

C. BODY WEIGHT

Body weight was not recorded.

D. NECROPSY

No gross abnormalities were observed at necropsy.

E. SKIN OBSERVATIONS

Two of three sites were free from irritation throughout the study. One hour after patch removal, one animal exhibited very slight erythema. Dermal irritation cleared from this animal by 24-hours. The mean for the 24, 48 and 72-hour readings were 0.0 for erythema and 0.0 for oedema.

Table 5.2.4-9 Glyphosate Acid Technical - Primary Skin Irritation Study in Rabbits ([redacted] 2005): Skin irritation scores (individual values)

Evaluation interval	Animal No.	Erythema	Oedema
1 hour	11807 (♂)	0	0
	11808 (♂)	1	0
	11809 (♂)	0	0
24-hours	11807 (♂)	0	0
	11808 (♂)	0	0
	11809 (♂)	0	0
48 hours	11807 (♂)	0	0
	11808 (♂)	0	0
	11809 (♂)	0	0
72-hours	11807 (♂)	0	0
	11808 (♂)	0	0
	11809 (♂)	0	0
Individual 24 – 72 h means	**11807 (♂)**	**0.0**	**0.0**
	11808 (♂)	**0.0**	**0.0**
	11809 (♂)	**0.0**	**0.0**

3. Assessment and conclusion

Assessment and conclusion by applicant:
Compared to the current OECD TG 404 (2015), deviations such as no step-wise approach, no report of body weights, water solubility and relative humidity are noted. These deviations are not considered to affect the study outcome. Therefore, the study is considered acceptable and the outcome can be reported as valid.
A single semi-occlusive application of glyphosate to intact rabbit skin for four hours provoke very slight erythema in one of three animals one hour after patch removal, which was cleared within 24-hours. The individual mean score over 24, 48 and 72-hours was 0.0 for erythema and 0.0 for oedema for all animals. Thus, glyphosate is not considered a skin irritant.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.4/010
Report author	[redacted]
Report year	1996
Report title	Glyphosate Acid: Skin Irritation to the Rabbit
Report No	[redacted]/P/4695
Document No	Not reported
Guidelines followed in study	OECD 404 (1992), 92/69/EEC B.4 (1992), US EPA 81-5
Deviations from current test guideline (OECD 404, 2015)	No step-wise approach, use of six instead of three animals simultaneously, the temperature was 17 ± 2 °C, no specific age of

	animals reported and occlusive dressing instead of semi-occlusive dressing. However, these deviations are considered to represent a worst-case scenario, not compromising the negative study outcome.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities[1,2]	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

In a primary dermal irritation study, six young adult, New Zealand White, female rabbits were dermally exposed to approximately 0.5 g of glyphosate acid (Batch: P24, Purity: 95.6 %), moistened with 0.5 mL deionised water, for 4 hours to an area (approximately 2.5 cm × 2.5 cm) on the left shorn flank, under an occlusive dressing. The patch was secured by two strips of surgical tape and the exposed skin was covered by impermeable rubber sheeting wrapped once around the trunk of the animal and secured with adhesive impermeable polyethylene tape. After removal of the patch, the application site was gently cleansed with warm water. Skin irritation was scored using the Draize scheme 0.5 – 1, 24, 48 and 72-hours after removal of the test substance.

Under the test conditions chosen, none of the animals showed any test item-related changes. The mean individual score for the 24, 48 and 72-hour readings were 0.0 for erythema and 0.0 for oedema.

Based on the study, glyphosate acid does not show a skin irritating potential under the test conditions chosen.

Materials and methods

A: Materials

1. Test material:

Identification:	Glyphosate acid
Description:	White, solid
Lot/Batch #:	P24
Purity:	95.6 % w/w
Stability of test compound:	The test substance was used within the expiry date (no date provided)

2. Vehicle and/or positive control: Deionised water

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White albino
Source:	Animals 1-5: [redacted]
	Animal 9: [redacted]
Age:	Young adults (not further specified)
Sex:	Female
Weight at dosing:	3.001 – 4.386 kg
Acclimation period:	At least 6 days

Housing:	Individually, in aluminium sheet cages in racks suitable for animals of this strain and the weight range expected during the course of the study	
Diet:	STANRAB SQC, (Special Diet Services Limited, Stepfield, Witham, Essex, UK), *ad libitum*	
Water:	Tap water, *ad libitum*	
Environmental conditions:	Temperature:	17 ± 2 °C
	Humidity:	40 – 70 %
	Air changes:	Approximately 25 / hour
	Photocycle:	12 hours light / dark cycle

4. Test conditions:

Patch site preparation technique:	Approximately 24-hours before application, an area approximately 7 cm x 13 cm on the left flank of each rabbit was clipped.
Patching technique:	Dermal application onto shaved, intact dorsal skin (approximate size 2.5 cm × 2.5 cm). The gauze was secured by two strips of surgical tape which was covered by a piece of impermeable rubber sheeting (approximate size 35 cm × 13 cm) wrapped around the trunk and is secured with adhesive impermeable polyethylene tape (7.5 cm wide).
Chemical preparation:	Approximately 0.5 g glyphosate was moistened with 0.5 mL of deionised water
Chemical application:	0.5 g / animal
Chemical removal:	Skin was gently cleaned using clean swabs of absorbent cotton wool soaked in clean warm water.

B: Study design and methods

In-life dates: 1995-03-09 to 1995-04-28

Animal assignment and treatment:

Approximately one day before treatment, the left flank was clipped with an electric clipper, exposing an area of approximately 7 cm × 13 cm. On the day of treatment, 0.5 g of glyphosate acid (95.6 % w/w) (moistened with approximately 0.5 mL deionised water) was applied to the test site (approximately 2.5 cm × 2.5 cm) on the left flank of six female rabbits. The treated area was covered with a piece of 8-ply surgical gauze (approximate size 2.5 cm × 2.5 cm), which was secured by two strips of surgical tape (approximate size 1 cm × 8 cm). This was covered by a piece of impermeable rubber sheeting (approximate size 35 cm × 13 cm) wrapped once around the trunk of the animal and secured with adhesive impermeable polyethylene tape (7.5 cm wide).

The dressings were left in position for approximately 4 hours. The application site was gently cleansed free of any residual test substance using clean swabs of absorbent cotton wool soaked in clean warm water and was then dried gently with clean tissue paper.

The Draize scale (see table below) was used to assess the degree of erythema and oedema at the application sites approximately 30 – 60 minutes, 1, 2 and 3 days after removal of the dressings. Any other signs of skin irritation were also recorded.

Skin reaction grading according to Draize criteria used by [redacted] (1996)

Skin Reaction	Grading
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef redness)	4
Oedema formation	
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well defined by definite raising)	2
Moderate oedema (raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre and extending beyond area of exposure)	4

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs reported.

C. BODY WEIGHT

Not reported.

D. NECROPSY

No necropsy was performed.

E. SKIN OBSERVATIONS

No skin reactions were observed at the application site of any animal at any observation time point (all scores were 0). The overall mean for the 24, 48 and 72-hour readings were 0.0 for erythema and 0.0 for oedema.

Table 5.2.4-10 Glyphosate Acid: Skin Irritation Study in Rabbits ([redacted] 1996): Skin irritation scores (individual values)

Evaluation interval	Animal No.	Erythema	Oedema
30 - 60 min	1 (♀)	0	0
	2 (♀)	0	0
	3 (♀)	0	0
	4 (♀)	0	0
	5 (♀)	0	0
	9 (♀)	0	0
24-hours	1 (♀)	0	0
	2 (♀)	0	0
	3 (♀)	0	0
	4 (♀)	0	0
	5 (♀)	0	0

Table 5.2.4-10 Glyphosate Acid: Skin Irritation Study in Rabbits ([redacted] 1996): Skin irritation scores (individual values)

Evaluation interval	Animal No.	Erythema	Oedema
	9 (♀)	0	0
48 hours	1 (♀)	0	0
	2 (♀)	0	0
	3 (♀)	0	0
	4 (♀)	0	0
	5 (♀)	0	0
	9 (♀)	0	0
72-hours	1 (♀)	0	0
	2 (♀)	0	0
	3 (♀)	0	0
	4 (♀)	0	0
	5 (♀)	0	0
	9 (♀)	0	0
Individual 24 – 72 h means	**1 (♀)**	**0.0**	**0.0**
	2 (♀)	**0.0**	**0.0**
	3 (♀)	**0.0**	**0.0**
	4 (♀)	**0.0**	**0.0**
	5 (♀)	**0.0**	**0.0**
	9 (♀)	**0.0**	**0.0**

3. Assessment and conclusion

Assessment and conclusion by applicant:
Compared to the current OECD TG 404 (2015), deviations such as no step-wise approach, use of six instead of three animals, occlusive instead of semi-occlusive dressing are noted.
Nevertheless, as the occlusive application is considered to represent a worst-case scenario, this deviation and all other deviations, considered as minor, do not compromise the negative study outcome. Therefore, the study is considered acceptable and the outcome can be reported as valid.
The occlusive application of glyphosate to intact rabbit skin for four hours elicited no skin reactions at the application site of any animal at any observation time. The individual mean score over 24, 48 and 72-hours was 0.0 for erythema and 0.0 for oedema for all animals.
Thus, glyphosate is not considered a skin irritant.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.4/011
Report author	[redacted]
Report year	1995
Report title	HR-001: Primary Dermal Irritation Study in Rabbits
Report No	[redacted] 95-0035
Document No	Not reported
Guidelines followed in study	OECD 404 (1992), US EPA FIFRA Guideline Subdivision F (1984), JMAFF 59 NohSan 4200 (1985)
Deviations from current test	No step-wise approach. Six instead of three animals were used

guideline (OECD 404, 2015)	simultaneously. It is not mentioned that only intact skin is used for treatment. No clinical signs were reported. Occlusive instead of semi-occlusive dressing. However, this is considered to represent a worst-case scenario, not compromising the negative study outcome.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities[1,2]	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

In a primary dermal irritation study, young adult New Zealand albino rabbits (six females) were dermally exposed to glyphosate technical (HR-001, Batch: T-941209, Purity: 97.56 %). The clipped dorso-lumbar region was exposed to 0.5 g of the solid test item, moistened with about 0.5 mL water, for 4 hours under occlusive conditions. The rabbits were observed for 72-hours. Skin irritation was scored using the Draize scheme 1, 24, 48 and 72-hours after removal of the test substance.

No skin reactions were observed at the application site of any animal at any observation time point. The mean for the 24, 48 and 72-hour readings for each animal were 0.0 for erythema and 0.0 for oedema, respectively.

Based on the study, glyphosate technical does not show a skin irritating potential under the test conditions chosen.

Materials and methods

A: Materials

1. Test material:

Identification:	HR-001 (glyphosate technical)
Description:	White crystal
Lot/Batch #:	T-941209
Purity:	97.56 %
Water solubility:	12 g/L (25 °C)
Stability of test compound:	Not reported

2. Vehicle and/or positive control: Deionised water

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White, Kbl:SPF
Source:	[redacted]
Age:	12 weeks
Sex:	Female
Weight at dosing:	2408 – 2686 g
Acclimation period:	18 days

Diet/Food:	Pellet Diet GC4 (Oriental Yeast Co., Ltd., Azusawa, Itabashi-ku, Tokyo)	
Water:	Water filtrated and sterilized, *ad libitum*	
Housing:	Individually in aluminium cages with wire-mesh floor	
Environmental conditions:	Temperature:	23.9 – 24 °C
	Humidity:	52.8 – 56.6 %
	Air changes:	15 / hour
	Photoperiod:	12 hours light / dark cycle

4. Test conditions:

Patch site preparation technique:	Dorsal fur of the trunk was removed with an electric clipper and a shaver approximately 24-hours before application (2.54 cm^2 × 2.54 cm^2, approximately 6 cm^2).
Patching technique:	Dermal application onto shaved, intact dorsal skin. The patch was held in position with a polyethylene sheet and non-irritating adhesive for exposure period duration.
Chemical preparation:	Glyphosate technical was finely ground in a mortar shortly before application.
Chemical application:	0.5 g in 0.5 mL deionised water
Chemical removal:	Washed off with deionised water.

B: Study design and methods

In life dates: 1995-05-16 to 1995-05-19

Animal assignment and treatment:

Glyphosate technical (HR-001) (0.5 g) moistened with 0.5 mL of deionised water was applied to the closely-clipped dorso-lumbar region (6 cm^2) of six female New Zealand rabbits and covered by a semi-occlusive gauze patch for 4 hours. The patch was held in place in an occlusive manner with a polyethylene sheet and non-irritating adhesive tape. At the end of the exposure period, the patch was removed, and the treatment site was washed with distilled water to remove any residual test substance. Thereafter, all animals were observed for primary dermal irritation 1, 24, 48 and 72-hours. Degree of erythema and oedema relative to treatment were recorded during a subsequent 72-hour observation period according to the criteria described in the Guideline of MAFF in Japan and the method of Draize. Body weights were measured prior to application, and after the final observation 72-hours after application.

Skin reaction grading according to Draize criteria used by [redacted] (1995)

Skin Reaction	Grading
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef redness) to slight eschar formation (injuries in depth)	4
Oedema formation	
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approximately 1 mm)	3
Severe oedema (raised more than 1 mm and extending beyond area of exposure)	4

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

Clinical signs were not observed.

C. BODY WEIGHT

All rabbits showed the expected body weight gain.

D. NECROPSY

No necropsy was performed.

E. SKIN OBSERVATIONS

No signs of erythema, eschar, oedema and any other evidence of irritation were observed in either the test substance treated site or the negative control site at any time during the observation period. The overall mean for the 24, 48 and 72-hour readings were 0.0 for erythema and 0.0 for oedema. The observation period was therefore completed after 72-hours.

Table 5.2.4-11 HR-001: Primary Dermal Irritation Study in Rabbits [redacted] 1995): Skin irritation scores (individual values)

Evaluation interval	Animal No.	Erythema and eschar formation	Oedema
1 hour	1 (♀)	0	0
	2 (♀)	0	0
	3 (♀)	0	0
	4 (♀)	0	0
	5 (♀)	0	0
	6 (♀)	0	0
24-hours	1 (♀)	0	0
	2 (♀)	0	0
	3 (♀)	0	0
	4 (♀)	0	0
	5 (♀)	0	0
	6 (♀)	0	0
48 hours	1 (♀)	0	0
	2 (♀)	0	0
	3 (♀)	0	0
	4 (♀)	0	0
	5 (♀)	0	0
	6 (♀)	0	0
72-hours	1 (♀)	0	0
	2 (♀)	0	0
	3 (♀)	0	0
	4 (♀)	0	0
	5 (♀)	0	0
	6 (♀)	0	0
Individual 24 – 72 h means	**1 (♀)**	**0.0**	**0.0**
	2 (♀)	**0.0**	**0.0**

Table 5.2.4-11 HR-001: Primary Dermal Irritation Study in Rabbits [redacted] 1995): Skin irritation scores (individual values)

Evaluation interval	Animal No.	Erythema and eschar formation	Oedema
	3 (♀)	0.0	0.0
	4 (♀)	0.0	0.0
	5 (♀)	0.0	0.0
	6 (♀)	0.0	0.0

3. Assessment and conclusion

Assessment and conclusion by applicant:
Compared to the current OECD TG 404 (2015), deviations such as no step-wise approach, use of six instead of three animals, occlusive instead of semi-occlusive dressing and no report of clinical signs are noted.
Nevertheless, as the occlusive application is considered to represent a worst-case scenario, this deviation as well as all other deviations is not compromising the negative study outcome. Therefore, the study is considered acceptable and the outcome can be reported as valid.
A single occlusive application of glyphosate to intact rabbit skin for four hours elicited no skin reactions at the application site of any animal at any observation time. The individual mean score over 24, 48 and 72-hours was 0.0 for erythema and 0.0 for oedema for all animals.
Thus, glyphosate is not considered a skin irritant.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.4/012
Report author	[redacted]
Report year	1994
Report title	Glyphosate Premix: Acute Dermal Irritation Test in the Rabbit
Report No	545/40
Document No	Not reported
Guidelines followed in study	US EPA 81-5 (1984), US EPA 798.4470
Deviations from current test guideline	Yes, step-wise approach by initial testing in one animal was not performed and animals were treated simultaneously. Six instead of three animals were used. Rationale for *in vivo* testing and consideration of pre-existing data was not documented. Water solubility and other physicochemical properties (with the exception of vapour pressure) were not documented.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive (low purity: 46.1 % glyphosate)
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

In an acute dermal irritation study, one male and five female New Zealand White rabbits were dermally exposed to glyphosate premix (Batch: 290-JaK-146-4; Purity: 46.1 % glyphosate). The fur was clipped from the dorsal / flank area of each rabbit on the day before treatment. A quantity of 0.5 mL of test material was applied to the intact skin of each rabbit under a 2.5 cm × 2.5 cm gauze patch for 4 hours under semi-occlusive conditions. Following removal of the patch the skin was gently swabbed with cotton wool soaked in distilled water to remove residual test material. The rabbits were observed for 72-hours. Skin reactions were evaluated 1, 24, 48 and 72-hours after patch removal and scored according to the Draize classification scheme.

Very slight erythema was noted in two animals 1 hour after patch removal. Very slight erythema persisted in one animal at the 24-hour observation time point and was also noted in one other animal. No other skin reactions were noted for any animal at any other observation time point. The mean score for the 24, 48 and 72-hour readings was 0.33 for erythema for two animals, 0.0 for erythema for four animals and 0.0 for oedema of all animals.

Based on the study, glyphosate premix does not show a skin irritating potential under the test conditions chosen.

Materials and methods

A: Materials

1. Test material:

Identification:	Glyphosate Premix (aqueous solution containing isopropylamine salt of glyphosate as the active ingredient)
Description:	Pale yellow liquid
Lot/Batch #:	290-JaK-146-4
Purity:	62.2 % as glyphosate isopropylamine salt (46.1 % as glyphosate)
Water solubility:	Not specified
Stability of test compound:	Expiry date: 1995-09-30

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Approximately 12 – 20 weeks old
Sex:	Male and female
Weight at start of dosing:	2.19 – 2.52 kg
Acclimation period:	At least 5 days
Diet/Food:	STANRAB SQC Rabbit Diet (Special Diet Services Ltd., Witham, Essex, UK) *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually housed in suspended metal cages

Environmental conditions:	Temperature:	18 – 22 °C
	Humidity:	46 – 59 %
	Air changes:	Approximately 15 changes per hour
	Photocycle:	12 hours light / dark cycle

4. Test conditions:

Patch site preparation technique:	The dorsal / flank area of each rabbit was clipped (area not specified), 24 h before application.
Patching technique:	Dermal application onto clipped, intact skin (2.5 cm × 2.5 cm gauze patch). The gauze patch was secured with surgical adhesive tape (BLENDERM) and wrapped in elasticated corset (TUBIGRIP) (semi-occlusive dressing).
Chemical preparation:	0.5 mL of test material was introduced under the 2.5 × 2.5 cm gauze patch.
Chemical application:	0.5 mL glyphosate premix
Chemical removal:	Gentle swabbing with cotton wool soaked in distilled water

B: Study design and methods

In life dates: 1994-03-29 to 1994-04-01

Animal assignment and treatment:
On the day before the test, each of a group of six New Zealand White rabbits (one male and five female) was clipped free of fur from the dorsal/flank area. Only animals with a healthy intact epidermis by gross observation were selected for the study. An amount of 0.5 mL of the test material was introduced to the intact skin of each rabbit under a 2.5 cm × 2.5 cm gauze patch for 4 hours under semi-occlusive conditions. The patch was secured with surgical adhesive tape (BLENDERM), and the trunk of each animal wrapped in an elasticated corset (TUBIGRIP). After 4 hours of exposure the patch was removed, and the skin was gently swabbed with cotton wool soaked in distilled water to remove residual test material.

Test sites were examined for evidence of primary skin irritation 1, 24, 48 and 72-hours after removal of the patch. Skin reactions were scored according to the Draize scale (see table below). Any other skin reactions, if present, were also recorded.

Skin reaction grading according to Draize criteria used by [redacted] (1994)

Skin Reaction	Grading
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Oedema formation	
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre and extending beyond area of exposure)	4

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical observations were recorded.

C. BODY WEIGHT

Body weight and body weight gain was not recorded.

D. NECROPSY

No necropsy was performed.

E. SKIN OBSERVATIONS

A single semi-occlusive application of glyphosate to intact rabbit skin for four hours elicited very slight erythema in two animals 1 hour after patch removal, which persisted in one animal at the 24-hour observation time point, and was also noted in one other animal at this time point. No other adverse skin reactions were noted for any other animal at any observation time point. The mean score for the 24, 48 and 72-hour readings was 0.33 for erythema for two animals, 0.0 for erythema for four animals and 0.0 for oedema of all animals. Neither alterations of the treated skin, nor corrosive effects were observed.

Table 5.2.4-12 Glyphosate Premix: Acute Dermal Irritation Test in the Rabbit ([redacted] 1994): Skin irritation scores (individual values)

Evaluation interval	Animal No.	Erythema	Oedema
1 hour	63 (♂)	1	0
	65 (♀)	0	0
	75 (♀)	0	0
	87 (♀)	0	0
	105 (♀)	1	0
	100 (♀)	0	0
24-hours	63 (♂)	1	0
	65 (♀)	1	0
	75 (♀)	0	0
	87 (♀)	0	0
	105 (♀)	0	0
	100 (♀)	0	0
48-hours	63 (♂)	0	0
	65 (♀)	0	0
	75 (♀)	0	0
	87 (♀)	0	0
	105 (♀)	0	0
	100 (♀)	0	0
72-hours	63 (♂)	0	0
	65 (♀)	0	0
	75 (♀)	0	0
	87 (♀)	0	0
	105 (♀)	0	0
	100 (♀)	0	0

Table 5.2.4-12 Glyphosate Premix: Acute Dermal Irritation Test in the Rabbit ([redacted] 1994): Skin irritation scores (individual values)

Evaluation interval	Animal No.	Erythema	Oedema
Individual 24 – 72 h means	63 (♂)	0.33	0.0
	65 (♀)	0.33	0.0
	75 (♀)	0.0	0.0
	87 (♀)	0.0	0.0
	105 (♀)	0.0	0.0
	100 (♀)	0.0	0.0

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study is with the exception of some minor deviations (no step-wise approach, 6 instead of 3 animals, water solubility and other physicochemical properties not provided) in concordance with the current OECD TG 404 (2015). Therefore, the study is considered acceptable and the outcome can be reported as valid. Nevertheless, due to the low purity of the test substance (46.1 % as glyphosate), the reliability of the study is considered supportive, only.

A single semi-occlusive application of glyphosate premix to intact rabbit skin for four hours elicited very slight erythema at the application site of two animals at the observation time of 24-hours. No skin reactions were noted with the remaining four animals at any observation time.

Individual mean scores over 24, 48 and 72-hours were 0.33 for two animals and 0.0 for four animals for erythema and 0.0 for oedema for all animals.

Thus, glyphosate is not considered a skin irritant.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.4/013
Report author	[redacted]
Report year	1994
Report title	Glyphosate 360 g/L: Acute dermal irritation test in the rabbit.
Report No	710/29
Document No	Not
Guidelines followed in study	No final conclusion possible.
GLP	No final conclusion possible.
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	0.5 mL of the test substance (glyphosate salt, purity 360 g/L formulation containing IPA) was applied to the intact skin of 2 male and 1 female rabbits.
Short description of results:	Non-irritant (not further specified)
Reasons for why the study is not considered relevant/reliable or not considered as key study:	Full study report is not available to the applicant. Only a short summary is provided in the Monograph, B5, 2000, *Category 4b*
Reasons why the study report is not available for submission	The notifier has not access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance" (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL.

Category study in AIR 5 dossier (L docs)	*Category 4b*

1. Information on the study

Data point:	CA 5.2.4/014
Report author	[redacted]
Report year	1994
Report title	Glyphosate (Alkaloida, Tiszavasvári): Primary Dermal Irritation Study in Rabbits
Report No	GHA-93-404/N
Document No	Not reported
Guidelines followed in study	OECD 404 (1981)
Deviations from current test guideline (OECD 404, 2015)	No information if a vehicle was used. According to OECD 404 (2015) solid test chemicals should be moistened sufficiently to ensure good skin contact. Occlusive instead of semi-occlusive dressing was used. Step-wise approach by initial testing in one animal was not performed. Rationale for *in vivo* testing and consideration of pre-existing data was not documented. No information was presented on water solubility. Four instead of three animals exposed simultaneously under occlusive instead of semi-occlusive conditions. Body weights were not recorded. Abraded skin was included in the test.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive (Sufficient moistening to ensure good skin contact is uncertain)
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

In a primary dermal irritation study, four New Zealand White rabbits were dermally exposed to glyphosate (Batch: 36300892; Purity: 99.6 %). The fur was clipped from the back of each rabbit the day prior to dosing. Five tenths (0.5) g of the test item was applied to two intact and two abraded test sites of each rabbit. Test sites were covered with Folpack foil and fixed with Leucoplast for 4 hours. Subsequently, the occlusive dressing was removed and the test sites were washed with warm water and gently dried with a towel.

Skin reactions were assessed 1, 24, 48 and 72-hours after removal of the binders. Skin reactions were scored according to the Draize method. Animals were observed daily for mortality and overt pharmacotoxic signs.

No skin reactions were observed at the application sites of any animal at any observation time point. The individual mean score for the 24, 48 and 72-hour readings were 0.0 for all animals for erythema and 0.0, 0.0, 0.0 and 0.333 for oedema. No mortality was observed and no clinical signs were noted in any animal.

Thus, glyphosate was not considered a skin irritant.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate
Description:	White or almost white crystalline powder
Lot/Batch #:	36300892
Purity:	99.6 %
Water solubility:	Not specified
Stability of test compound:	Expiry date: 1994-09-01

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Source:	Not specified
Age:	Not specified
Sex:	Not specified
Weight at dosing:	Not specified
Acclimation period:	At least 5 days
Diet/Food:	Standard rabbit chow (not further specified) with fresh carrots, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Housed in wise box
Environmental conditions:	Temperature: 18 ± 2 °C Humidity: 40 – 70 % Air changes: 10 times / hour Photocycle: 12 hours light / dark cycle

4. Test conditions:

Patch site/preparation technique:	The back of each rabbit was clipped and distributed into distinct areas (two intact sites and two abraded sites) 24 h before application. Remaining hair was removed with MELT, an intimate depilating product for human use.
Patching technique:	Dermal application onto clipped, non-abraded skin (right-hand / forquarter and left-hand / hindquarter) and abraded skin (right-hand / hindquarter and right-hand / forquarter). Test sites were covered with Folpack fixed with Leucoplast.
Chemical preparation:	Not specified
Chemical application:	Test article (0.5 g) was topically applied to two intact and two abraded dorsal test sites
Chemical removal:	Exposure sites were washed with abundant warm water and dried carefully with a towel

B: Study design and methods

In life dates: 1993-11-29 to 1993-12-02

Animal assignment and treatment:
The test was conducted using four New Zealand White rabbits. Test article (0.5 g) was applied to two intact and two abraded test sites on the back of each of the four animals. Test sites were covered with Folpack foil and fixed with Leucoplast for 4 hours. After exposure the tape and covering were removed and the skin was washed with abundant warm water and gently dried with a towel.

Skin reactions were assessed 1, 24, 48 and 72-hours after removal of the covering. Skin reactions were scored according to the Draize method. Animals were observed daily for mortality and clinical signs.

Skin reaction grading according to Draize criteria used by [redacted] (1994)

Skin Reaction	Grading
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Oedema formation	
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well defined by definite raising)	2
Moderate oedema (raised approximately 1 mm)	3
Severe oedema (raised more than 1 mm and extending beyond area of exposure)	4

II. RESULTS AND DISUSSION

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical observations were noted.

C. BODY WEIGHT

Body weight and body weight gain were not recorded.

D. NECROPSY

No necropsies were performed.

E. SKIN OBSERVATIONS

Very slight oedema (barely perceptible) was observed at one site in one animal after 24-hours. However, this finding was not observed at any time point thereafter. All other animals did not show any signs of erythema or oedema in response to the application of glyphosate technical for 4 hours. The mean score for the 24, 48 and 72-hour readings were 0.0 for all animals for erythema and 0.0, 0.0, 0.333 and 0.0 for oedema.

Table 5.2.4-13 Glyphosate (Alkaloida, Tiszavasvári): Primary dermal irritation study in rabbits ([redacted] 1994): Skin irritation scores for intact skin (individual values)

Evaluation interval	Animal	Erythema (L/R)	Oedema (L/R)
1 hour	1	0/0	0/0
	2	0/0	0/0
	3	0/0	0/0
	4	0/0	0/0
24-hours	1	0/0	0/0
	2	0/0	0/0
	3	0/0	0/1
	4	0/0	0/0
48 hours	1	0/0	0/0
	2	0/0	0/0
	3	0/0	0/0
	4	0/0	0/0
72-hours	1	0/0	0/0
	2	0/0	0/0
	3	0/0	0/0
	4	0/0	0/0
Individual 24 – 72 h means	1	**0.0**	**0.0**
	2	**0.0**	**0.0**
	3	**0.0**	**0.333**
	4	**0.0**	**0.0**

III. Conclusions

Based on the experimental results the test substance glyphosate is considered as non-irritant to skin.

3. Assessment and conclusion

Assessment and conclusion by applicant:

As the occlusive application is considered to represent a worst-case scenario, this deviation does not compromise the negative study outcome. Nevertheless, as the solid test chemical is not known if moistened and sufficient exposure is uncertain, the study is considered supplementary, only.

A single occlusive application of glyphosate to intact rabbit skin for four hours elicited a very slight reaction in one site at 24-hours; all other sites had no skin reactions at any observation time. The individual mean score over 24, 48 and 72-hours was 0.0 for erythema for all animals and 0.333 for one animal for oedema, and 0.0 for all other animals.

Thus, glyphosate is not considered a skin irritant.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.4/015
Report author	[redacted]
Report year	1991
Report title	Primary Skin Irritation Study with Glyphosate Technical (FSG 03090 h/05 March 90) in New Zealand White Rabbits
Report No	ES.878.SKIN
Document No	Not reported
Guidelines followed in study	OECD 404 (1987)
Deviations from current test guideline (OECD 404, 2015)	Step-wise approach by initial testing in one animal was not performed and three animals were treated simultaneously. Rationale for *in vivo* testing and consideration of pre-existing data was not documented. It is not mentioned that only intact skin is used for treatment. Water solubility and physicochemical properties were not documented. Volume of the vehicle distilled water used to form slurry was not reported. Occlusive (aluminium foil) instead of semi-occlusive dressing. However, this is considered to represent a worst-case scenario, not compromising the negative study outcome.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

A primary skin irritation test was carried out using two male and one female New Zealand White rabbits to determine the potential of glyphosate technical (Batch: 60; Purity: 96.8 %) to produce dermal irritation after single topical exposure. The test compound (0.5 g mixed with distilled water into a slurry) was applied for four hours to the skin of the rabbits using an aluminium foil patch of 3 cm × 3 cm (occlusive exposure conditions). After removal of the patch, the application area was washed off with distilled water. Skin reactions were scored according to the Draize method and recorded 1, 24, 48 and 72-hours after removal of the test-patch. Animals were observed daily for mortality and clinical signs. Body weights were recorded at the start of acclimatisation, the day of application and at study termination. The study was terminated on day 3, since no cutaneous reaction was present in any animal. Animals were sacrificed and subjected to necropsy.

No mortalities occurred. No signs of systemic toxicity and no noteworthy changes in body weight were noted. There was no skin reactions observed in any animal at any observation time-point. Therefore, the study was terminated after the 72-hour reading time-point. No gross pathological findings were noted at necropsy.

The mean for the 24, 48 and 72-hour readings for each animal were 0.0 for erythema and 0.0 for oedema, respectively.
Based on the study, glyphosate technical does not show a skin irritating potential under the test conditions chosen.

Materials and methods

A: Materials

1. Test material:

Identification:	Glyphosate Technical (FSG 03090 h/05 March 90)
Description:	Solid white coloured crystals, odourless
Lot/Batch #:	60
Purity:	96.8 %
Water solubility:	Not soluble / suspendable in water
Stability of test compound:	Expiry date: 1992-07

2. Vehicle and/or positive control: Distilled water

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Approximately 14 weeks
Sex:	Male and female
Weight at dosing:	1.8 – 2.1 kg
Acclimation period:	At least 4 days
Diet/Food:	Standard "Gold Mohur" pelleted rabbit maintenance diet (M/s Lipton India Ltd., Bangalore 560 052, India), *ad libitum*
Water:	Deep borewell water passed through activated charcoal filter and exposed to UV rays, *ad libitum*
Housing:	Individually housed in stainless steel / aluminium cages
Environmental conditions:	Temperature: 23 ± 2 °C
	Humidity: 68 ± 6 %
	Air changes: 10 – 15 air changes per hour
	Photocycle: 12 hours light / dark cycle

4. Test conditions:

Patch site preparation technique:	The dorsal fur of each rabbit was clipped (approximate area 10 × 10 cm), 24 h before application.
Patching technique:	Test patch dermally applied to dorsolateral thoracic skin. The patch was secured in position with adhesive tape and wrapped with an elastic bandage around the abdomen.
Chemical preparation:	0.5 g of test compound was finely ground and mixed with distilled water (volume not specified) to form a slurry and spread evenly over a 3 × 3 cm aluminium foil patch.
Chemical application:	Foil patch with slurry (0.5 g of test compound) was topically applied to dorsolateral thoracic skin. Animals were fixed in pyrogen test cages for the 4 hour exposure period.
Chemical removal:	Skin flushed with distilled water

B: Study design and methods

In life dates: 1990-09 (not further specified)

Animal assignment and treatment:

The test was conducted using two male and one female New Zealand White rabbits. Test compound (0.5 g glyphosate technical mixed with distilled water [amount not specified] into a slurry) was applied to the dorsolateral thoracic skin of each rabbit using an aluminium foil patch (3 × 3 cm). A control patch of bare aluminium foil was applied 3 – 4 cm posterior to the test patch (occlusive). Both patches were secured in position with adhesive tape and wrapped with an elastic bandage around abdomen. Animals were fixed in pyrogen test cages for a 4 hour exposure period. Thereafter, the dressing was removed, and the skin flushed with distilled water.

Skin reactions were assessed 1, 24, 48 and 72-hours after removal of the dressing. Skin reactions were scored according to the Draize method (see table below). Animals were observed for mortality and clinical signs daily. Body weights were recorded at the start of the acclimatisation period, on the day of application, and at termination. Necropsy was performed in animals sacrificed at termination.

Skin reaction grading according to Draize criteria used by [redacted] (1991d)

Skin Reaction	Grading
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Oedema formation	
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well defined by definite raising)	2
Moderate oedema (raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre and extending beyond area of exposure)	4

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical observations or symptoms of toxicity were noted.

C. BODY WEIGHT

There were no noteworthy changes in body weight.

D. NECROPSY

No gross abnormalities were observed at necropsy.

E. SKIN OBSERVATIONS

No skin reactions were observed at the application site of any animal at any observation time point (all scores were 0) following 4 hours occlusive exposure to glyphosate technical. The overall mean for the 24, 48 and 72-hour readings were 0.0 for erythema and 0.0 for oedema.

Table 5.2.4-14 Primary Skin Irritation Study with Glyphosate Technical (FSG 03090 h/05 March 90) in New Zealand White Rabbits ([redacted] 1991): Skin irritation scores (individual values)

Evaluation interval	Animal No.	Erythema	Oedema
1 hour	RS 007 (♂) RS 008 (♂) RS 009 (♀)	0 0 0	0 0 0
24-hours	RS 007 (♂) RS 008 (♂) RS 009 (♀)	0 0 0	0 0 0
48 hours	RS 007 (♂) RS 008 (♂) RS 009 (♀)	0 0 0	0 0 0
72-hours	RS 007 (♂) RS 008 (♂) RS 009 (♀)	0 0 0	0 0 0
Individual 24 – 72 h means	**RS 007 (♂)** **RS 008 (♂)** **RS 009 (♀)**	**0.0** **0.0** **0.0**	**0.0** **0.0** **0.0**

3. Assessment and conclusion

Assessment and conclusion by applicant:

Compared to the current OECD TG 404 (2015), deviations such as no step-wise approach, no report of water solubility, physicochemical properties, volume of the vehicle, occlusive (aluminium foil) instead of semi-occlusive dressing are noted. Nevertheless, as the occlusive application is considered to represent a worst-case scenario, this deviation and all other deviations, considered as minor, do not compromise the negative study outcome. Therefore, the study is considered acceptable and the outcome can be reported as valid.

The occlusive application of glyphosate to rabbit skin for four hours elicited no skin reactions at the application site of any animal at any observation time. The individual mean score over 24, 48 and 72-hours was 0.0 for erythema and 0.0 for oedema for all animals.

Thus, glyphosate is not considered a skin irritant.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.4/016
Report author	[redacted]
Report year	1991
Report title	Acute dermal irritation study in New Zealand White rabbits treated with the test article glyphosate technico 98 %
Report No	910259
Document No	Not reported
Guidelines followed in study	Not reported

GLP	Not reported
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	0.5 g test material (glyphosate acid, purity 98 %, moistened with saline) applied onto intact skin of 3 male rabbits
Short description of results:	Very slight irritation (not further specified)
Reasons for why the study is not considered relevant/reliable or not considered as key study:	Full study report is not available to the applicant. Only a short summary is provided in the Monograph, B5, 2000, *Category 4b*
Reasons why the study report is not available for submission	The notifier has not access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance" (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL.
Category study in AIR 5 dossier (L docs)	Category 4b

1. Information on the study

Data point:	CA 5.2.4/017
Report author	[redacted]
Report year	1990
Report title	Acute Dermal Irritation/Corrosion of Glyphosate Technical in the Rabbit (Intact and Abraded Skin)
Report No	AGC-900822A
Document No	Not reported
Guidelines followed in study	OECD 404 (1981), Method B.4 84/449/EEC
Deviations from current test guideline (OECD 404, 2015)	Yes, step-wise approach by initial testing in one animal was not performed and three animals were treated simultaneously. Rationale for *in vivo* testing and consideration of pre-existing data was not documented. Volume of saline used as vehicle to moisten test substance was not reported. Body weights at start and termination of study, age of animals, acclimation period, and environmental conditions were not reported. These deviations are not considered to affect the study outcome.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

A primary skin irritation test was carried out using three male New Zealand White rabbits to evaluate the potential of glyphosate technical (Batch: 0190 A; Purity: 98.1 %) to produce dermal irritation after single topical exposure on intact and abraded dorsal skin for 4 hours. The test compound (0.5 g moistened with saline) was applied for four hours to the skin of the rabbits using gauze pad (6 cm^2) secured with non-irritating tape (semi-occlusive exposure conditions). Untreated shaved skin and abraded skin sites served

as comparator controls for each animal. After removal of the dressing, the application area was washed off with sterile physiological saline. Skin reactions were scored according to the Draize method and recorded 1, 24, 48 and 72-hours after removal of the patch. Animals were observed for mortality and clinical signs. Body weights were recorded at the start of application.

No mortalities occurred. No signs of toxicity were noted. There were no signs of erythema or oedema observed in any animal at any observation time-point on either intact or abraded skin. The mean for the 24, 48 and 72-hour readings for each animal was 0.0 for erythema and 0.0 for oedema, respectively.

Based on the study, glyphosate technical does not show a skin irritating potential under the test conditions chosen. Thus, glyphosate technical does not warrant classification as being irritating / corrosive to the skin.

Materials and methods

A: Materials

1. Test material:

Identification:	Glyphosate Technical
Description:	Yellowish (transparent)
Lot/Batch #:	0190 A
Purity:	98.1 %
Water solubility:	Not specified
Stability of test compound:	Not specified

2. Vehicle and/or positive control: 0.9 % sterile physiological saline

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Adult (not further specified)
Sex:	Male
Weight at dosing:	2.2 – 2.4 kg
Acclimation period:	Not specified
Diet/Food:	Standard rabbit diet (Redmills, Goresbridge, Co. Kilkenny, Ireland), *ad libitum*
Water:	Drinking water, *ad libitum*
Housing:	Individually housed in standard grid bottom rabbit cages
Environmental conditions:	Temperature: Not specified Humidity: Not specified Air changes: Not specified Photocycle: 12 hours light / dark cycle

4. Test conditions:

Patch site preparation technique:	The dorsal fur of each rabbit was clipped (approximate area 6 × 6 cm), 24 h before application. A sterile 20 gauge Microlance needle was used to abrade the skin prior to application of the test substance.

Patching technique: Gauze pads (6 cm^2) with test substance were applied directly to the intact and abraded test sites and secured with non-irritating tape (Elastoplast elastic adhesive bandage B.P.).

Chemical preparation: Test substance (0.5 g) was moistened with saline (volume not specified) and applied to a gauze pad ("Propax" gauze pads).

Chemical application: Gauze pads (6 cm^2) with 0.5 g of test substance were applied directly to the intact and abraded test sites for 4 hours.

Chemical removal: Skin washed with sterile physiological saline

B: Study design and methods

In life dates: 1990-03-30 – 1990-08-22 (completion of final report)

Animal assignment and treatment:

The test was conducted using three male adult New Zealand White rabbits. Fur was clipped (approximately 6 × 6 cm) from each animal 24-hours before testing. Skin was abraded using a sterile 20 gauge microlance needle prior to application of test substance. Test compound (0.5 g moistened with saline) applied on a gauze pad (6 cm^2) was directly applied to the intact or abraded skin test sites of each animal. Gauze pads were secured using Elastoplast elastic adhesive bandage. Untreated shaved skin and abraded skin sites served as controls for each animal. After 4 hours of exposure the adhesive dressings were removed, and residual test substance was washed off using sterile physiological saline.

Animals were examined for signs of erythema or oedema 1, 24, 48 and 72-hours after patch removal. Additionally, animals were observed for any other lesions or signs of toxic effects. Irritation responses were scored according to Draize criteria provided in the table below.

Skin reaction grading according to Draize criteria used by [redacted] (1990)

Skin Reaction	Grading
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Oedema formation	
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well defined by definite raising)	2
Moderate oedema (raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre and extending beyond area of exposure)	4

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical observations or symptoms of toxicity were noted.

C. BODY WEIGHT

Body weights were not reported.

D. NECROPSY

Necropsy was not performed.

E. SKIN OBSERVATIONS

No erythema or oedema were observed at the application or control sites of any animal for any observation time point (all scores were 0). The mean for the 24, 48 and 72-hour readings of each animal were 0.0 for erythema and 0.0 for oedema, respectively.

Table 5.2.4-15 Acute Dermal Irritation/Corrosion of Glyphosate Technical in the Rabbit (Intact and Abraded Skin) [redacted] 1990): Skin irritation scores (individual values for intact skin)

Evaluation interval	Animal No.	Erythema*	Oedema*
1 hour	BL-751 (♂)	0/0	0/0
	BL-761 (♂)	0/0	0/0
	BL-802 (♂)	0/0	0/0
24-hours	BL-751 (♂)	0/0	0/0
	BL-761 (♂)	0/0	0/0
	BL-802 (♂)	0/0	0/0
48 hours	BL-751 (♂)	0/0	0/0
	BL-761 (♂)	0/0	0/0
	BL-802 (♂)	0/0	0/0
72-hours	BL-751 (♂)	0/0	0/0
	BL-761 (♂)	0/0	0/0
	BL-802 (♂)	0/0	0/0
Individual 24 – 72 h means	**BL-751 (♂)**	**0.0**	**0.0**
	BL-761 (♂)	**0.0**	**0.0**
	BL-802 (♂)	**0.0**	**0.0**

*: skin reaction score of treated site (intact skin) / and control site

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in accordance with the current OECD TG 404 (2015). Therefore, the study is considered acceptable and the outcome can be reported as valid.
A single semi-occlusive application of glyphosate to intact rabbit skin for four hours elicited no skin reactions at the application site of any animal at any observation time. The individual mean score over 24, 48 and 72-hours was 0.0 for erythema and 0.0 for oedema for all animals.
Thus, glyphosate is not considered a skin irritant.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.4/018
Report author	[redacted]
Report year	1989
Report title	Glyphosate Technical: Primary Skin Irritation in Rabbits
Report No	5885
Document No	Not reported
Guidelines followed in study	US EPA 81-5
Deviations from current test guideline (OECD 404, 2015)	Yes, step-wise approach by initial testing in one animal was not performed and animals were treated simultaneously. Six instead of three animals used. Rationale for *in vivo* testing and consideration of pre-existing data was not documented. Purity of the test substance not provided. Nevertheless, from the batch number a purity of 98.6 % was concluded. Water solubility and physicochemical properties were not documented. Volume of vehicle for moistening the test material was not recorded. Stability of the test material was not documented. Animal age and body weights were not recorded. Temperature range outside the required range which is not considered to affect the outcome of the study. These deviations are not considered to affect the study outcome.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

In a primary dermal irritation study, two male and four female nulliparous and non-pregnant young adult New Zealand White rabbits were dermally exposed to glyphosate technical (Batch: 206-Jak-25-1; Purity: 98.6 %). The hair was clipped from the dorsal area of the trunk of each rabbit. The test material (0.5 g moistened with water), was applied to the intact skin of each rabbit under a 2.5 cm × 2.5 cm patch of gauze for 4 hours under semi-occlusive conditions. Following removal of the patch the skin was wiped with a damp tissue to remove surplus test material. The rabbits were observed for 72-hours. Skin reactions were evaluated 1, 24, 48 and 72-hours after patch removal and graded according to the EPA recommended scoring system.

No skin reactions were observed at the application site of any animal at any observation time point. The overall mean for the 24, 48 and 72-hour readings were 0.0 for erythema and 0.0 for oedema.

Based on the study, glyphosate technical does not show a skin irritating potential under the test conditions chosen.

Materials and methods

A: Materials

1. Test material:

Identification:	Glyphosate Technical (PMG)
Description:	White powder
Lot/Batch #:	206-Jak-25-1
Purity:	Not specified in the study report Batch 206-JaK-25-1 reported with 98.6 %, see [redacted], 1991.
Water solubility:	Not specified
Stability of test compound:	Not specified

2. Vehicle and/or positive control: Water

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Young adults (not further specified)
Sex:	Male and female (nulliparous and non-pregnant)
Weight at dosing:	Not specified
Acclimation period:	8 days
Diet/Food:	Standard rabbit diet (Special Diet Services, 1 Stepfield, Witham, Essex, CM8 3AD, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in aluminium cages with grid floors and peat moss filled trays beneath
Environmental conditions:	Temperature: 19 – 24 °C (mean minimum and maximum)
	Humidity: 57 % (mean relative humidity)
	Air changes: Not specified
	Photoperiod: 12 hours light / dark cycle

4. Test conditions:

Patch site preparation technique:	The dorsal area of the trunk of each rabbit was clipped (area not specified), 24 h before application.
Patching technique:	Dermal application onto clipped, intact skin (2.5 cm × 2.5 cm gauze patch). The patch was covered with Micropore tape and the trunk was loosely bound with Elastoplast Elastic Bandage (semi-occlusive dressing).
Chemical preparation:	0.5 g glyphosate / animal was weighed and moistened with water (amount not specified) before application.
Chemical application:	0.5 g glyphosate technical
Chemical removal:	Skin was wiped with damp tissue

B: Study design and methods

In life dates: 1989-06-14 to 1989-06-18

Animal assignment and treatment:

The hair was clipped from the dorsal area of the trunk of each rabbit approximately 24-hours before treatment. Care was taken to avoid abrading the skin. The test was conducted using two male and four female young adult New Zealand White rabbits. An amount of 0.5 g of the test material (moistened with water) was applied to the intact skin of each rabbit under a 2.5 cm × 2.5 cm patch of gauze under semi-occlusive conditions. After 4 hours of exposure, the patch was removed, and the skin was wiped with damp tissue to remove surplus test material.

Skin reactions were assessed 1, 24, 48 and 72-hours after removal of the patch. Skin reactions were graded according to the EPA Recommended Scoring System (see table below).

Skin reaction grading according to EPA Recommended Scoring System used by [redacted] (1989)

Skin Reaction	Grading
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Oedema formation	
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well defined by definite raising)	2
Moderate oedema (area raised approximately 1 mm)	3
Severe oedema (raised more than 1 mm and extending beyond area of exposure)	4

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical observations were recorded.

C. BODY WEIGHT

Body weight and body weight gain was not recorded.

D. NECROPSY

Necropsy was not performed.

E. SKIN OBSERVATIONS

No erythema or oedema were observed at the application site of any animal for any observation time point (all scores were 0) following 4 hours semi-occlusive exposure to glyphosate technical. The mean for the 24, 48 and 72-hour readings were 0.0 for erythema and 0.0 for oedema.

Table 5.2.4-16 Glyphosate Technical: Primary Skin Irritation in Rabbits ([redacted], 1989): Skin irritation scores (individual values)

Evaluation interval	Animal No.	Erythema	Oedema
1 hour	1 (♀)	0	0
	2 (♀)	0	0
	3 (♀)	0	0
	4 (♂)	0	0
	5 (♀)	0	0
	6 (♂)	0	0
24-hours	1 (♀)	0	0
	2 (♀)	0	0
	3 (♀)	0	0
	4 (♂)	0	0
	5 (♀)	0	0
	6 (♂)	0	0
48 hours	1 (♀)	0	0
	2 (♀)	0	0
	3 (♀)	0	0
	4 (♂)	0	0
	5 (♀)	0	0
	6 (♂)	0	0
72-hours	1 (♀)	0	0
	2 (♀)	0	0
	3 (♀)	0	0
	4 (♂)	0	0
	5 (♀)	0	0
	6 (♂)	0	0
Individual 24 – 72 h means	**1 (♀)**	**0.0**	**0.0**
	2 (♀)	**0.0**	**0.0**
	3 (♀)	**0.0**	**0.0**
	4 (♂)	**0.0**	**0.0**
	5 (♀)	**0.0**	**0.0**
	6 (♂)	**0.0**	**0.0**

3. Assessment and conclusion

Assessment and conclusion by applicant:

Compared to the current OECD TG 404 (2015), deviations noted were no step-wise approach, use of six instead of three animals, no purity of the test substance provided (concluded from the batch number), no information on water solubility, physicochemical properties, volume of vehicle for moistening the test material, stability of the test material, animal age, and body weights were not recorded, and temperature range outside the required range.

Nevertheless, the deviations are not considered to affect the outcome of the study. Therefore, the study is considered acceptable and the outcome can be reported as valid.

A single semi-occlusive application of glyphosate to intact rabbit skin for four hours elicited no skin reactions at the application site of any animal at any observation time. The individual mean score over 24, 48 and 72-hours was 0.0 for erythema and 0.0 for oedema for all animals.

Thus, glyphosate is not considered a skin irritant.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.4/019
Report author	[redacted]
Report year	1989
Report title	Primary skin irritation study with glyphosate technical (isopropylamine salt 62 % in water equivalent to 46 % of N-phosphonomethylglycine acid) in rabbits (4-hour semi-occlusive application on intact and abraded skin)
Report No	238072
Document No	[redacted]438
Guidelines followed in study	No final conclusion possible.
GLP	No final conclusion possible.
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	0.5 mL of the pure test substance (glyphosate salt, purity 62 %) was applied onto the intact skin of 3 female and onto the abraded skin of 3 male albino rabbits.
Short description of results:	Slight irritation (not further specified)
Reasons for why the study is not considered relevant/reliable or not considered as key study:	Full study report is not available to the applicant. Only a short summary is provided in the Monograph, B5, 2000, *Category 4b*
Reasons why the study report is not available for submission	The notifier has not access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a “request for administrative assistance” (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL.
Category study in AIR 5 dossier (L docs)	Category 4b

1. Information on the study

Data point:	CA 5.2.4/020
Report author	[redacted]
Report year	1988
Report title	Primary Dermal Irritation Study of Glyphosate Batch/lot/nbr no. XLI-55 in New Zealand White Rabbits
Report No	88.2053.010
Document No	Not reported
Guidelines followed in study	US EPA 81-5
Deviations from current test guideline (OECD 404, 2015)	The dermal irritation was evaluated at 0.5, 24, 48 and 72-hours instead of 1, 24, 48 and 72-hours. Six instead of three animals were used. Animals were treated simultaneously. Age of animals and number of air changes were not specified. These deviations are not considered to affect the study outcome.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5	Category 2a

dossier (L docs)	

2. Full summary

In a primary dermal irritation study, young adult New Zealand White rabbits (three males and three females) were dermally exposed to glyphosate (Batch: XLI-55, Purity: 97.76 %). Each rabbit was administered 0.5 g of the test article (moistened with 0.5 mL of physiological saline) to the clipped back. Each test site was semi-occluded with a one-inch square gauze patch held in place with Micropore® tape. The patches were removed 4 hours following dose administration. Dermal irritation was scored according to the Draize method at 0.5, 24, 48 and 72-hours after patch removal.

Under the test conditions chosen, none of six male rabbits exposed for 4 hours to 0.5 g glyphosate / patch (semi-occlusive conditions) showed any test item-related changes. The overall mean for the 0.5, 24, 48 and 72-hour readings were 0.0 for erythema and 0.0 for oedema.
Based on the study, glyphosate does not show a skin irritating potential under the test conditions chosen.

Materials and methods

A: Materials

1. Test material:

Identification:	Glyphosate (*N*-phosphonomethylglycine)
Description:	White powder
Lot/Batch #:	XLI-55
Purity:	97.76 %
Water solubility:	Not mentioned in the report
Stability of test compound:	Stored at room temperature

2. Vehicle and/or positive control: Physiological saline

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Young adult (not further specified)
Sex:	Male and female
Weight at dosing:	2 – 3 kg
Acclimation period:	At least five days
Diet/Food:	NIH 09 Rabbit Ration certified feed (Zeigler Brothers, Inc., Gardners, PA, US), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in wire mesh cages
Environmental conditions:	Temperature: 20 – 23.9 °C
	Humidity: 40 – 60 %
	Air changes: Not specified
	Photocycle: 12 hour light / dark cycle

4. Test conditions:

Patch site preparation technique:	The fur on the back was clipped with an electric clipper, 24-hours before application
Patching technique:	Dermal application onto shaved, intact dorsal skin; patch was held on the test site with Micropore®
Chemical preparation:	0.5 g glyphosate was moistened with 0.5 mL of 0.9 % saline
Chemical application:	0.5 g / animal
Chemical removal:	Skin was wiped with gauze

B: Study design and methods

In life dates: 1998-04-11 to 1998-04-14

Animal assignment and treatment:

Six healthy animals (three males and three females) weighing between 2 – 3 kg were selected randomly from the acclimated colony and assigned to the test group. Selection suitability was based on health, weight requirement and of dorsal skin for testing. The fur on the back of each rabbit was clipped with an electric clipper on the day prior to dose administration. The test article (0.5 g moistened with 0.5 mL physiological saline) was applied topically to each of two intact dorsal test sites per rabbit. Immediately after dosing, the test sites were semi-occluded with a one-inch square gauze patch held in place with tape. The animals were collared during the exposure period to prevent removal of the patches. The patches and collars were removed 4-hours after dose administration and the exposure sites gently wiped with gauze to remove as much non-absorbed test article as possible.

Dermal irritation was evaluated at 0.5, 24, 48 and 72-hours after patch removal. Erythema and oedema were scored separately according to the Draize method (see table below). The animals were observed twice daily for mortality at least five hours apart. Body weights were obtained on study day 1 prior to dose administration. At study termination, the animals were euthanised by intra-cardiac injection of sodium pentobarbital and discarded.

Individual animal scores were obtained at each scoring interval by adding the total erythema and eschar formation scores from both application sites to the total oedema formation scores from both sites and dividing by two.

Skin reaction grading according to Draize criteria used by [redacted] (1988)

Skin Reaction	Grading
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef redness) to slight eschar formation (injuries in depth)	4
Oedema formation	
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well defined by definite raising)	2
Moderate oedema (raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre and extending beyond area of exposure)	4

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

Not reported.

C. BODY WEIGHT

Not reported.

D. NECROPSY

No necropsy was performed.

E. SKIN OBSERVATIONS

No dermal irritation was noted following test substance application.

Table 5.2.4-17 Primary Dermal Irritation Study of Glyphosate ([redacted] 1988): Skin irritation scores (individual values)

Evaluation interval	Animal No.	Erythema	Oedema
0.5 hour	1167 (♂)	0	0
	1176 (♂)	0	0
	1177 (♂)	0	0
	1264 (♀)	0	0
	1252 (♀)	0	0
	1259 (♀)	0	0
24-hours	1167 (♂)	0	0
	1176 (♂)	0	0
	1177 (♂)	0	0
	1264 (♀)	0	0
	1252 (♀)	0	0
	1259 (♀)	0	0
48 hours	1167 (♂)	0	0
	1176 (♂)	0	0
	1177 (♂)	0	0
	1264 (♀)	0	0
	1252 (♀)	0	0
	1259 (♀)	0	0
72-hours	1167 (♂)	0	0
	1176 (♂)	0	0
	1177 (♂)	0	0
	1264 (♀)	0	0
	1252 (♀)	0	0
	1259 (♀)	0	0
Individual 24 – 72 h means	1167 (♂)	**0.0**	**0.0**
	1176 (♂)	**0.0**	**0.0**
	1177 (♂)	**0.0**	**0.0**
	1264 (♀)	**0.0**	**0.0**
	1252 (♀)	**0.0**	**0.0**
	1259 (♀)	**0.0**	**0.0**

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD TG 404 (2015). Therefore, the study is considered acceptable and the outcome can be reported as valid.

A single semi-occlusive application of glyphosate to intact rabbit skin for 4 hours elicited no skin reactions at the application site of any animal at any observation time. The individual mean score over 24, 48 and 72-hours was 0.0 for erythema and 0.0 for oedema for all animals.

Thus, glyphosate is not considered a skin irritant.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.4/021
Report author	[redacted]
Report year	1987
Report title	Primary Dermal Irritation Study of MON 8750 in New Zealand White Rabbits
Report No	[redacted]-86-431/9308A
Document No	Not reported
Guidelines followed in study	US EPA 81-5 (1982)
Deviations from current test guideline	Yes, step-wise approach by initial testing in one animal was not performed. Rationale for *in vivo* testing and consideration of pre-existing data was not documented. No information was presented on water solubility and stability of the test material. Six instead of three animals exposed simultaneously. Body weights were only recorded on day 1 before dosing. Skin reactions were not evaluated at 1 hour.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

In a primary dermal irritation study, six male young adult New Zealand White rabbits were dermally exposed to MON 8750. The fur was clipped from the back of each rabbit the day prior to dosing. Test article (0.5 g MON 8750 moistened with 0.5 mL saline) was applied to two non-abraded test sites of each rabbit. Test sites were wrapped with semi-occlusive binders consisting of a 1-inch square gauze patch and Micropore tape for 4 hours. Animals were collared throughout the 4-hour exposure period. After 4 hours of exposure the semi-occlusive binders were removed, and the skin was gently wiped with gauze to remove any non-absorbed test article. Skin reactions were assessed 4, 24, 48 and 72-hours after removal of the binders. Skin reactions were scored according to the Draize method. Animals were observed daily for mortality and overt pharmacotoxic signs.

No skin reactions were observed at the application sites of any animal at any observation time point. The mean score for the 4, 24, 48 and 72-hour readings were 0.0 for erythema and 0.0 for oedema. No pharmacotoxic signs were noted in any animal.

Thus, MON 8750 is considered to be not irritating to skin.

Materials and methods

A: Materials

1. Test material:

Identification:	MON 8750, FD-86-431
Description:	White powder
Lot/Batch #:	XLG-225 (Assigned FDRL Identification 86-0616)
Purity:	90.8 %
Water solubility:	Not specified
Stability of test compound:	Not specified

2. Vehicle and/or positive control: Saline

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Not specified
Sex:	Male
Weight at dosing:	2 – 3 kg
Acclimation period:	At least 5 days
Diet/Food:	NIH 09 Rabbit Ration, certified feed (Zeigler Brothers, Inc., Gardners, PA, US) *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually housed in stainless steel mesh cages
Environmental conditions:	Temperature: 20.0 – 23.9 °C Humidity: 40 – 60 % Air changes: Not specified Photocycle: 12 hours light / dark cycle

4. Test conditions:

Patch site preparation technique:	The back of each rabbit was clipped (area not specified), 24 h before application.
Patching technique:	Dermal application onto clipped, non-abraded skin (two test sites). Test sites were wrapped in semi-occlusive binders consisting of 1 inch square gauze patch and Micropore tape.
Chemical preparation:	Test article was ground with a pestle and mortar before dosing. 0.5 g MON 8750 / animal / test site was weighed and moistened with 0.5 mL physiological saline before application.
Chemical application:	Test article (0.5 g moistened with 0.5 mL saline) was topically applied to two non-abraded dorsal test sites. Animals were collared during the exposure period.
Chemical removal:	Exposure sites gently wiped with gauze to remove non-absorbed test article.

B: Study design and methods

In life dates: 1986-11-06 to 1986-11-09

Animal assignment and treatment:

The test was conducted using six male young adult New Zealand White rabbits. Test article (0.5 g MON 8750 moistened with 0.5 mL saline) was applied to two non-abraded test sites on the dorsal of each rabbit. Test sites were wrapped with semi-occlusive binders consisting of a 1-inch square gauze patch and Micropore tape for 4 hours. Animals were collared throughout the 4-hour exposure period to prevent removal of the patches. After exposure the semi-occlusive binders were removed, and the skin was gently wiped with gauze to remove any non-absorbed test article.

Skin reactions were assessed 4, 24, 48 and 72-hours after removal of the binders. Skin reactions were scored according to the Draize method (see table below). Animals were observed daily for mortality and overt pharmacotoxic signs. Body weights were obtained on study day 1 prior to dose administration.

Skin reaction grading according to Draize criteria used by [redacted] (1987)

Skin Reaction	Grading
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Oedema formation	
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well defined by definite raising)	2
Moderate oedema (raised approximately 1 mm)	3
Severe oedema (raised more than 1 mm and extending beyond area of exposure)	4

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical observations were noted.

C. BODY WEIGHT

Body weight and body weight gain were not recorded.

D. NECROPSY

No necropsies were performed.

E. SKIN OBSERVATIONS

No erythema or oedema were observed at the application sites of any animal for any observation time point (all scores were 0) following 4 hours' semi-occlusive exposure to MON 8750. The mean for the 24, 48 and

72-hour readings were 0.0 for erythema and 0.0 for oedema.

Table 5.2.4-18 Primary Dermal Irritation Study of MON 8750 in New Zealand White Rabbits ([redacted] 1987): Skin irritation scores (individual values)

Evaluation interval	Animal No.	Erythema (L/R)	Oedema (L/R)
4 hours	86-3383 (♂)	0/0	0/0
	86-3384 (♂)	0/0	0/0
	86-3385 (♂)	0/0	0/0
	86-3386 (♂)	0/0	0/0
	86-3387 (♂)	0/0	0/0
	86-3388 (♂)	0/0	0/0
24-hours	86-3383 (♂)	0/0	0/0
	86-3384 (♂)	0/0	0/0
	86-3385 (♂)	0/0	0/0
	86-3386 (♂)	0/0	0/0
	86-3387 (♂)	0/0	0/0
	86-3388 (♂)	0/0	0/0
48 hours	86-3383 (♂)	0/0	0/0
	86-3384 (♂)	0/0	0/0
	86-3385 (♂)	0/0	0/0
	86-3386 (♂)	0/0	0/0
	86-3387 (♂)	0/0	0/0
	86-3388 (♂)	0/0	0/0
72-hours	86-3383 (♂)	0/0	0/0
	86-3384 (♂)	0/0	0/0
	86-3385 (♂)	0/0	0/0
	86-3386 (♂)	0/0	0/0
	86-3387 (♂)	0/0	0/0
	86-3388 (♂)	0/0	0/0
Individual 24 – 72 h means	**86-3383 (♂)**	**0.0**	**0.0**
	86-3384 (♂)	**0.0**	**0.0**
	86-3385 (♂)	**0.0**	**0.0**
	86-3386 (♂)	**0.0**	**0.0**
	86-3387 (♂)	**0.0**	**0.0**
	86-3388 (♂)	**0.0**	**0.0**

3. Assessment and conclusion

Assessment and conclusion by applicant:

Compared to the current OECD TG 404 (2015), deviations such as no step-wise approach, use of six instead of three animals, no report of body weight and no 1-hour evaluation of skin irritation are noted.
A single semi-occlusive application of glyphosate to intact rabbit skin for four hours elicited no skin reactions at the application site of any animal at any observation time. The mean individual score over 24, 48 and 72-hours was 0.0 for erythema and 0.0 for oedema for all animals.
Thus, glyphosate is not considered a skin irritant.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.4/022
Report author	[redacted]
Report year	1983
Report title	Primary skin irritation test on rabbits with glyphosate (tech) of Excel Industries, Ltd.
Report No	400060
Document No	Not reported
Guidelines followed in study	None
Deviations from current test guideline (OECD 404 (2015))	Step-wise approach by initial testing in one animal not performed. Rationale for *in vivo* testing and consideration of pre-existing data not documented. No information provided on the Batch number, the description of the test substance, CAS-number, animal age, in life dates, environmental conditions. Six instead of three animals were used, 24-hours instead of 4 hours' exposure. Additionally, abraded skin was exposed. Scoring of skin reactions not performed at reading time-point of 48 hours after application. Additionally, systemic effects, like clinical signs of toxicity as well as body weight were not recorded.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Supportive (limited reporting of test conditions, limited scoring time points)
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

In a primary skin irritation study, three male and three female New Zealand White rabbits were dermally exposed to glyphosate technical. The fur was clipped from both sides of each rabbit the day prior to dosing. Test article (0.5 g moistened with saline) was applied to abraded and intact test site of each rabbit. A gauze patch was applied on top of the test material and hold in place with an adhesive tape. Animals were restrained throughout the 24-hour exposure period. After 24-hours of exposure, the patches and unabsorbed test material were removed. Skin reactions were assessed 24 and 72-hours after removal of the patches. Skin reactions were scored according to the Draize method.

No skin reactions were observed at the application sites of any animal at any observation time point. The mean score for the 24 and 72-hour readings were 0.0 for erythema and 0.0 for oedema.

Thus, glyphosate technical does not show a skin irritating potential under the test conditions.

Materials and methods

A: Materials

1. Test material:

Identification:	Glyphosate technical, sample 8.7.83
Description:	White amorphous powder
Lot/Batch #:	R&D Sample
Purity:	95 %
Water solubility:	Not specified
Stability of test compound:	Not specified

2. Vehicle and/or positive control: Saline

3. Test animals:

Species:	Rabbit
Strain:	NWS
Source:	[redacted]
Age:	Not specified
Sex:	Male and female
Weight at dosing:	1.5 – 2.5 kg
Acclimation period:	Not specified
Diet/Food:	Lucerne grass, carrots, germinated grams with wheat bran, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually housed in air-conditioned room with regular lighting condition except during exposure; during exposure animals were housed singly in restrain cages
Environmental conditions:	Not specified

4. Test conditions:

Patch site preparation technique:	Both sides of each rabbit (back skin) were clipped (area size not specified), 24 h before application. Skin was abraded on one site of the animal (penetration of s*tratum corneum*, but not dermis)
Patching technique:	Dermal application onto clipped, intact and abraded skin (two test sites). Gauze patch was secured over treated area with adhesive tape.
Chemical preparation:	0.5 g glyphosate / animal / test site was moistened with saline before application.
Chemical application:	Test article (0.5 g moistened with saline [amount not specified]) was topically applied to two test sites (intact and abraded). Animals were restrained during the exposure period (24-hours).
Chemical removal:	Patches and unabsorbed test material were removed at 24-hours.

B: Study design and methods

In life dates: Not specified; finalisation date: 1983-10-18

Animal assignment and treatment:

The test was conducted using three male and three female rabbits. Test article (0.5 g moistened with saline) was applied to the test sites of intact and abraded skin (abrasions penetrating the *stratum corneum* but not the dermis) of each rabbit. A gauze patch was placed on the test site and hold in place with an adhesive tape. Animals were restrained throughout the 24-hour exposure period. After 24-hours of exposure the patches and unabsorbed test material were removed.

Skin reactions were assessed 24 and 72-hours after removal of the patches. Skin reactions were scored according to the Draize method.

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

Clinical observations were not reported.

C. BODY WEIGHT

Body weight and body weight gain was not reported.

D. NECROPSY

No necropsies were performed.

E. SKIN OBSERVATIONS

No erythema or oedema were observed at the application sites of any animal for any observation time point (all scores were 0) following a 24-hour exposure to glyphosate technical. The individual mean score for 24 and 72-hour readings were 0.0 for erythema and 0.0 for oedema.

Table 5.2.4-19 Primary skin irritation test on rabbits with glyphosate (tech) ([redacted], 1983): Skin irritation scores of intact skin (individual values)

Evaluation interval	Animal No.	Erythema	Oedema
24-hours	1	0	0
	2	0	0
	3	0	0
	4	0	0
	5	0	0
	6	0	0
72-hours	1	0	0
	2	0	0
	3	0	0
	4	0	0
	5	0	0
	6	0	0
Individual 24 – 72 h means	**1**	**0.0**	**0.0**
	2	**0.0**	**0.0**
	3	**0.0**	**0.0**
	4	**0.0**	**0.0**
	5	**0.0**	**0.0**
	6	**0.0**	**0.0**

3. Assessment and conclusion

Assessment and conclusion by applicant:

Compared to the current OECD TG 404 (2015), deviations such as no step-wise approach, 6 instead of 3 animals, 24-hours exposure instead of 4 hours, no scoring of skin effects at reading time points of 48 hours after application were noted. No information is provided on the batch number, the description of the test substance, CAS number, animal age, in life dates, environmental conditions, clinical signs of toxicity and body weights.

As the 24-hour application is considered to represent a worst-case scenario, this deviation is not compromising the negative study outcome. Nevertheless, due to the deviations, the study is considered to be used as supplemental information. Therefore, the study is presented here for the sake of completeness, only.

A single semi-occlusive application of glyphosate to intact rabbit skin for 24-hours elicited no skin reactions at the application site of any animal at any observation time. The mean score over 24 and 72-hours was 0.0 for erythema and 0.0 for oedema for all animals.

Thus, glyphosate is not considered a skin irritant.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.4/023
Report author	[redacted]
Report year	1981
Report title	Primary skin irritation of MON 0139 to rabbits
Report No	800259
Document No	Not reported
Guidelines followed in study	None; study conducted prior to test guidelines existence
Deviations from current test guideline (OECD 404 (2015))	Yes, step-wise approach by initial testing in one animal was not performed. Rationale for in-vivo testing and consideration of pre-existing data was not documented. No information provided on the environmental conditions. Six instead of three animals exposed simultaneously for 24-hours instead of 4 hours under occlusive instead of semi-occlusive conditions. Additionally, abraded skin was exposed. Scoring of skin reactions was only performed at reading time-points of 24 and 72-hours, not at 48 hours after application. Additionally, body weight was not recorded.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

In a primary dermal irritation study, three male and three female young adult New Zealand White rabbits were dermally exposed to MON 0139. The fur was clipped from the back of each rabbit prior to dosing. Test article (0.5 mL MON 0139) was applied to two intact test sites and two abraded test sites of each rabbit and covered with gauze patches. Test sites were wrapped with an occlusive dressing consisting of a latex rubber wrap secured by bandaging and elastic tape. After 24-hours of exposure the occlusive patches were removed, and the skin was any non-absorbed test material was wiped from the treated test sites. Skin reactions were assessed 24 and 72-hours after removal of the patches. Skin reactions were scored according to the Draize method.

Erythema was observed on intact skin in one of six animals 24-hours after patch removal. No oedema was observed at any time point. No erythema was observed 72-hours after patch removal. As exposure and examination of abraded skin is not in concordance with the current guideline, results of this part will not be elaborated on.

Due to the lack of scoring data at the 48 hour time point, for the calculation of the mean individual score over 24 - 72-hours (as required for classification purpose according to Regulation (EC) 1272/2008), persistence of irritating effects over the 48 hour time point was assumed (worst case).

The calculated mean individual scores for the 24 – 72-hour readings were 0.67 for one animal and 0.0 for the remaining five animals for erythema and 0.0 for oedema.

Thus, MON 0139 is considered non-irritant to skin.

Materials and methods

A: Materials

1. Test material:

Identification:	MON 0139
Description:	Amber liquid
Lot/Batch #:	SSRT-11012
Purity:	Not specified (IPA, 65 %, according to Monograph 2001)
Water solubility:	Not specified
Stability of test compound:	Not specified

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White (Isf: (NZW))
Source:	[redacted]
Age:	Young adult (not further specified)
Sex:	Male and female
Weight at dosing:	1.87 – 2.73 kg
Acclimation period:	Not specified
Diet/Food:	Not specified, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually housed (not further specified)

Environmental conditions: Not specified

4. Test conditions:

Patch site preparation technique:	The back of each rabbit was clipped (area not specified) before application. The skin of one of the test sites was abraded, the other one intact. Abrasion was achieved with a hypodermic needle, sufficiently deep to penetrate the *stratum corneum* but not deep enough to produce bleeding.
Patching technique:	Dermal application onto clipped sites, of which two were abraded and two were intact (4 test sites in total). Test sites were wrapped in an occlusive dressing consisting of 1 inch square gauze patch and a wrap of latex rubber on top held in place by an elastic tape.
Chemical preparation:	Test article was used undiluted.
Chemical application:	Test article (0.5 mL glyphosate) was topically applied to four test sites of each animal and wrapped by an occlusive dressing.
Chemical removal:	Exposure sites wiped to remove excess test material.

B: Study design and methods

In life dates: 1980-08-26 to 1980-08-29

Animal assignment and treatment:

The test was conducted using three male and three female young adult New Zealand White rabbits. Test material (0.5 mL MON 0139) was applied to four test sites (two abraded and two non-abraded) on the dorsal part of each rabbit under one-inch square gauze patches, covered by means of an occlusive dressing consisting of latex rubber wrap, which was held in place by an elastic band. After 24-hours of exposure, the occlusive dressing was removed, and the skin was wiped to remove any excess of test material.

Skin reactions were assessed 24 and 72-hours after removal of the patches. Skin reactions were scored according to the Draize method.

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

Clinical observations were not recorded.

C. BODY WEIGHT

Body weight and body weight gain were not recorded.

D. NECROPSY

No necropsies were performed.

E. SKIN OBSERVATIONS

Very slight erythema (grade 1) was observed in 1/6 animals after 24-hours at one of two test sites with intact skin. No oedema was observed at the application sites of any animal at any observation time point. After 72-hours no erythema was observed in any of the animals.

Due to the lack of scoring data at the 48 hour time point, for the calculation of the mean individual score over 24 - 72-hours (as required for classification purpose according to Regulation (EC) 1272/2008), persistence of irritating effects over the 48 hour time point was assumed (worst case).

The calculated mean individual scores over the 24 - 72-hours readings were 0.67 for one animal and 0.0 for the remaining 5 animals for erythema and 0.0 for oedema.

Table 5.2.4-20 Primary skin irritation of MON 0139 to rabbits [redacted] 1981): Skin irritation scores of intact skin (individual values)

Evaluation interval	Animal No.	Erythema R/L§	Oedema R/L
24-hours	01M01 (♂)	0/1	0/0
	01M02 (♂)	0/0	0/0
	01M03 (♂)	0/0	0/0
	01F01 (♀)	0/0	0/0
	01F02 (♀)	0/0	0/0
	01F03 (♀)	0/0	0/0
72-hours	01M01 (♂)	0/0	0/0
	01M02 (♂)	0/0	0/0
	01M03 (♂)	0/0	0/0
	01F01 (♀)	0/0	0/0
	01F02 (♀)	0/0	0/0
	01F03 (♀)	0/0	0/0
Individual 24-72 h means#	**01M01 (♂)**	**0.67***	**0.0**
	01M02 (♂)	**0.0**	**0.0**
	01M03 (♂)	**0.0**	**0.0**
	01F01 (♀)	**0.0**	**0.0**
	01F02 (♀)	**0.0**	**0.0**
	01F03 (♀)	**0.0**	**0.0**

§ Two test sites have been evaluated (right test site (R) and left test site (L))
# For calculation, persistence of 24-hour scores over 48 hours is assumed
* Calculation based on score 1 at one test site, mean calculated per test site.

3. Assessment and conclusion

Assessment and conclusion by applicant:
Compared to the current OECD TG 404 (2015), deviations such as no step-wise approach, six instead of three animals, 24-hours exposure instead of 4 hours, occlusive instead of semi-occlusive conditions, no scoring of skin effects at reading time points of 48 hours after application.
As the occlusive application and the 24-hour exposure are considered to represent a worst-case scenario, these deviations do not compromise the negative study outcome. Nevertheless, due to the deviations, the study is considered to be used as supplemental information. Therefore, the study is presented here for the sake of completeness, only.
The occlusive application of glyphosate to rabbit skin for 24-hours elicited only slight skin reactions at the application site of one animal at one observation time. The mean individual score over 24 - 72-hours was 0.67 for erythema for one animal (assuming persistence of the 24-hour score over 48 hours) and 0.0 for six animals and 0.0 for oedema for all animals. Thus, glyphosate is not considered a skin irritant.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.4/024
Report author	[redacted]
Report year	1979
Report title	Primary Dermal Irritation Study in Rabbits
Report No	[redacted]-77-428
Document No	Not reported
Guidelines followed in study	None; study conducted prior to test guidelines existence
Deviations from current test guideline (OECD 404, 2015)	Six instead of three rabbits were used, no step-wise approach, animals were treated simultaneously. Rationale for *in vivo* testing and consideration of pre-existing data was not documented. No time point is mentioned when the skin was clipped. In addition, abraded skin was exposed. The test item was administered as 0.125 per test site, with four test sites per animal (total of 0.5 g) rather than one dose of 0.5 g to one site. The test item was administered for 24-hours instead of 4 hours and occlusive (Dermicel® tape wrapped with plastic sheeting) instead of semi-occlusive dressing. No data on removal of residual test material was reported. Signs of dermal irritation were recorded only at 24 and 72-hours. No clinical signs and body weights were reported. Additionally, no information was provided about food and drinking, the humidity and housing of the rabbits.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Supportive (no GLP-study, no guideline, clinical signs & body weight not reported)
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Glyphosate (Batch: XLI-180, Purity: 99 %) was evaluated for potential primary dermal irritation using six New Zealand White rabbits (three males and three females). The skin was clipped over the back and sides. Each rabbit was administered 0.5 mL of the test article (applied as 25 % w/v solution in distilled water, equivalent to 0.125 g glyphosate per test site) to two intact test sites and two abraded sites. Each test site was covered occlusive for 24-hours following dose administration. Dermal irritation was scored according to the Draize method at 24 and 72-hours after patch removal.

In the intact skin, one male had very slight erythema (one site) at 24-hours following test substance application. The irritation was recovered at the 72-hour time point. There was no other irritation noted at 24 or 72-hours for the other animals.

Due to the lack of scoring data at the 48-hour time point, for the calculation of the mean individual score over 24 - 72-hours (as required for classification purpose according to Regulation (EC) 1272/2008),

persistence of irritating effects over the 48 hour time point was assumed (worst case). The calculated mean individual scores (per site) were 0.67 for one male and 0.0 for the remaining animals for erythema, and 0.0 for oedema all animals.
Thus, glyphosate is considered not irritant.

Due to the deviations from the current OECD TG 404 (2015), the study can be used as supportive information only.

Materials and methods

A: Materials

1. Test material:

Identification:	Glyphosate Technical
Description:	Fine white powder
Lot/Batch #:	XLI-180
Purity:	99 %
Stability of test compound:	Not specified

2. Vehicle and/or positive control: Distilled water

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Not specified
Sex:	Male and female
Weight at dosing:	2.25 – 2.80 kg
Acclimation period:	Not specified
Diet/Food:	Not specified
Water:	Not specified
Housing:	Not specified
Environmental conditions:	Temperature: Not specified
	Humidity: Not specified
	Air changes: Not specified
	Photocycle: Not specified

4. Test conditions:

Patch site preparation technique:	The back and sides were closely clipped with an electric clipper. Four test sites per rabbit, each site 1" × 1" in area. Two sites, one on each side of the spinal column were abraded, while the remaining two sites were left intact.
Patching technique:	Test substance was applied beneath a surgical gauze square, 1" × 1", eight single layers thick, placed directly on the test site and secured with Dermicel® tape. The animals were then wrapped with plastic sheeting secured with masking tape to help contain the test material (occlusive dressing).
Chemical preparation:	25 % w/v solution in distilled water
Chemical application:	0.5 mL / test site [equivalent to 0.125 g glyphosate / test site]

Chemical removal: After 24-hours the sheeting and gauze patches were removed. No removal of test substance reported.

B: Study design and methods

In life dates: Not specified

Animal assignment and treatment:
Six albino rabbits were closely clipped over the back and sides with an electric clipper. There were four test sites per rabbit, each site 1" × 1" in area. Two sites, one on each side of the spinal column were abraded, while the remaining two sites were left intact. The abrasions were sufficiently deep so as to penetrate the stratum corneum, but not so deep as to disturb the derma or produce bleeding.

The test material was administered as a 25 % w/v solution in distilled water. In all cases 0.5 mL of the test substance was applied beneath a surgical gauze square, 1" × 1", eight single layers thick, placed directly on the test site and secured with Dermicel® tape. The animals were then wrapped with plastic sheeting secured with masking tape to help contain the test material. After 24-hours' exposure period the occlusive dressing was removed.

Observations for signs of dermal irritation or systemic toxicity were recorded at 24 and 72-hours after application according to the table below. At each observation all treated sites were scored for erythema, oedema, and eschar formation.

Skin reaction grading according to Draize criteria used by [redacted] (1979)

Skin Reaction	Grading
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef redness) to slight eschar formation (injuries in depth)	4
Oedema formation	
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approximately 1 mm)	3
Severe oedema (raised more than 1 mm and extending beyond area of exposure)	4

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

Not reported.

C. BODY WEIGHT

Not reported.

D. NECROPSY

No necropsy was performed.

E. SKIN OBSERVATIONS

One male had very slight erythema (one site) at 24-hours following test substance application. The irritation was recovered at the 72-hour time point. There was no other irritation noted at 24 or 72-hours for the other animals.

Due to the lack of scoring data at the 48 hour time point, for the calculation of the mean individual score over 24 - 72-hours (as required for classification purpose according to Regulation (EC) 1272/2008), persistence of irritating effects over the 48 hour time point was assumed (worst case). The calculated mean individual scores (per site) were 0.67 for one male and 0.0 for the remaining animals for erythema, and 0.0 for oedema all animals.

Table 5.2.4-21 Primary Dermal Irritation Study in Rabbits ([redacted] 1979): Skin irritation scores of intact skin (individual values)

Evaluation interval	Animal No.	Erythema A/B§	Oedema A/B§
24-hours	655 (♀)	0/0	0/0
	657 (♂)	0/1	0/0
	658 (♀)	0/0	0/0
	659 (♂)	0/0	0/0
	660 (♂)	0/0	0/0
	661 (♀)	0/0	0/0
72-hours	655 (♀)	0/0	0/0
	657 (♂)	0/0	0/0
	658 (♀)	0/0	0/0
	659 (♂)	0/0	0/0
	660 (♂)	0/0	0/0
	661 (♀)	0/0	0/0
Individual 24 – 72 h means#	**655 (♀)**	**0.0**	**0.0**
	657 (♂)	**0.67***	**0.0**
	658 (♀)	**0.0**	**0.0**
	659 (♂)	**0.0**	**0.0**
	660 (♂)	**0.0**	**0.0**
	661 (♀)	**0.0**	**0.0**

§ Two test sites have been evaluated (test site A and test site B)
# For calculation, persistence of 24-hour scores over 48 hours is assumed
* Calculation based on score 1 at one test site, mean calculated per test site.

3. Assessment and conclusion

Assessment and conclusion by applicant:
Compared to the current OECD TG 404 (2015), deviations such as no step-wise approach, use of 6 instead of 3 animal, occlusive instead of semi-occlusive dressing, exposure 24 instead of 4 hours, record of skin reactions at 24 and 72-hours, only, no report of body weights and clinical signs, no information about food and water supply, housing and environmental conditions of the rabbits are noted.
Due to the deviations from the current OECD TG 404 (2015), the non-GLP study not following any guideline can be used as supplementary information, only.
Occlusive application of glyphosate to intact rabbit skin for 24-hours elicited skin reactions at the application site of two animals at the 24-hour observation time. The calculated individual mean score for 24 - 72-hours was 0.67 (assuming persistence of the 24-hour score over 48 hours) for one male for erythema and 0.0 for the remaining animals for erythema and all oedema values, respectively.
Thus, glyphosate is not considered a skin irritant.

Assessment and conclusion by RMS:

CA 5.2.5 Eye irritation

Eye irritation was examined in numerous studies in rabbits that were performed either with the acid or its different salts.

Glyphosate acid

Due to the rapid occurrence (less than one hour), persistence (cornea opacity and conjunctival lesions not reversible within a period of up to 21 days) and severity (marked cornea opacity up to score 4, sloughing of the cornea, haemorrhage of the lower conjunctival membrane, blood stained discharge) of ocular effects, the previous EU evaluation already concluded glyphosate acid to be strongly irritating to rabbit eyes ([redacted] (1994; CA 5.2.5/015), [redacted]. (1994; CA 5.2.5/016), [redacted] (1991; CA 5.2.5/017), [redacted] (1991; CA 5.2.5/018), [redacted] (1990; CA 5.2.5/019) and [redacted] (1989; CA 5.2.5/020 see Table 5.2.5-1). Therefore, in the sense of animal welfare considerations, it is impossible to understand why such a high number of additional studies (14 studies) on eye irritating potential in rabbits have been conducted afterwards.

Nevertheless, most of the studies submitted for re-evaluation in 2015 on eye irritating potential of glyphosate acid supported the previous evaluation. In contrast, two studies, by [redacted] (2009; CA 5.2.5/004) and [redacted] (2007; CA 5.2.5/008) failed to produce irritating effects as significant as the majority of the studies. However, in the [redacted] study the instilled test substance was washed out after one hour instead of 24-hours according to the current OECD Guideline 405. One of the submitted studies for re-evaluation in 2015 ([redacted], 2011; CA 5.2.5/001) was conducted in one male rabbit only. Due to severe effects observed up to 24-hours after instillation, the substance was considered to cause severe eye damage/corrosion, and the study was therefore terminated. Another study ([redacted], 2009; CA 5.2.5/005) revealed a corrosive potential, as the pH value was determined to be below 2 and therefore, no *in vivo* study was conducted.

For the current re-evaluation (2020) no new studies for glyphosate acid were submitted.
In conclusion, the former EU evaluation of glyphosate considering glyphosate acid to be severely irritant to rabbit eyes are still valid.

Therefore, according to the criteria laid down in the CLP Regulation (EC No. 1272/2008), glyphosate acid needs to be labelled and classified with 'Causes irreversible effects on the eye / Causes serious damage to eyes (Category 1)', H318.

Glyphosate salts

In contrast to glyphosate acid, its isopropylamine, sodium, or ammonium salts caused no or only slight eye irritating effects ([redacted] (1994; CA 5.2.5/014), [redacted] (1989; CA 5.2.5/021), [redacted] (1987; CA 5.2.5/023), [redacted] (1987; CA 5.2.5/024), [redacted] (1981; CA 5.2.5/026)).
Based on the results of available studies, classification according to criteria of the CLP Regulation (EC No. 1272/2008) is considered not needed for the glyphosate salts.

Table 5.2.5-1: Studies on eye irritation with glyphosate

Annex Point	Study	Study type	Substance(s)	Reference list-related category[a]	Result
CA 5.2.5/001	[redacted], 2011	Eye irritation *in vivo*: New Zealand White rabbit, ♂	Glyphosate technical (Batch: 569753, Purity: 96.3 %)	Valid, Category 2a	Corrosive/serious eye damage
CA 5.2.5/002	[redacted], 2010	Eye irritation *in vivo:* Himalayan rabbits, ♂	Glyphosate TC (Batch: 20090506, Purity: 97.3 %)	Valid[#], Category 2a	Irritating
CA 5.2.5/003	[redacted] 2009	Eye irritation *in vivo:* Himalayan rabbits, ♂	Glyphosate TC (Batch: 20090151501, Purity: 96.4 %)	Valid[#], Category 2a	Irritating
CA 5.2.5/004	[redacted], 2009	Eye irritation *in vivo:* Himalayan rabbits, ♂	Glyphosate TC (Batch: 20080801, Purity: 98.8 %)	Valid[#], Category 2a	Not irritating
CA 5.2.5/005	[redacted] 2009	no study performed (pH ≤2)	Glyphosate technical	Valid, Category 2a	Corrosive (pH ≤2)
CA 5.2.5/006	[redacted], 2009	Eye irritation *in vivo*: New Zealand White rabbit, ♂ / ♀	Glyphosate tech grade mixed 5-batch (Batch: 080704-1 thru 5, Purity: 96.4 %)	Valid, Category 2a	Irritating
CA 5.2.5/007	[redacted] 2008	Eye irritation *in vivo*: New Zealand White rabbit, ♂ / ♀	Glyphosate technical (Batch: 20070606, Purity: 98.05 %)	Valid, Category 2a	Serious eye damage
CA 5.2.5/008	[redacted] 2007	Eye irritation *in vivo*: New Zealand White (SPF) rabbit, ♂ / ♀	Glyphosate technical (Batch: 0507, Purity: 96.1 %)	Valid, Category 2a	Not irritating
CA 5.2.5/009	[redacted], 2007	Eye irritation *in vivo*: New Zealand White (SPF) rabbit, ♂ / ♀	Glyphosate technical (Batch: 200609062, Purity: 95.1 %)	Valid, Category 2a	Irritating
CA 5.2.5/010	[redacted] 2005	Eye irritation *in vivo*: New Zealand albino rabbit, ♂	Glyphosate acid technical (Batch: 040205, Purity: 97.23 %)	Valid, Category 2a	Irritating
CA 5.2.5/011	[redacted] 1997	Eye irritation *in vivo*: New Zealand White rabbit, ♀	Glyphosate acid (Batch: P24, Purity: 95.6 % (w/w))	Valid, Category 2a	Irritating

Table 5.2.5-1: Studies on eye irritation with glyphosate

Annex Point	Study	Study type	Substance(s)	Reference list-related category§	Result
CA 5.2.5/012	[redacted] 1996	Eye irritation *in vivo*: New Zealand White rabbit, ♂ / ♀	Glyphosate technical (Batch: 120594, Purity: 98.2 %)	Valid, Category 2a	Serious eye damage
CA 5.2.5/013	[redacted], 1995	Eye irritation *in vivo*: New Zealand White Kbl:NZW rabbit, ♀	HR-001 (glyphosate technical, Batch: T-941209, Purity: 97.56 %)	Valid, Category 2a	Serious eye damage
CA 5.2.5/014	[redacted]1994	Eye irritation *in vivo*: New Zealand White rabbit, ♂ / ♀	Glyphosate premix (technical concentrate of glyphosate isopropylamine salt, Batch: 290-JaK-146-4, Purity: 46.1 % glyphosate acid)	Valid, Category 3a	Not irritating
CA 5.2.5/015	[redacted] 1994	Eye irritation *in vivo*: New Zealand White rabbit, ♀	Glyphosate acid (Batch: not reported, Purity: 95 %)	Invalid, Category 4b	Severe eye damage
CA 5.2.5/016	[redacted] 1994	Eye irritation *in vivo*: New Zealand rabbit, ♀	Glyphosate technical (Batch: 36300892, Purity: 99.6 %)	Valid, Category 2a	Severe eye damage
CA 5.2.5/017	[redacted], 1991	Eye irritation *in vivo*: New Zealand White rabbit, ♂ / ♀	Glyphosate technical (Batch: 60, Purity: 96.8 %)	Valid, Category 3a	Severe eye damage
CA 5.2.5/018	[redacted] 1991	Eye irritation *in vivo*: New Zealand White rabbit	Glyphosate acid (Batch: not reported, Purity: 98.0 %)	Invalid, Category 4b	Irritating
CA 5.2.5/019	[redacted] 1990	Eye irritation *in vivo*: New Zealand White rabbit, ♀	Glyphosate technical (Batch: 0190 A, Purity: 98.1 %)	Valid, Category 3a	Irritating
CA 5.2.5/020	[redacted] 1989	Eye irritation *in vivo*: New Zealand White rabbit, ♂ / ♀	Glyphosate technical (Batch: 206-Jak-25-1; Purity: 98.6 %)	Valid, Category 3a	Serious eye damage
CA 5.2.5/021	[redacted] 1989	Eye irritation *in vivo*: rabbit, ♂ / ♀	Glyphosate technical; isopropylamine salt (Purity: 62 %, 46 % glyphosate acid)	Invalid, Category 4b	Irritating
CA 5.2.5/022	[redacted] 1988	Eye irritation *in vivo*: New Zealand White rabbit, ♂ / ♀	Glyphosate (Batch: XLI-55, Purity: 97.76 %)	Valid, Category 2a	Serious eye damage
CA 5.2.5/023	[redacted]1987	Eye irritation *in vivo*: New Zealand White rabbit	Glyphosate sodium salt (MON 8722, Batch: XLG-256, Purity: 70.7 %)	Valid, Category 3a	Not irritating

Table 5.2.5-1: Studies on eye irritation with glyphosate

Annex Point	Study	Study type	Substance(s)	Reference list-related category§	Result
CA 5.2.5/024	[redacted] 1987	Eye irritation *in vivo*: New Zealand White rabbit	Glyphosate ammonium salt (MON 8750, Batch: XLG-255, Purity: 90.8 %)	Valid, Category 3a	Not irritating
CA 5.2.5/025	[redacted] 1983	Eye irritation *in vivo*: NWS rabbit, ♂ / ♀	Glyphosate (Tech, Batch: 8.7.83, Purity: 95 %)	Invalid, Category 3b	Not applicable
CA 5.2.5/026	[redacted] 1981	Eye irritation *in vivo*: New Zealand White rabbit, ♂ / ♀	Glyphosate IPA salt (MON 0139) (Batch: SSRT-11012, Purity: not reported)	Valid, Category 3a	Not irritating

[#] performed at [redacted]

§: The category describes the acceptability/reliability of the study within the AIR 5 submission. This category does not describe hazard classification. (for details please refer to the Doc ID: 110054-B-GRG-Jun_2020)

1. Information on the study

Data point:	CA 5.2.5/001
Report author	[redacted]
Report year	2011
Report title	Glyphosate technical: Acute eye irritation study in rabbits
Report No	10/218-005N
Document No	NOT REPORTED
Guidelines followed in study	OECD 405 (2002), US EPA OPPTS 870.2400 (1998), 2004/73/EC B.5 (2008)
Deviations from current test guideline (OECD 405, 2017)	No treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application. Based on the information provided by the Sponsor, treatment was performed even though the pH of glyphosate technical was measured at 1.99. The relative humidity was out of the guideline range during the study.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

In a primary eye irritation study, 0.1 g of glyphosate technical (Batch: 569753, Purity: 96.3 %) was instilled into the conjunctival sac of the left eye of one adult male New Zealand White rabbit. The untreated right eye served as control. Immediately following instillation, an assessment of initial pain was made. Scoring of irritation effects was performed approximately 1 and 24-hours after test material instillation. Irritation

was scored according to the Draize numerical evaluation.

Initial Pain Reaction (IPR) scores were taken after instillation into the eye and a score of 3 (on a 0 – 5 scale) was observed. Conjunctival redness, chemosis and conjunctival discharge, as well as corneal opacity were observed in the rabbit 1 and 24-hours after application. Additionally, corneal erosion, redness of the conjunctiva with pale areas, pink, clean ocular discharge, oedema of the eyelids, a few black points on the conjunctiva and dry surface of the eye were noted at one hour after the treatment. Fluorescein staining was positive at the 24-hour observation. No clinical signs of systemic toxicity were observed in the animal during the study and no mortality occurred.

Based on the symptoms, no further animals were dosed and the study was terminated after the 24-hour observation (Regulation (EC) No 440/2008).

Glyphosate technical is considered to cause serious damage/corrosion to the eyes under the chosen test conditions.

Materials and methods

A: Materials

1. Test material:

Identification:	Glyphosate technical
Description:	Dry white powder
Lot/Batch number:	569753
Purity:	96.3 % w/w
Stability of test compound:	Stable under storage conditions are <30 °C, Recertification date end of August 2011

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age at dosing:	Approximately 14 weeks
Sex:	Male
Weight at dosing:	3035 g
Housing:	Individually in metal cage
Acclimation period:	13 days
Diet:	Purina Base – Lap gr. diet (Agribrands Europe Hungary PLC, H-5300 Karcag, Madarasi út, Hungary), *ad libitum*
Water:	Tap water, *ad libitum*
Environmental conditions:	Temperature: 20 ± 3 °C Humidity: 24 – 64 % Air changes: 15 – 20 / hour Photoperiod: 12 hours light / dark cycle

B: Study design and methods

In-life dates: 2011-12-21 to 2011-12-22

Animal assignment and treatment:

Approximately 1 hour before the start of the test, the eyes of the provisionally selected test rabbits were examined for evidence of ocular irritation or defect using a hand-held slit-lamp. The animal used in the study was free of ocular damage. Initially, a single rabbit was treated.

An amount of 0.1 g of the test material was placed into the conjunctival sac of the left eye, formed by gently pulling the lower lid away from the eyeball. The upper and lower eyelids were held together for about 1 second immediately after treatment, to prevent loss of the test material, and then released. The right eye remained untreated and was therefore used as control.

Immediately after administration of the test material, an assessment of the initial pain reaction was made according to a 0-5 scale. Following review of the ocular responses produced in the first treated animal, no further animals were treated. The treated eyes were not rinsed after instillation.

The ocular reaction (i.e. corneal opacity, iridic effects, conjunctivae and chemosis) was assessed approximately 1 and 24-hours following treatment, according to the numerical evaluation described by Draize. The treated eye was further examined using 2 % fluorescein solution before treatment and 24-hours after treatment. Additionally, any other signs of eye irritation were recorded.

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed in the animal during the study.

C. BODY WEIGHT

The body weight was considered to be within the normal range of variability.

D. NECROPSY

Not reported.

E. EYE OBSERVATIONS

An initial pain reaction score of 3 (on a 0 – 5 scale) was recorded.

Conjunctival redness, chemosis, and conjunctival discharge, as well as corneal opacity, were observed in the rabbit 1 and 24-hours after application. Additionally, corneal erosion, redness of the conjunctiva with pale areas, pink, clean ocular discharge, oedema of the eyelids, a few black points on the conjunctiva, and dry surface of the eye were noted one hour after the treatment. Fluorescein staining was positive at the 24-hours' observation.

Based on the symptoms, no further animals were tested and the study was terminated after the 24-hour observation (Regulation (EC) No 440/2008).

Table 5.2.5-2 Glyphosate technical: Acute eye irritation study in rabbits. ([redacted] 2011): Eye irritation – Individual irritation scores

Time	Cornea		Iris	Conjunctiva		
	Opacity	Area		Redness	Chemosis	Discharge
1 hour	2	4	0	2	3	3
24-hours	3	3	1	3	4	3
Individual mean (1, 24 h)	**2.5**	**--**	**0.5**	**2.5**	**3.5**	**--**

3. Assessment and conclusion

Assessment and conclusion by applicant:

Except the deviation of no treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application and the use of test material with a pH of 1.99, the study is in concordance with the current OECD TG 405 (2017). The deviations did not compromise the acceptability of the study. Therefore, the study is considered acceptable and the outcome can be reported as valid.

Instillation of glyphosate technical into the rabbit eye elicited strong response in treated animal. The individual scores at 24-hours for the animal were 3 for corneal opacity, 1 for iris lesions, 3 for conjunctival redness, and 4 for conjunctival chemosis, and therefore, the study was terminated at 24-hours.

Thus, under test conditions of the study, glyphosate technical is considered to cause serious damage/corrosion to the eyes.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.5/002
Report author	[redacted]
Report year	2010
Report title	Acute Eye Irritation/Corrosion Test of Glyphosate TC in Rabbits
Report No	24606
Document No	Not reported
Guidelines followed in study	OECD 405 (2002), US EPA OPPTS 870.2400 (1998), EC method B.5. (2004/73/EC)
Deviations from current test guideline (OECD 405, 2017)	No treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application. This deviation is not considered to affect the study outcome.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid[#]
Category study in AIR 5 dossier (L docs)	Category 2a

#: Following the feedback from the Assessment Group Glyphosate in March 2020, the Notifier is aware of concerns regarding work conducted at this specific contract laboratory [redacted]. For the sake of completeness, this study is included in the dossier

submission since it was part of the data package of previous EU evaluation processes.

2. Full summary

In an eye irritation study, 0.1 g of the undiluted, solid glyphosate TC (Batch: 20090506, Purity: 97.3 %) was instilled into the right conjunctival sac of three male Himalayan rabbits in a stepwise manner. One hour after instillation the eyes were rinsed with sodium chloride solution. Animals were observed for 7 days. Eye irritation was scored 1, 24, 48 and 72-hours and 4 and 7 days after test item instillation. Body weight of all animals was measured at the beginning and at the end of the study. Behaviour and food consumption were monitored.

Corneal opacity (grade 1) was observed in all animals 24 – 72-hours, in animal no. 1 until 4 days and in animal no. 3 until 5 days after instillation. The fluorescein test performed 24-hours after instillation revealed cornea1 staining in all animals (½ to ¾ of the surface). Irritation of the iris (grade 1) was observed in all animals 24 and 48 hours, in animal no. 3 until 72-hours after instillation. Conjunctival redness (grade 1 or 2) was observed in all animals 1 hour – 4 days, in animal no. 3 until 6 days after instillation. Chemosis (grade 1) was observed in all animals 1 hour and 24-hours after instillation. In addition, secretion was observed in all animals 1 hour and 24-hours after instillation. These effects were fully reversible within 7 days. There were no systemic intolerance reactions.

The individual mean irritation scores (24 – 72-hours) of the three rabbits were as follows:

- for corneal opacity: 1.00, 1.00, 1.00
- for iris lesions: 0.67, 0.67, 1.00
- for conjunctival redness: 1.00, 1.33, 2.00
- for conjunctival chemosis: 0.33, 0.33, 0.33

Materials and methods

A: Materials

1. Test material:

Identification:	Glyphosate TC
Description:	White solid powder
Lot/Batch #:	20090506
Purity:	97.3 % (w/w)
Stability of test compound:	Expiry date: 2011-05 (when stored at room temperature in the dark)

2. Vehicle and/or positive control: No vehicle was used

3. Test animals:

Species:	Rabbit
Strain:	Himalayan
Source:	[redacted]
Age:	Approximately 6 – 8 months
Sex:	Males
Weight at dosing:	2.5 – 2.7 kg
Acclimation period:	At least 20 adaptation days
Diet/Food:	ssniff® K-H V2333 (ssniff Spezialdiäten GmbH, Soest, Germany), *ad libitum* before and after the exposure period

Water: Tap water, *ad libitum,* before and after the exposure period

Housing: The animals were kept singly in cages measuring 380 mm × 425 mm × 600 mm (manufacturer: Dipl. Ing. W. EHRET GmbH, 16352 Schönwalde, Germany). After test item application, animals were held in restrainers for 8 hours to prevent a complete body turn, wiping of the eyes with paws, and excluded irritation by excrements/urine.

Environmental conditions:
- Temperature: 20 ± 3 °C
- Humidity: 30 – 70 %
- Air changes: Not reported
- Photoperiod: 12-hour light / dark cycle

B: Study design and methods

In life dates: 2009-10-26 to 2009-11-12 (Start of treatment to study completion)

Animal assignment and treatment:
A quantity of 0.1 g of the test item was administered into one eye each of three animals. The test item was placed into the conjunctival sac of the right eye of each animal after gently pulling the lower lid away from the eyeball. The lids were then gently held together for about one second in order to prevent loss of the material. The left eye, which remained untreated, served as a control. The test was performed initially using one animal. As no corrosive or severe irritant effects were observed in this animal, two further animals were employed 24-hours after start of the initial test.

One hour after instillation the eyes were rinsed with 20 mL sodium chloride solution. The eyes were examined ophthalmoscopically with a slit lamp prior to the administration and 1, 24, 48, 72-hours and 4 – 7 days after the administration. Twenty-four hours and 7 days after administration, fluorescein was applied to the eyes before being examined to aid evaluation of the cornea for possible lesions. The eye reactions were observed, registered and scored according to the Draize scheme (see table below).

Numerical scoring system (grading of ocular lesions)

Cornea	
Opacity: degree of density (readings should be taken from most dense area)*	
No ulceration or opacity	0
Scattered or diffuse areas of opacity (other than slight dulling of normal lustre), details of iris clearly visible	1
Easily discernible translucent area, details of iris slightly obscured	2
Nacreous areas, no details of iris visible, size of pupil barely discernible	3
Opaque cornea; iris not discernible through the opacity	4
Iris	
Normal	0
Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperaemia or injection, iris reacting to light (a sluggish reaction is considered to be an effect)	1
Haemorrhage, gross destruction, or no reaction to light	2
Conjunctivae, Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)	
Normal	0
Some blood vessels hyperaemic (injected)	1
Diffuse, crimson colour; individual vessels not easily discernible	2
Diffuse beefy red	3
Conjunctivae, Chemosis (refers to lids and/or nictitating membranes)	
Normal	0
Some swelling above normal	1
Obvious swelling with partial eversion of lids	2
Swelling with lids about half-closed	3

Numerical scoring system (grading of ocular lesions)

Swelling with lids more than half-closed	4

* The area of corneal opacity should be noted

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed during the study.

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance.

D. NECROPSY

No data on necropsy were reported.

E. EYE OBSERVATIONS

Corneal opacity (grade 1) was observed in all animals 24 – 72-hours, in animal no. 1 until 4 days and in animal no. 3 until 5 days after instillation. The fluorescein test performed 24-hours after instillation revealed corneal staining in all animals (½ – ¾ of the surface). Irritation of the iris (grade 1) was observed in all animals 24 and 48 hours, in animal no. 3 until 72-hours after instillation. Conjunctival redness (grade 1 or 2) was observed in all animals 1 hour – 4 days, in animal no. 3 until 6 days after instillation. Chemosis (grade 1) was observed in all animals 1 hour and 24-hours after instillation. In addition, secretion was observed in all animals 1 hour and 24-hours after instillation.

The individual mean irritation scores (24 – 72-hours) of the three rabbits were 1.00 for all animals for corneal opacity, 0.67, 0.67, 1.00 for iris lesions, 1.00, 1.33, 2.00 for conjunctival redness and 0.33 for all animals for conjunctival chemosis. These effects were fully reversible within 7 days.

Table 5.2.5-3 Acute Eye Irritation/Corrosion Test of Glyphosate TC in Rabbits. ([redacted] 2010): Eye irritation – Individual irritation scores

Animal	Scoring [h]	Cornea opacity	Iris	Conjunctiva	
				Redness	Chemosis
Rabbit 1 (male)	1	0	0	1[a]	1
	24	**1**	**1**	**1[a]**	**1**
	48	**1**	**1**	**1**	**0**
	72	**1**	**0**	**1**	**0**
	Day 4	1	0	1	0
	Day 5	0	0	0	0
	Day 6	--	--	--	--
	Day 7	--	--	--	--
Individual mean (24, 48, 72 h)		**1.00**	**0.67**	**1.00**	**0.33**
Rabbit 2 (male)	1	0	0	1[a]	1
	24	**1**	**1**	**2[a]**	**1**
	48	**1**	**1**	**1**	**0**

Table 5.2.5-3 Acute Eye Irritation/Corrosion Test of Glyphosate TC in Rabbits. ([redacted] 2010): Eye irritation – Individual irritation scores

	72	**1**	**0**	**1**	**0**
	Day 4	0	0	1	0
	Day 5	0	0	0	0
	Day 6	--	--	--	--
	Day 7	--	--	--	--
Individual mean (24, 48, 72 h)		**1.00**	**0.67**	**1.33**	**0.33**
Rabbit 3 (male)	1	0	0	1[a]	1
	24	**1**	**1**	**2[a]**	**1**
	48	**1**	**1**	**2**	**0**
	72	**1**	**1**	**2**	**0**
	Day 4	1	0	1	0
	Day 5	1	0	1	0
	Day 6	0	0	1	0
	Day 7	0	0	0	0
Individual mean (24, 48, 72 h)		**1.00**	**1.00**	**2.00**	**0.33**

[a] = secretion; -- = no examination

3. Assessment and conclusion

Assessment and conclusion by applicant:

Except the deviation of no treatment with systemic analgesic or topical anaesthesia of the animals/animal eyes prior, during or after test substance application the study is in concordance with the current OECD TG 405 (2017). This deviation did not compromise the acceptability of the study. Therefore, the study is considered acceptable and the outcome can be reported as valid.

Instillation of glyphosate TC into the rabbit eye elicited slight to moderate irritation in all animals. The mean scores were 1.00 for cornea opacity for all animals, 0.67, 0.67, 1.0 for iris effects, 1.00, 1.33, 2.00 for conjunctivae redness, and 0.33 for chemosis for all animals. These effects were fully reversible within 7 days. Thus, glyphosate is considered irritant to the eye.

Nevertheless, for classification purposes all eye irritation studies should be taken into account.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.5/003
Report author	[redacted]
Report year	2009
Report title	Acute Eye Irritation/Corrosion Test of Glyphosate TC in Rabbits
Report No	24878
Document No	Not reported
Guidelines followed in study	OECD 405 (2002); Commission Directive 2004/73/EC B.5 (2004), OPPTS 870.2400 (1998)
Deviations from current test guideline (OECD 405, 2017)	No treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application. This deviation is not considered to affect the study outcome.
Previous evaluation	Yes, accepted in RAR (2015)

GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid[#]
Category study in AIR 5 dossier (L docs)	Category 2a

#: Following the feedback from the Assessment Group Glyphosate in March 2020, the Notifier is aware of concerns regarding work conducted at this specific contract laboratory [redacted]. For the sake of completeness, this study is included in the dossier submission since it was part of the data package of previous EU evaluation processes.

2. Full summary

In an eye irritation study, 0.1 g of the undiluted test substance glyphosate TC (Batch: 2009051501, Purity: 96.4 %) was instilled into the right conjunctival sac of three young male adult Himalayan rabbits. Animals were observed for 8 days. Eye irritation was scored using the Draize scheme 1, 24, 48 and 72-hours and 4, 5, 6, 7 and 8 days after test item instillation. Application of glyphosate TC into the rabbit eye resulted in slight and fully reversible ocular changes, not exceeding Grade 1. All eye effects were reversible within 8 days after instillation. No signs of corrosion or staining were observed in any eye. The individual mean irritation scores (24 – 72-hours) of the three rabbits were as follows:

- for corneal opacity: 1.00, 1.00, 1.00
- for iris lesions: 1.00, 0.67, 0.33
- for conjunctival redness: 1.00, 1.00, 1.00
- for chemosis of the conjunctiva: 0.67, 0.33, 0.00

Based on the study, glyphosate TC has the potential for irritating effects to the eye under the test conditions chosen.

Materials and methods

A: Materials

1. Test material:

Identification:	Glyphosate TC
Description:	White solid powder
Lot/Batch #:	2009051501
Purity:	96.4 %
Stability of test compound:	Expiry date: 15 May 2011

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rabbit
Strain:	Himalayan
Source:	[redacted]
Age:	Approximately 6.5 – 7.5 months
Sex:	Males
Weight at dosing:	2.5 – 2.8 kg
Acclimation period:	At least 20 days

Diet/Food:	ssniff® K-H V2333 (ssniff Spezialdiäten GmbH, Soest, Germany), *ad libitum* before and after the exposure period	
Water:	Tap water, *ad libitum*	
Housing:	Individual housing during acclimatisation period and for study period starting 8 hours after test item instillation. At test item instillation animals were kept in restrainers for 8 hours to prevent wiping of the eyes and exposure to urine or excrements.	
Environmental conditions:	Temperature:	20 ± 3 °C
	Humidity:	30 – 70 %
	Air changes:	Not reported
	Photoperiod:	12 hours light / dark cycle

B: Study design and methods

In life dates: 2009-10-15 to 2009-10-29

Animal assignment and treatment:

The test was conducted using three young male adult Himalayan albino rabbits. The test was performed in a sequential manner, first using one animal. Since no corrosive or severe eye effects were observed in the first animal the test was completed using the remaining two rabbits. An amount of 0.1 g of the solid test substance was applied into the conjunctival sac of the right eye of the rabbits. The lids were then gently held together for about one second. One hour after instillation the eyes were rinsed with 20 mL saline solution. The left eye remained untreated and served as the reference control. Eye reactions were assessed according to the scoring system listed in Commission Directive 2004/73/EC approximately 1, 24, 48 and 72-hours, as well as 4 – 8 days after instillation. Twenty-four hours and seven days after administration, fluorescein was applied to the eyes before being examined to aid evaluation of the cornea for possible lesions. Additionally, behaviour and food consumption were monitored. Body weights were determined at beginning of the study and at termination.

Numerical scoring system (Draize scale for scoring ocular irritation)

Cornea	
Opacity: degree of density (readings should be taken from most dense area)*	
No ulceration or opacity	0
Scattered or diffuse areas of opacity (other than slight dulling of normal lustre), details of iris clearly visible	1
Easily discernible translucent areas, details of iris slightly obscured	2
Nacreous areas, no details of iris visible, size of pupil barely discernible	3
Opaque cornea; iris not discernible through the opacity	4
Iris	
Normal	0
Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperaemia, or injection, iris reactive to light (a sluggish reaction is considered to be an effect)	1
Haemorrhage, gross destruction, or no reaction to light	2
Conjunctivae, Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)	
Normal	0
Some blood vessels hyperaemic (injected)	1
Diffuse, crimson colour; individual vessels not easily discernible	2
Diffuse, beefy red	3
Conjunctivae, Chemosis (lids and/or nictitating membranes)	
Normal	0
Some swelling above normal	1

Numerical scoring system (Draize scale for scoring ocular irritation)

Obvious swelling with partial eversion of lids	2
Swelling with lids about half-closed	3
Swelling with lids more than half-closed	4

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed during the study.

C. BODY WEIGHT

No adverse effects on body weight were noted.

D. NECROPSY

No necropsy was performed.

E. EYE OBSERVATIONS

Corneal opacity (grade 1) was observed in all animals 24 – 72-hours, in animal no. 2 until 4 days and in animal no. 1 until 7 days after instillation. The fluorescein test demonstrated corneal staining in all animals when performed 24-hours after instillation and only in animal no. 1 when performed 7 days after instillation. The fluorescein test performed 24-hours after instillation revealed corneal staining in animal nos. 1 and 2 (½ – ¾ of the surface) and in animal no. 3 (¼ – ½ of the surface). The fluorescein test performed 7 days after instillation revealed staining in animal no. 1 (up to ¼ of the surface).
Irritation of the iris (grade 1) was observed in all animals at the 24-hour observation, in animal no. 2 until 48 hours and in animal no. 1 until 72-hours after test item instillation.

Conjunctival redness (grade 1) was noticed in all animals in the observation period from 1 hour to 72-hours as well as in animal no. 1 until 4 days and in animal no. 2 until 5 days after instillation.
Chemosis (grade 1) was observed in all animals at 1 hour, in animal no. 2 until 24-hours, and in animal no. 1 until 48 hours after instillation.

In addition, secretion was observed in all animals 1 hour after instillation. There were no systemic reactions. All rabbits were free of ocular signs by Day 8 after instillation.

The individual mean irritation scores (24 – 72-hours) were calculated to be 1.00 for corneal opacity and for conjunctiva redness for all three animals, 1.00, 0.67 and 0.33 for iris lesions, and 0.67, 0.33 and 0.00 for chemosis of the conjunctiva (see table below).

Table 5.2.5-4 Acute Eye Irritation/Corrosion Test of Glyphosate TC in Rabbits. ([redacted] 2009): Eye irritation in rabbits – Individual irritation scores

Animal	Scoring [h]	Cornea opacity	Iris	Conjunctiva	
				Redness	Chemosis
Rabbit 1 (male)	1	0	0	1	1[a]
	24	**1**	**1**	**1**	**1**
	48	**1**	**1**	**1**	**1**

Table 5.2.5-4 Acute Eye Irritation/Corrosion Test of Glyphosate TC in Rabbits. ([redacted] 2009): Eye irritation in rabbits – Individual irritation scores

	72	**1**	**1**	**1**	**0**
	Day 4	1	0	1	0
	Day 5	1	0	0	0
	Day 6	1	0	0	0
	Day 7	1	0	0	0
	Day 8	0	0	0	0
Individual mean (24, 48, 72 h)		**1.00**	**1.0**	**1.00**	**0.67**
Rabbit 2 (male)	1	0	0	1	1[a]
	24	**1**	**1**	**1**	**1**
	48	**1**	**1**	**1**	**0**
	72	**1**	**0**	**1**	**0**
	Day 4	1	0	1	0
	Day 5	0	0	1	0
	Day 6	0	0	0	0
	Day 7	—	—	—	—
	Day 8	—	—	—	—
Individual mean (24, 48, 72 h)		**1.00**	**0.67**	**1.00**	**0.33**
Rabbit 3 (male)	1	0	0	1	1[a]
	24	**1**	**1**	**1**	**0**
	48	**1**	**0**	**1**	**0**
	72	**1**	**0**	**1**	**0**
	Day 4	0	0	0	0
	Day 5	—	—	—	—
	Day 6	—	—	—	—
	Day 7	—	—	—	—
	Day 8	—	—	—	—
Individual mean (24, 48, 72 h)		**1.00**	**0.33**	**1.00**	**0.00**

[a] secretion

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study is in concordance with the current OECD 405 (2017). Therefore, the study is considered acceptable and the outcome can be reported as valid.

A single instillation of glyphosate into the rabbit eye revealed slight and fully reversible ocular changes (≤ grade 1); all eye effects were reversible within 8 days after instillation.

It is concluded that the test item is 'irritant' to the eyes of Himalayan rabbits based on the individual mean scores over 24, 48 and 72 h, which were 1.00 for all animals for the endpoints corneal opacity and conjunctival redness, 0.33, 0.67, 1.00 for iris lesions and 0.00, 0.33, 0.67 for chemosis. Nevertheless, for classification purposes the serious eye damage observed in another study should be taken into account.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.5/004
Report author	[redacted]
Report year	2009
Report title	Acute Eye Irritation/Corrosion Test of Glyphosate TC in Rabbits

Report No	23914
Document No	NOT REPORTED
Guidelines followed in study	OECD 405 (2002), US EPA OPPTS 870.2400 (1998), EC method B.5 (2004/73/EC)
Deviations from current test guideline (OECD 405, 2017)	No treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application. This deviation is not considered to affect the study outcome.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid[#]
Category study in AIR 5 dossier (L docs)	Category 2a

#: Following the feedback from the Assessment Group Glyphosate in March 2020, the Notifier is aware of concerns regarding work conducted at this specific contract laboratory [redacted]. For the sake of completeness, this study is included in the dossier submission since it was part of the data package of previous EU evaluation processes.

2. Full summary

Under the present test conditions, a single instillation of 0.1 g glyphosate TC (Batch: 20080801, Purity: 98.8 %) per animal into the conjunctival sac of the right eye of three rabbits caused the following changes:

Corneal opacity (grade 1) was observed in animal no. 1 (24 – 72-hours) and in animal no. 3 (24 and 48 hours) after instillation. The fluorescein test performed 24-hours after instillation revealed corneal staining in animal no. 1 and 3 (up to 1/4 of the surface).

Conjunctival redness (grade 1) was observed in all animals 60 minutes – 48 hours, in animal no. 1 until 72-hours after instillation.

Chemosis (grade 1) was observed in animal no. 1 (24 and 48 hours) after instillation. In addition, secretion was observed in all animals 60 minutes after instillation. The irises were not affected by instillation of the test item.

There were no systemic reactions. All animals were free of signs of ocular irritation by Day 4.

The individual mean irritation scores (24 – 72-hours) of the three rabbits were as follows:

- for corneal opacity: 1.00, 0,00, 0.67
- for iris lesions: 0.00, 0.00, 0.00
- for conjunctival redness: 1.00, 0.67, 0.67
- for conjunctival chemosis: 0.67, 0.00, 0.00

Based on the study, glyphosate TC was not irritating to the eyes under the test conditions chosen.

Materials and methods

A: Materials

1. Test material:

Identification:	Glyphosate TC
Description:	White solid powder
Lot/Batch #:	20080801
Purity:	98.8 %
Stability of test compound:	Expiry date: 2010-08-01

2. Vehicle and/or positive control: No vehicle used

3. Test animals:

Species:	Rabbit
Strain:	Himalayan
Source:	[redacted]
Age:	Approximately 4 – 32.5 months at dosing
Sex:	Males
Weight at dosing:	3.9 – 4.1 kg
Acclimation period:	At least 20 days
Diet/Food:	ssniff® K-H V2333 (ssniff Spezialdiäten GmbH, Soest, Germany), *ad libitum* before and after the exposure period
Water:	Tap water, *ad libitum*
Housing:	Individual housing during acclimatisation period and for study period starting 8 hours after test item instillation. After test item instillation, animals were kept in restrainers for 8 hours to prevent wiping of the eyes and exposure to urine or excrements.
Environmental conditions:	Temperature: 20 ± 3 °C Humidity: 30 – 70 % Air changes: Not reported Photoperiod: 12-hour light / dark cycle

B: Study design and methods

In life dates: 2009-02-04 to 2009-02-15 (Start of treatment to study completion)

Animal assignment and treatment:
A quantity of 0.1 g of the test item was administered into the right eye each of three animals. The test item was placed into the conjunctival sac of the right eye of each animal after gently pulling the lower lid away from the eyeball. The lids were then gently held together for about one second in order to prevent loss of the material. The left eye, which remained untreated, served as a control. The test was performed initially using one animal. As no corrosive or severe irritant effects were observed in this animal, two further animals were employed 24-hours after start of the initial test.

One hour after instillation the eyes were rinsed with 20 mL sodium chloride solution. The eyes were examined ophthalmoscopically with a slit lamp prior to the administration and l, 24, 48, 72-hours and 4

days after the administration. The eye reactions were observed and registered. 24-hours after administration, fluorescein was applied to the eyes before being examined to aid evaluation of the cornea for possible lesions.

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed during the study.

C. BODY WEIGHT

All rabbits showed the expected body weight gain.

D. NECROPSY

No necropsy was performed.

E. EYE OBSERVATIONS

A single instillation of 0.1 g test item per animal into the conjunctival sac of the right eye of three rabbits caused the following changes:

Corneal opacity (grade 1) was observed in animal no. 1 (24 – 72) hours and in animal no. 3 (24 and 48 hours) after instillation. The fluorescein test performed 24-hours after instillation revealed corneal staining in animal no. 1 and 3 (up to 1/4 of the surface).

Conjunctival redness (grade 1) was observed in all animals 60 – 48 hours, in animal no. 1 until 72-hours after instillation.

Chemosis (grade 1) was observed in animal no. 1 (24 and 48 hours) after instillation.

In addition, secretion was observed in all animals 60 minutes after instillation.

The irises were not affected by instillation of the test item and there were no systemic reactions.

All animals were free of signs of ocular irritation by Day 4.

The individual mean irritation scores (24 – 72-hours) of the three rabbits were 1.00, 0,00, 0.67 for corneal opacity, 0.00 for all animals for iris lesions, 1.00, 0.67, 0.67 for conjunctival redness and 0.67, 0.00, 0.00 for conjunctival chemosis.

Table 5.2.5-5 Acute Eye Irritation/Corrosion Test of Glyphosate TC in Rabbits. ([redacted] 2009): Eye irritation with eye irrigation 1 hour after application – Individual irritation scores

Animal	Scoring [h]	Cornea opacity	Iris	Conjunctiva	
				Redness	Chemosis
Rabbit 1	1	0	0	1[a]	0
	24	**1**	**0**	**1**	**1**
	48	**1**	**0**	**1**	**1**
	72	**1**	**0**	**1**	**0**
	Day 4	0	0	0	0
Individual mean (24, 48, 72 h)		**1.00**	**0.00**	**1.00**	**0.67**
Rabbit 2	1	0	0	1[a]	0
	24	**0**	**0**	**1**	**0**
	48	**0**	**0**	**1**	**0**
	72	**0**	**0**	**0**	**0**
	Day 4	--	--	--	--
Individual mean (24, 48, 72 h)		**0.00**	**0.00**	**0.67**	**0.00**
Rabbit 3	1	0	0	1[a]	0
	24	**1**	**0**	**1**	**0**
	48	**1**	**0**	**1**	**0**
	72	**0**	**0**	**0**	**0**
	Day 4	--	--	--	--
Individual mean (24, 48, 72 h)		**0.67**	**0.00**	**0.67**	**0.00**

[a] = secretion; -- = no examination

3. Assessment and conclusion

Assessment and conclusion by applicant:

Except the deviation of no treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application, the study follows the current OECD TG 405 (2017). Therefore, the study is considered acceptable and the outcome can be reported as valid.

Instillation of glyphosate TC into the rabbit eye elicited slight response in treated animals. The individual mean scores over 24, 48 and 72-hours for the three animals were 1.00, 0,00, 0.67 for corneal opacity, 0.0 for all animals for iris lesions, 1.00, 0.67, 0.67 for conjunctival redness, and 0.67, 0.00, 0.00 for conjunctival chemosis.

Thus, under test conditions of the study, glyphosate technical is considered not irritating to the eye mucosa of rabbits.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.5/005
Report author	[redacted]
Report year	2009
Report title	Expert Statement Glyphosate technical: Primary eye irritation study in rabbits
Report No	C22897
Document No	Not reported
Guidelines followed in study	OECD 405 (2002) Council Regulation (EC) No 440/2008 (2008)
Deviations from current test guideline (OECD 405, 2017)	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities[1,2]	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

A pH measurement was performed with the test item in a 1 % (w/w) solution in purified water before the study initiation. The pH of the test item was found to be 1.93.

According to the OECD Guidelines 405 and Council Regulation (EC) No 440/2008 B.5:
Physicochemical properties and chemical reactivity – Substances exhibiting pH extremes such as ≤2.0 may have strong local effects. If extreme pH is the basis for identifying a substance as corrosive or irritant to the eye, then its acid reserve (buffering capacity) may also be taken into consideration.

It is assumed that the test substance item has corrosive properties; therefore, no eye irritation study in rabbits with glyphosate technical was performed.

3. Assessment and conclusion

Assessment and conclusion by applicant:
Based on the extreme pH, the test item requires classification as Eye damaging, Category 1, H318 (Causes serious eye damage) according to the criteria laid down in the CLP Regulation (EC No. 1272/2008).

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.5/006
Report author	[redacted]
Report year	2009
Report title	Glyphosate – Acute Eye Irritation Study in Rabbits
Report No	12172-08
Document No	Not reported
Guidelines followed in study	US EPA OPPTS 870.2400, Equivalent to OECD 405 (2002)
Deviations from current test guideline (OECD 405, 2017)	No treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application. Temperature and humidity were outside the recommended range (16 – 23 °C instead of 20 ± 3 °C and 33 – 92 % instead of 30 – 70 %). All animals were treated at once instead of stepwise, as requested by the current OECD guideline 405 (2017). Systemic adverse effects and record of clinical signs were not performed. These deviations did not affect the study outcome.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

An acute eye irritation study was conducted on three albino rabbits using test substance glyphosate (Batch: 080704-1 thru 5, Purity: 96.4 %). The test substance, 0.1 mL by volume (93.2 mg), was placed into the conjunctival sac of the right eye of each animal. All treated eyes were washed with water after recording the 24-hour observation. Eye irritation was scored 1, 24, 48 and 72-hours and 4, 7, 10, 14 and 17 days after test item instillation. No further observations on clinical signs were recorded.

Grades for opacity were ranging from 0 (after 1 hour) to grade 1 or 2 from 24-hour to Day 4, returning to score 0 at Day 7 and 10 for two animals, respectively. One animal did not show any effect on opacity. The iris grading was 1 between Day 1 to 4 in only one animal and did not show any effect in the remaining two rabbits. Conjunctival redness was noted one hour after removal of test item (score 1) in all three animals. An increase to grade 2 was observed in two animals at 24-hours, which decreased to grade 1 at Day 4 and Day 7, respectively. Clearance of redness (score 0) was observed after 72-hours (one animal) and at Day 17 (two animals). For chemosis, the highest grade observed was 3 for one animal between Days 1 and 3. In the other two animals the effect was not as pronounced and fully reversible in all animals latest at Day 17. Discharge was also observed with the highest grade 3 on Day 1 but was also fully reversible in all animals latest at Day 7. No necrosis or ulceration was seen in any of the treated animals. All effects observed in any eye were fully reversible within 7 days.

The individual mean irritation scores (24 – 72-hours) of the three rabbits were as follows:

- for corneal opacity: 1.00, 0.00, 2.00
- for iris lesions: 0.00, 0.00, 1.00
- for conjunctival redness: 2.00, 0.67, 2.00
- for chemosis of the conjunctiva: 1.67, 0.00, 3.00

Based on the study, glyphosate TC has irritating properties onto the eye under the test conditions chosen.

Materials and methods

A: Materials

1. Test material:

Identification:	Glyphosate Tech Grade Mixed 5-Batch
Description:	White powder
Lot/Batch #:	080704-1 thru 5
Purity:	96.4 % (2008-10-17); 96.71 % (2009-01-08)
Stability of test compound:	No data given in the report

2. Vehicle and/or positive control: No vehicle was used

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Approximately 3 months
Sex:	Males (2) and female (1; nulliparous and non-pregnant)
Weight at dosing:	Males: 2.2 – 2.4 kg; female: 2.3 kg
Acclimation period:	5 days
Diet/Food:	PMI Feeds, Inc.™ Lab Rabbit Diet #5321, 8 oz. daily
Water:	Tap water, *ad libitum*
Housing:	Individual housing in suspended, wire bottom, stainless steel cages
Environmental conditions:	Temperature: 16 – 23 °C *
	Humidity: 33 – 92 % *
	Air changes: 10 – 12 / hour
	Photoperiod: 12-hour light / dark cycle

* Temperature and humidity were outside of protocol range, but did not affect study outcome.

B: Study design and methods

In life dates: 2008-11-08 to 2008-11-27 (Start of treatment to study completion)

Animal assignment and treatment:
Healthy albino rabbits were released from quarantine and both eyes of each animal were carefully examined within 24-hours prior to treatment with a fluorescein sodium ophthalmic solution and cobalt-filtered light. Both eyes of each animal were again carefully examined just prior to treatment, but without the fluorescein sodium ophthalmic solution. Only those animals without eye defects or irritation were selected for testing. On Day 0, a dose of 0.1 mL by volume (93.2 mg) of the undiluted test substance was placed into the conjunctival sac of the right eye of each animal by gently pulling the lower lid away from the eyeball to form a cup into which the test substance was dropped. The lids were gently held together for one second to prevent loss of material. The untreated left eyes served as comparative controls. The grades of ocular reaction were recorded at 1, 24, 48 and 72-hours, and at 4, 7, 10, 14 and 17 days after treatment. The corneas

of all treated eyes were examined immediately after the 24-hour observation with a fluorescein sodium ophthalmic solution. All treated eyes were washed with room temperature deionized water for one minute immediately after recording the 24-hour observation.

Results

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

Clinical signs were not recorded.

C. BODY WEIGHT

All animals gained weight over the duration of the study.

D. NECROPSY

No gross abnormalities were recorded at necropsy.

E. EYE OBSERVATIONS

Corneal opacity scores ranged from 0 (after 1 hour) to grade 1 (one animal) or grade 2 (one animal) from 24-hour to Day 4 reading time points, returning to score 0 at Day 7 and 10 for these two animals, respectively. One animal did not show any effect on opacity.

The iris grading was 1 between Day 1 – 4 in only one animal and did not show any effect in the remaining two rabbits.

Conjunctival redness was noted one hour after removal of test item (score 1) in all three animals. An increase to grade 2 was observed in two animals at 24-hours, which decreased to grade 1 at Day 4 and Day 7, respectively. Clearance of redness (score 0) was observed after 72-hours (one animal) and at Day 17 (two animals).

For chemosis, the highest grade observed was 3 for one animal between Days 1 and 3. In the other two animals the effect was not as pronounced and fully reversible in all animals latest at Day 17. Discharge was also observed, with the highest grade 3 on Day 1 but was also fully reversible in all animals latest at Day 7.

Fluorescein staining was observed in two of three eyes at 24-hours after treatment and was not observed in any eyes on Day 10 after treatment. No necrosis or ulceration was seen in any of the treated animals.

All effects observed in any eye were fully reversible within 17 days.

The individual mean irritation scores (24, 48 and 72-hours) of the three rabbits were 1.00, 0.00 and 2.00 for corneal opacity, 0.00, 0.00, 1.00 for iris lesions, 2.00, 0.67, 2.00 for conjunctival redness and 1.67, 0.00, 3.00 for chemosis.

Table 5.2.5-6 Glyphosate – Acute Eye Irritation Study in Rabbits. ([redacted] 2009): Eye irritation – Individual irritation scores

Animal	Scoring [h]	Cornea		Iris	Conjunctiva		
		Opacity	Area		Redness	Chemosis	Discharge
Rabbit 3144 (male)	1	0	0	0	1	1	2
	24	**1**	4	**0**	**2**	**2**	2
	48	**1**	4	**0**	**2**	**2**	2
	72	**1**	4	**0**	**2**	**1**	1
	Day 4	1	4	0	1	1	1
	Day 7	1	4	0	1	1	0
	Day 10	0	0	0	1	1	0
	Day 14	0	0	0	1	1	0
	Day 17	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**1.00**	**---**	**0.00**	**2.00**	**1.67**	**---**
Rabbit 3202 (male)	1	0	0	0	1	1	1
	24	**0**	0	**0**	**1**	**0**	0
	48	**0**	0	**0**	**1**	**0**	0
	72	**0**	0	**0**	**0**	**0**	0
	Day 4	0	0	0	0	0	0
	Day 7	0	0	0	0	0	0
	Day 10	0	0	0	0	0	0
	Day 14	0	0	0	0	0	0
	Day 17	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.00**	**---**	**0.00**	**0.67**	**0.00**	**---**
Rabbit 3201 (female)	1	0	0	0	1	2	2
	24	**2**	4	**1**	**2**	**3**	3
	48	**2**	4	**1**	**2**	**3**	2
	72	**2**	2	**1**	**2**	**3**	1
	Day 4	2	2	1	2	2	1
	Day 7	0	0	0	1	0	0
	Day 10	0	0	0	1	0	0
	Day 14	0	0	0	1	0	0
	Day 17	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**2.00**	**---**	**1.00**	**2.00**	**3.00**	**---**

3. Assessment and conclusion

Assessment and conclusion by applicant:

Except the deviation of no treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application the study is in concordance with the current OECD TG 405 (2017). This deviation did not compromise the acceptability of the study. Therefore, the study is considered acceptable and the outcome can be reported as valid.

Instillation of glyphosate TC into the rabbit eye elicited positive response in all animals. The mean scores were 1.00, 0.00 and 2.00 for corneal opacity, 0.00, 0.00 and 1.00 for iris lesions, 2.00, 0.67 and 2.00 for conjunctival redness and 1.67, 0.00 and 3.00 for chemosis. These effects were fully reversible within 17 days. Thus, glyphosate is considered irritant to the eye.

Nevertheless, for classification purposes all eye irritation studies should be taken into account.

Assessment and conclusion by RMS:

RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.5/007
Report author	[redacted]
Report year	2008
Report title	Acute Eye Irritation/Corrosion Study in Rabbits with Glyphosate Technical
Report No	[redacted]-3996.312.599.07
Document No	Not reported
Guidelines followed in study	OECD 405 (2002)
Deviations from current test guideline (OECD 405, 2017)	No treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application. This deviation is not considered to affect the study outcome.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The acute eye irritation / corrosion potential of glyphosate technical (Batch: 20070606, Purity: 98.05 %) was evaluated in New Zealand White rabbits. The test was initially planned for three animals but conducted using one rabbit first. Due to severe ocular reactions observed in the initial test, only one additional animal was tested to confirm the response. One tenth (0.1) g of the undiluted solid test item was instilled into the left conjunctival sac of the eye of each animal. After application, both animals were examined at approximately 1, 24, 48, 72-hours and 7 and 14 days, and one of the animals was also examined at approximately 21 days to verify the presence of lesions in the cornea, iris, eyelid and eyeball conjunctivae, and for behavioural and clinical alterations. The untreated right eye was used as a negative control.

The test item applied in the eye of the rabbits produced corneal opacity, iritis (circumcorneal injection), conjunctival hyperaemia, oedema and secretion in both tested eyes. Corneal opacity and conjunctival hyperaemia were still noted at the end of the observation period in one of two tested eyes. All irritation signs had returned to normal by the 14-day time point following treatment to one of two tested eyes. Fluorescein sodium dye detected treatment-related changes of the surface of the cornea in both tested eyes. Additional ocular changes observed included: blepharitis and a small raised-off area on the corneal surface.

The individual mean irritation scores (24, 48 and 72-hours) of the two rabbits were as follows:

- for corneal opacity: 3.33 and 3.67
- for iris lesions: 1.00 and 1.00
- for conjunctival redness: 3.00 and 2.67
- for chemosis of the conjunctiva: 2.00 and 1.33

Based on the study, glyphosate TC has the potential to seriously damage the eyes under the test conditions chosen.

Materials and methods

A. Materials

1. Test material:

Identification:	Glyphosate Technical
Description:	Solid
Lot/Batch #:	20070606
Purity:	98.05 %
pH:	2.20 (at 1 %, 23.1 °C)
Stability of test compound:	No data given in the report.

2. Vehicle and/or positive control: No vehicle was used

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	18 weeks old
Sex:	Male and female (nulliparous and non-pregnant)
Weight at dosing:	Male: 3.346; female: 3.624 kg
Acclimation period:	5 – 6 days
Diet/Food:	Pelleted and autoclaved commercial diet for rabbits (Guabi, Mogiana Alimentos S.A. - Brazil), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in galvanised steel cages. Autoclaved wood shavings were placed in a tray below the cages to collect excrements.
Environmental conditions: Temperature:	17 – 22 °C
Humidity:	30 – 70 %
Air changes:	10 – 15 / hour
Photoperiod:	12-hour light / dark cycle

B: Study design and methods

In life dates: 2008-05-26 to 2008-06-17

Animal assignment and treatment:

On tenth (0.1) g of the test item was applied to the eye of each animal. The test item was applied into the conjunctival sac of the left eye of each animal after gently pulling the lower lid away from the eyeball. Following application, the eyelids were gently held together for about one second in order to prevent test item loss. The right eye remained untreated and was used as a negative control.

The test was performed initially using one animal for evaluation of any irritant / corrosive effect of the test item to the eye. Because some severe ocular reactions were observed in the initial test, only one additional animal was tested to confirm the response.

The animals' eyes were grossly examined with the aid of an auxiliary light source for signs of irritation in

the cornea, iris, eyelid and eyeball conjunctivae at approximately 1, 24, 48, 72-hours and 7 and 14 days after test item application to the first rabbit, and at approximately 1, 24, 48, 72-hours and 7, 14 and 21 days after test item application to the second rabbit. The grading of the ocular reactions was done according to the Draize scheme. After recording the observations at 24-hour time point for each animal, the corneal surface on all test and control eyes was examined using a fluorescein sodium dye to detect abnormalities which are not grossly observable. The fluorescein examinations were conducted to each subsequent interval until two negative responses were obtained. In addition, a clinical examination was accomplished to verify the presence or absence of any local or systemic toxic effects.

Results

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

No data on clinical signs reported.

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance.

D. NECROPSY

No data on necropsy were reported.

E. EYE OBSERVATIONS

Corneal opacity grade 1 – 4, with affected area varying from 1 – 3, was noted at all observation time points and persisted until the end of the observation period for rabbit #05.

Iritis grade 1 (circumcorneal injection) was noted from the 1 – 72-hour time points for both tested rabbits.

Conjunctival effects included: hyperaemia grade 1 – 3, from the 1-hour to the 7-day time points for rabbit #04 and persisted until day 21 in rabbit #05.

Edema grade of 1 – 3 were observed from the 1-hour to the 14-day time points.
For secretion, grades 1 – 2 were noted until the 72-hour time point for one rabbit and until the 14-day time point for the second rabbit.

All eye irritation signs had returned to normal by the 14-day time point for rabbit #04.

Fluorescein sodium dye detected treatment-related changes to the surface of the cornea at the 24 – 72-hour time points for rabbit #05, and at the 24-hour time point for rabbit #04. Blepharitis was observed in the initial treated animal at the 48- and 72-hour time points and in the subsequently treated animal at the 24-, 48- and 72-hour and 7- and 14-day time points. In the second animal a small raised off area on the corneal surface in the right inferior quadrant at the 21-day time point was noted.

Individual mean scores over 24, 48 and 72-hours for each animal were 3.33 and 3.67 for corneal opacity, 1.00 and 1.00 for iris lesions, 1.33 and 2.00 for chemosis as well as 3.00 and 2.67 for redness of the conjunctiva.

Table 5.2.5-7 Acute Eye Irritation/Corrosion Study in Rabbits with Glyphosate Technical. ([redacted] 2008): Eye irritation – Individual irritation scores

Animal	Scoring [h]	Cornea		Iris	Conjunctiva		
		Opacity	Area		Hyperaemia	Oedema	Secretion
Rabbit 04 (male)	1	1	2	1*	2	3	2
	24	**3**	2	**1***	**3**	**3**	2
	48	**3**	2	**1***	**3**	**2**	2
	72	**4**	1	**1***	**3**	**1**	1
	Day 7	0	0	0	1	1	0
	Day 14	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**3.33**	**---**	**1.00**	**3.00**	**2.00**	**---**
Rabbit 05 (female)	1	3	2	1*	2	1	2
	24	**4**	2	**1***	**2**	**2**	2
	48	**3**	3	**1***	**3**	**1**	2
	72	**4**	3	**1***	**3**	**1**	2
	Day 7	4	2	0	2	1	2
	Day 14	4	2	0	2	1	1
	Day 21	4	1	0	1	0	0
Individual mean (24, 48, 72 h)		**3.67**	**---**	**1.00**	**2.67**	**1.33**	**---**

* Circumcorneal injection

3. Assessment and conclusion

Assessment and conclusion by applicant:

Except the deviation of no treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application the study is in concordance with the current OECD TG 405 (2017). This deviation did not compromise the acceptability of the study. Therefore, the study is considered acceptable and the outcome can be reported as valid.

Instillation of glyphosate technical into the rabbit eye elicited strong response in treated animals. The individual mean scores over 24, 48 and 72-hours for each animal were 3.33 and 3.67 for corneal opacity, 1.00 and 1.00 for iris lesions, 1.33 and 2.00 for chemosis, as well as 3.00 and 2.67 for redness of the conjunctiva. These effects were not reversible within 21 days.

Thus, glyphosate is considered to have the potential to seriously damage the eye.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.5/008
Report author	[redacted]
Report year	2007
Report title	Glyphosate Technical Material: Primary Eye Irritation Study in Rabbits
Report No	B02788
Document No	Not reported
Guidelines followed in study	OECD 405 (2002), US EPA OPPTS 870.2400 (1998), 2004/73/EC B.5 (2004), JMAFF 12 NohSan No. 8147 (2000)

Deviations from current test guideline (OECD 405, 2017)	No treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application, animals were previously used in a primary skin irritation study. This deviation is not considered to affect the study outcome.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

In a primary eye irritation study, 0.1 g of glyphosate technical (Batch: 0507, Purity: 96.1 %) was instilled into the conjunctival sac of the left eye of each of three young adult New Zealand albino rabbits (one male and two females). The animals were observed for seven days. The ocular reaction was assessed according to the numerical scoring system listed in the Commission Directive 2004/73/EC, 29 April 2004, at approximately 1, 24, 48 and 72-hours as well as 7 days after instillation.

The instillation of glyphosate technical into the eye resulted in mild, early-onset and transient ocular changes, such as reddening of the conjunctivae and sclera, discharge and chemosis. These effects were reversible and were no longer evident seven days after treatment. No abnormal findings were observed in the cornea or iris of any animal at any of the examinations. No corrosion was observed at any of the measuring intervals. No staining of the treated eyes by the test item was observed and no clinical signs were observed.

The individual mean irritation scores (24 – 72-hours) of the three rabbits were as follows:

- for corneal opacity: 0.00, 0.00, 00.0
- for iris lesions: 0.00, 0.00, 0.00
- for conjunctival redness: 0.67, 1.67, 1.67
- for conjunctival chemosis: 0.00, 0.33, 1.00

Based on the study, glyphosate technical was not irritating to the eyes under the test conditions chosen.

Materials and methods

A: Materials

1. Test material:

Identification:	Glyphosate Technical Material
Description:	White powder
Lot/Batch number:	0507
Purity:	96.1 % w/w glyphosate acid
Stability of test compound:	Stable under storage conditions at room temperature (range of 20 ± 5 °C), protected from light and humidity.

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White (SPF)
Source:	[redacted]
Age:	Male: 11 – 12 weeks: females: 14 – 16 weeks
Sex:	Male and females

Weight at Dosing:	Male: 2640 g; females: 2990 – 3001 g	
Acclimation period:	5/6 days (the animals were previously used in the Primary Skin Irritation Study [redacted] B02777)	
Diet:	Pelleted standard Provimi Kliba 3418 rabbit maintenance diet (Provimi Kliba AG, CH-Kaiseraugust, Switzerland), *ad libitum*	
Water:	Tap water, *ad libitum*	
Housing:	Individually in stainless steel cages equipped with feed hoppers and drinking water bowls. Woodblocks and haysticks provided for gnawing	
Environmental conditions:	Temperature:	17 – 23 °C
	Humidity:	30 – 70 %
	Air changes:	10 – 15 per hour
	Photoperiod:	12 hours light / dark cycle

B: Study design and methods

In-life dates: 2006-12-27 to 2007-01-04

Animal assignment and treatment:
On the day of treatment, 0.1 g of the test item was placed into the conjunctival sac of the left eye of each animal after gently pulling the lid away from the eyeball. The lids were then gently held together for about one second to prevent loss of the test substance. The right eye remained untreated and acted as the reference control. The treated eyes were not rinsed after instillation of the test substance.

As it was suspected that the test substance might produce irritancy, a single female was treated first. As neither a corrosive effect nor a severe irritant effect was observed after 1- and 24- hour examinations, the test was completed using the two remaining animals.

The ocular reaction (i.e. corneal opacity, iridic effects, conjunctivae and chemosis) was assessed according to the numerical scoring system listed in the Commission Directive 2004/73/EC, 29 April 2004, at approximately 1, 24, 48 and 72-hours, as well as 7 days after instillation. Additionally, ocular discharge, reddening of the sclera and staining of conjunctivae, sclera, and cornea by the test substance was assessed according to the scheme presented in the guideline.

The animals were observed daily throughout the study for viability, mortality and clinical signs. Body weights were measured at the start of acclimatisation, on the day of treatment and at termination of the observation period. No necropsy was performed at termination of the study.

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed in the animals during the study

C. BODY WEIGHT

The body weights of all rabbits were considered to be within the normal range of variability.

D. NECROPSY

Not reported.

E. EYE OBSERVATIONS

No abnormal findings were observed in the cornea or iris of any animal at any of the measurement intervals. Moderate reddening of the conjunctivae was noted in all animals at the 1-hour reading and persisted in one animal as slight until the 48-hour reading and in two animals as moderate to slight until 72-hours after treatment. Slight to obvious swelling (chemosis) with partial eversion of the lids was observed in all three animals at the 1-hour reading and persisted with the same severity in two animals at the 24-hours and in one animal as slight until the 48-hour reading. Slight to moderate reddening of the sclera was noted in all animals at the 1- and 24-hour reading and persisted as slight reddening in two animals until the 48-hour reading. Slight to moderate ocular discharge was seen in all animals at the 1-hour reading and persisted as slight to moderate discharge in two animals at the 24-hour reading. No abnormal findings were observed in the treated eye of any animal 7 days after treatment, the end of the observation period for all animals. No staining of the treated eyes produced by the test substance was observed and no corrosion of the cornea was observed at any of the reading times.

The individual mean irritation scores (24 – 72-hours) were calculated to be 0.00 for all animals for corneal opacity and iris lesions, 0.67, 1.67, 1.67 for conjunctival redness, and 0.00, 0.33, 1.00 for conjunctival chemosis. The individual scores for each time point, individual mean and group mean scores (24 – 72-hours) are presented in the table below.

Table 5.2.5-8 Glyphosate Technical Material: Primary Eye Irritation Study in Rabbits. ([redacted], 2007): Eye irritation – Individual irritation scores

Animal	Scoring [h]	Cornea		Iris	Conjunctiva			Sclera
		Opacity	Area		Redness	Chemosis	Discharge	
Rabbit 31 (male)	1	0	0	0	2	1	1	2
	24	**0**	0	**0**	**1**	**0**	0	1
	48	**0**	0	**0**	**1**	**0**	0	0
	72	**0**	0	**0**	**0**	**0**	0	0
	Day 7	0	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.00**	---	**0.00**	**0.67**	**0.00**	---	---
Rabbit 32 (female)	1	0	0	0	2	2	1	2
	24	**0**	0	**0**	**2**	**1**	1	2
	48	**0**	0	**0**	**2**	**0**	0	1
	72	**0**	0	**0**	**1**	**0**	0	0
	Day 7	0	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.00**	---	**0.00**	**1.67**	**0.33**	---	---
Rabbit 33 (female)	1	0	0	0	2	2	2	2
	24	**0**	0	**0**	**2**	**2**	2	2
	48	**0**	0	**0**	**2**	**1**	0	1
	72	**0**	0	**0**	**1**	**0**	0	0
	Day 7	0	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.00**	---	**0.00**	**1.67**	**1.0**	---	---

3. Assessment and conclusion

Assessment and conclusion by applicant:

Except the deviation of no treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application and the previously use of the animals in a skin irritation study, the study is in concordance with the current OECD TG 405 (2017). The deviations did not compromise the acceptability of the study. Therefore, the study is considered acceptable and the outcome can be reported as valid.

Instillation of glyphosate technical into the rabbit eye elicited slight response in treated animals. The individual mean scores over 24, 48 and 72-hours for each animal were 0.00 for all animals for corneal opacity and iris lesions, 0.67, 1.67, 1.67 for conjunctival redness, and 0.00, 0.33, 1.00 for conjunctival chemosis.

These effects were reversible within 7 days. Thus, under test conditions of the study, glyphosate technical is considered not irritating to the eye mucosa of rabbits.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.5/009
Report author	[redacted]
Report year	2007
Report title	Glyphosate Technical (NUP 05068): Primary Eye Irritation Study in Rabbits
Report No	B02305
Document No	Not reported
Guidelines followed in study	OECD 405 (2002), Commission Directive 2004/73/EC B.5 (2004), JMAFF guideline 2-1-5 (2005)
Deviations from current test guideline (OECD 405, 2017)	No treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application. Treated eyes were not rinsed after instillation.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

In an eye irritation study, 0.1 g of the undiluted, solid glyphosate technical (Batch: 200609062, Purity: 95.1 %) was instilled into the left conjunctival sac of three young adult New Zealand albino rabbits in a stepwise manner. Animals were observed for 14 days. Eye irritation was scored using the Draize scheme 1, 24, 48 and 72-hours and 7, 10, and 14 days after test item instillation. Application of glyphosate technical (NUP 05068) into the rabbit eye resulted in marked, early onset and transient ocular changes of very slight to slight corneal opacity, slight to marked conjunctival redness, conjunctival chemosis, reddening of the sclera, and discharge. All eye effects were reversible within 10 days after instillation. No abnormal findings were observed in the iris of any animal at any of the examinations. No signs of corrosion or staining were observed in any eye. The individual mean irritation scores (24 – 72-hours) of the three rabbits were as

follows:

- for corneal opacity: 1.67, 2.00 and 0.67;
- for iris lesions: 0.00, 0.00, 0.00
- for conjunctival redness: 2.67, 2.00, 2.00
- for chemosis of the conjunctiva: 2.00, 2.00, 1.00.

Based on the study, glyphosate TC has the potential for irritating the eye under the test conditions chosen.

I. MATERIAL AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate Technical (NUP 05068)
Description:	Solid
Lot/Batch #:	200609062
Purity:	95.1 %
Stability of test compound:	Stable under storage conditions (20 ± 5 °C), light protected; Expiry date: 2008-09-14

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White, SPF
Source:	[redacted]
Age:	Male: 15 weeks; females: 12 and 15 weeks
Sex:	Male and females
Weight at dosing:	Male: 2.969 kg; females: 2.605 and 3.416 kg
Acclimation period:	At least five days
Diet/Food:	Pelleted standard Provimi Kliba 3418 rabbit maintenance diet (Provimi Kliba AG, CH-Kaiseraugust, Switzerland), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in stainless steel cages with feed hoppers and drinking water bowls. Wood blooks and haysticks were provided for gnawing.
Environmental conditions:	Temperature: 17 – 23 °C Humidity: 30 – 70 % Air changes: 10 – 15 / hour Photoperiod: 12 hours light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2007-01-22 to 2007-02-26 (Start of treatment to study completion)

Animal assignment and treatment:
The test was conducted using young adult New Zealand albino rabbits (one male, two females). The test was performed in a sequential manner, first using one animal. Since no corrosive or severe eye effects were

observed in the first animal the test was completed using the remaining two rabbits. An amount of 0.1 g of the solid test substance was applied into the conjunctival sac of the left eye of the rabbits. The lids were then gently held together for about one second. The treated eyes were not rinsed after instillation. The right eye remained untreated and served as the reference control. Eye reactions were assessed according to the scoring system listed in Commission Directive 2004/73/EC approximately 1, 24, 48 and 72-hours, as well as 7, 10 and 14 days after instillation. Scleral reddening and ocular discharge were also assessed. Eye examinations were made using a diagnostic lamp. The animals were observed for mortality and clinical signs daily. Body weights were determined at beginning of acclimatisation, on the day of application and at termination.

Numerical scoring system (Draize scale for scoring ocular irritation):

Cornea	
Opacity: degree of density (readings should be taken from most dense area)*	
No ulceration or opacity	0
Scattered or diffuse areas of opacity (other than slight dulling of normal lustre), details of iris clearly visible	1
Easily discernible translucent areas, details of iris slightly obscured	2
Nacreous areas, no details of iris visible, size of pupil barely discernible	3
Opaque cornea; iris not discernible through the opacity	4
Area of cornea involved	
Zero	0
One quarter (or less) but not zero	1
Greater than one quarter, but less than half	2
Greater than half, but less than three quarters	3
Greater than three quarters, up to whole area	4
Iris	
Normal	0
Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperaemia or injection (any of these or combination or any thereof), iris still reacting to light (sluggish reaction is positive)	1
No reaction to light, haemorrhage, gross destruction (any or all of these)	2
Conjunctivae, Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)	
Blood vessels normal	0
Some blood vessels definitely hyperaemic (injected)	1
Diffuse, crimson colour, individual vessels not easily discernible	2
Diffuse, beefy red	3
Conjunctivae, Chemosis (lids and/or nictitating membranes)	
No swelling	0
Any swelling above normal (including nictitating membranes)	1
Obvious swelling with partial eversion of lids	2
Swelling with lids about half-closed	3
Swelling with lids more than half-closed	4
Discharge	
No discharge	0
Slight: Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)	1
Moderate: Discharge with moistening of the lids and hairs just adjacent to lids	2
Marked: Discharge with moistening of the lids and hairs and considerable area around the eye	3

* The area of corneal opacity should be noted

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed during the study.

C. BODY WEIGHT

All rabbits showed the expected body weight gain.

D. NECROPSY

No necropsy was performed.

E. EYE OBSERVATIONS

Very slight to slight corneal opacity was observed in all rabbits from 1 hour after instillation up to 72-hours. No signs of iritis, corrosion, or staining were observed in any animal throughout the study period. One hour after instillation slight to moderate conjunctival redness was observed in the treated eyes of all rabbits. By 24-hours the redness increased to marked in two animals and to moderate in one rabbit and were still persistent at the 48-hour evaluation time point. At 72-hours slight or moderate reddening of the conjunctivae was seen, and a slight reddening persisted in all three animals until Day 7. Moderate to marked chemosis of the conjunctivae was observed from 1 hour after instillation up to 24-hours. The swelling decreased with time. Seventy-two hours after treatment slight swelling was still present in two animals. Moderate ocular discharge was noted in two rabbits one hour after instillation and moderate or marked discharge in all animals at the 24-hour reading time point, which persisted at the 48-hour reading as slight or moderate in all rabbits. After 72-hour slight discharge was still present in one rabbit.

Reddening of the sclera was observed in all animals. However, one hour after instillation sclera of one animal was not assessable due to conjunctival swelling. In two animals moderate or marked reddening of the sclera was observed at this time point. After 24-hours all rabbits showed marked reddening of the sclera. This sign persisted in the rabbits as moderate or marked at the 48- and 72-hour readings. In one animal slight reddening was still present after 7 days.

All rabbits were free of ocular signs by Day 10 after instillation.

Individual mean scores calculated for the three animals over 24, 48 and 72-hours were 1.67, 2.00 and 0.67 for corneal opacity, 0.00, 0.00 and 0.00 for iris lesions, 2.67, 2.00 and 2.00 for redness of the conjunctiva, and 2.00, 2.00 and 1.0 for chemosis, respectively.

Table 5.2.5-9 Glyphosate Technical (NUP 05068): Primary Eye Irritation Study in Rabbits. ([redacted] 2007): Eye irritation in rabbits – Individual irritation scores

Animal	Scoring [h]	Cornea		Iris	Conjunctiva			Sclera
		Opacity	Area		Redness	Chemosis	Discharge	
Rabbit 10 (male)	1	1	1	0	2	3	2	3
	24	**2**	2	**0**	**3**	**3**	2	3
	48	**2**	2	**0**	**3**	**2**	1	3
	72	**1**	1	**0**	**2**	**1**	0	2
	Day 7	0	0	0	1	0	0	1

Table 5.2.5-9 Glyphosate Technical (NUP 05068): Primary Eye Irritation Study in Rabbits. ([redacted] 2007): Eye irritation in rabbits – Individual irritation scores

Animal	Scoring [h]	Cornea		Iris	Conjunctiva			Sclera
		Opacity	Area		Redness	Chemosis	Discharge	
	Day 10	0	0	0	0	0	0	0
	Day 14	0	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**1.67**	**---**	**0.0**	**2.67**	**2.00**	**---**	**---**
Rabbit 11 (female)	1	1	1	0	1	2	0	2
	24	**2**	4	**0**	**2**	**3**	3	3
	48	**2**	4	**0**	**2**	**2**	2	3
	72	**2**	4	**0**	**2**	**1**	1	2
	Day 7	0	0	0	1	0	0	0
	Day 10	0	0	0	0	0	0	0
	Day 14	0	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**2.00**	**---**	**0.0**	**2.00**	**2.00**	**---**	**---**
Rabbit 12 (female)	1	1	4	0	2	3	2	n.a.
	24	**1**	4	**0**	**3**	**2**	2	3
	48	**1**	4	**0**	**2**	**1**	1	2
	72	**0**	0	**0**	**1**	**0**	0	2
	Day 7	0	0	0	1	0	0	0
	Day 10	0	0	0	0	0	0	0
	Day 14	0	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.67**	**---**	**0.0**	**2.00**	**1.00**	**---**	**---**

n.a. = not assessable due to swelling of the conjunctivae

3. Assessment and conclusion

Assessment and conclusion by applicant:

Compared to the current OECD TG 405 (2017), minor deviations such as no treatment with systemic analgesic or topical anaesthesia of the animals, no rinsing after test substance instillation are noted.

As the lack of rinsing is considered as worst-case, this deviation is not considered to underestimate the results the study outcome. Therefore, the study is considered acceptable and the outcome can be reported as valid.

A single instillation of glyphosate into the rabbit eye revealed marked, early onset and transient ocular changes, such as corneal opacity, reddening of the conjunctivae and sclera, discharge and chemosis. All eye effects were reversible within 10 days after instillation. The mean individual score over 24, 48 and 72-hours was 1.67, 2.00 and 0.67 for corneal opacity, 0.00, 0.00 and 0.00 for iris lesions, 2.67, 2.00 and 2.00 for redness of the conjunctiva, and 2.00, 2.00 and 1.00 for chemosis.

Thus, based on the study results, the test substance glyphosate technical (NUP 05068) is considered to be irritating to the rabbit eye. Nevertheless, for classification purposes the serious eye damage observed in another study should be taken into account.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.5/010
Report author	[redacted]
Report year	2005
Report title	Glyphosate Acid Technical - Primary Eye Irritation Study in Rabbits
Report No	15277
Document No	Not reported
Guidelines followed in study	OECD 405 (2002), US EPA OPPTS 870.2400 (1998), JMAFF 59 NohSan No. 4200 (1985)
Deviations from current test guideline (OECD 405, 2017)	No treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application. All three animals were treated at once instead of a stepwise manner. Animal weights and age were not recorded.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

A primary eye irritation test was conducted with rabbits to determine the potential for glyphosate acid technical (Batch: 040205, Purity: 97.23 %) to produce irritation from a single instillation via the ocular route. Prior to use, the test substance was ground to a powder and 0.06 g of the ground test substance was instilled into the right eye of three healthy rabbits. The left eye remained and served as control. Animals were observed for 10 days. Ocular irritation was evaluated by the Draize scheme 1, 24, 48 and 72-hours and 4, 7 and 10 days after test item instillation. Application of glyphosate acid technical into the rabbit eye elicited corneal opacity, iritis, and conjunctivitis one hour after test substance instillation in all three animals. The overall incidence and severity of irritation decreased gradually over time. All eye irritation effects were fully reversible by Day 10.

The individual mean irritation scores (24, 48 and 72-hours) of the three rabbits were as follows:

- for corneal opacity: 1.00, 1.00 and 1.00
- for iris lesions: 1.00, 1.00 and 1.00
- for conjunctival redness: 2.33, 2.67 and 2.67
- for chemosis of the conjunctiva: 1.67, 2.00 and 2.00.

Based on the study, glyphosate TC was irritating the eye under the chosen test conditions.

Materials and methods

A: Materials

1. Test material:

Identification: Glyphosate Acid Technical
Description: White crystalline powder
Lot/Batch #: 040205

Purity:	97.23 %
Water solubility:	12 g/L
pH:	2.5 in 1 % solution
Stability of test compound:	No data given in the report. Test substance was expected to be stable for the duration of testing.

2. Vehicle and/or positive control: No vehicle was used

3. Test animals:

Species:	Rabbit
Strain:	New Zealand albino
Source:	[redacted]
Age:	Young adult
Sex:	Males
Weight at dosing:	No data given in the report
Acclimation period:	7 days
Diet/Food:	Pelleted Purina Rabbit Chow #5326
Water:	Tap water, *ad libitum*
Housing:	Individually in suspended stainless steel cages with mesh floors which conform to the size recommendations in the most recent *Guide for the Care and Use of Laboratory Animals DHEW (NIH)*. Litter paper was placed beneath the cage and was changed at least three times per week.
Environmental conditions:	Temperature: 18 – 22 °C Humidity: Not reported Air changes: Not reported Photoperiod: 12-hour light / dark cycle

B: Study design and methods

In life dates: 2004-05-26 to 2004-06-05

Animal assignment and treatment:

Prior to use, the test substance was ground to a powder. One-tenth of a millilitre (0.06 g) of the ground test substance was instilled into the right eye of three healthy rabbits, which were selected based on fluorescein dye examinations of their eyes prior to instillation of the test substance. The left eye remained untreated and served as control. Ocular irritation was evaluated by the Draize method at 1, 24, 48 and 72-hours as well as at 4, 7 and 10 days post-instillation. The fluorescein dye evaluation procedure was used at 24-hours and as needed at subsequent scoring intervals to evaluate the extent of corneal damage or to verify reversibility of effects. Individual scores were recorded for each animal. In addition to observations of the cornea, iris, and conjunctivae, any other observed lesions were noted. The average individual score for all rabbits at 24, 48 and 72-hours was calculated. Additionally, animals were observed for signs of gross toxicity and behavioural changes at least once daily during the test period. Observations included gross evaluation of skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somato-motor activity and behaviour pattern. Particular attention was directed to observation of tremors, convulsions, salivation, diarrhoea and coma.

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

There were no other signs of gross toxicity, adverse pharmacologic effects or abnormal behaviour.

C. BODY WEIGHT

Body weight not reported.

D. NECROPSY

No necropsy was performed.

E. EYE OBSERVATIONS

Results of the eye irritation assessment are presented in the table below. One hour after test substance instillation, all three treated eyes exhibited corneal opacity, iritis, and conjunctivitis. The overall incidence and severity of irritation decreased gradually over time. All animals were free of ocular irritation by Day 10 (study termination).

Individual mean scores over 24, 48 and 72-hours for each animal were 1.00 for corneal opacity and iris lesions, 2.33, 2.67 and 2.67 for redness of the conjunctiva, and 1.67, 2.00 and 2.00 for chemosis.

Table 5.2.5-10 Primary Eye Irritation Study in Rabbits. ([redacted]., 2005): Eye irritation – Individual irritation scores

Animal	Scoring [h]	Cornea		Iris	Conjunctiva		
		Opacity	Area		Redness	Chemosis	Discharge
Rabbit 11915 (male)	1	1	1	1	3	2	2
	24	**1[1]**	4	**1**	**3**	**2**	2[2]
	48	**1**	4	**1**	**2**	**2**	2[2]
	72	**1[1]**	4	**1**	**2**	**1**	2
	Day 4	1[1]	4	1	2	1	1
	Day 7	1[1]	2	0	1	0	0
	Day 10	0[1]	4	0	0	0	0
Individual mean (24, 48, 72 h)		**1.00**	**---**	**1.00**	**2.33**	**1.67**	**---**
Rabbit 11916 (male)	1	1	1	1	3	2	2
	24	**1[1]**	4	**1**	**3**	**3**	2
	48	**1**	4	**1**	**3**	**2**	2
	72	**1[1]**	4	**1**	**2**	**1**	2
	Day 4	1[1]	4	1	2	1	1
	Day 7	1[1]	2	0	2	0	0
	Day 10	0[1]	4	0	0	0	0
Individual mean (24, 48, 72 h)		**1.00**	**---**	**1.00**	**2.67**	**2.00**	**---**
Rabbit 11917 (male)	1	1	1	1	2	2	2
	24	**1[1]**	4	**1**	**3**	**3**	2
	48	**1**	4	**1**	**3**	**2**	2
	72	**1[1]**	4	**1**	**2**	**1**	2
	Day 4	1[1]	4	1	2	1	1
	Day 7	1[1]	2	0	1	0	0

Table 5.2.5-10 Primary Eye Irritation Study in Rabbits. ([redacted] 2005): Eye irritation – Individual irritation scores

	Day 10	0[1]	4	0	0	0	0
Individual mean (24, 48, 72 h)		**1.00**	**---**	**1.00**	**2.67**	**2.00**	**---**

[1] 2 % ophthalmic fluorescein sodium used to evaluate the extent or verify the absence of corneal opacity.
[2] Cream white-coloured discharge noted.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Except the deviation of no treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application the study is in concordance with the current OECD TG 405 (2017). These deviations did not compromise the acceptability of the study. Therefore, the study is considered acceptable and the outcome can be reported as valid.
Nevertheless, due to the low amount of the test substance (0.06 g), the reliability of the study is considered supplementary, only.
Instillation of glyphosate TC into the rabbit eye elicited positive response in all animals. The individual mean scores over 24, 48 and 72-hours for each animal were 1.00 for all animals for corneal opacity and iris lesions, 2.33, 2.67 and 2.67 for redness of the conjunctiva, and 1.67, 2.00 and 2.00 for chemosis. These effects were fully reversible within 10 days. Thus, glyphosate is considered irritant to the eye.
Nevertheless, for classification purposes all eye irritation studies should be taken into account.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.5/01
Report author	[redacted]
Report year	1997
Report title	Glyphosate Acid: Eye Irritation to the Rabbit
Report No	[redacted]/P/5138
Document No	Not reported
Guidelines followed in study	OECD 405 (1987), 92/69/EEC B.5 (1992), EPA 81-4
Deviations from current test guideline (OECD 405, 2017)	Six instead of three animals, no treatment with systemic analgesic prior, during, or after test substance application, only local anaesthetic ophthaine was used in five of six animals prior dosing. These deviations are not considered to affect the study outcome.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

In a primary eye irritation study, 0.1 g of glyphosate acid (Batch: P24, Purity: 95.6 % (w/w)) was instilled

into the conjunctival sac of the left eye of one of six young adult New Zealand White albino female rabbits. When the eye irritation potential had been fully assessed in the first animal, the test substance was applied into the test eye of the remaining five animals, as described previously.

The eyes were examined using the Draize scale to assess the grade of ocular reaction approximately at 1, 24, 48, 72-hour time points and after 4, 7 and 8 days after application. In addition, as an aid in the assessment of corneal damage, fluorescein staining was used at all readings from 1 day after application. A modified form of the Kay and Calandra system was used to interpret and classify the numerical scores. Body weight was determined on the day of dosing.

Corneal, iridial and conjunctival effects were seen in all animals for up to 4 days. All signs of irritation had completely regressed in five animals 7 days after application. Slight conjunctival redness was seen in the remaining animal on Day 7; the animal had completely recovered by Day 8.

The individual mean irritation scores (24 – 72-hours) of the six rabbits were as follows:

- for corneal opacity: 0.67, 1.00, 1.33, 2.00, 1.00, 2.00,
- for iris lesions: 0.33, 0.67, 0.67, 0.67, 1.00, 1.00,
- for conjunctival redness: 1.67, 2.00, 2.00, 2.00, 2.00, 2.00
- for conjunctival chemosis: 1.33, 1.33, 1.67, 2.00, 2.00, 2.00

Based on the study, glyphosate acid has the potential for irritation of the eyes under the test conditions chosen.

Materials and methods

A: Materials

1. Test material:

Identification:	Glyphosate acid
Description:	White solid
Lot/Batch number:	P24
Purity:	95.6 % (w/w)
CAS#:	Not reported
Stability of test compound:	The test substance was used within the expiry date (expiry date Not reported)

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White albino
Source:	[redacted]
Age:	Young adult (age Not reported)
Sex:	Females
Weight at dosing:	2951 – 3702 g
Acclimation period:	At least 6 days
Diet:	STANRAB SQC, (Special Diet Services Limited, Stepfield, Witham, Essex, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in aluminium sheet cages in racks suitable for animals of this strain and the weight range expected during the course of the

study.

Environmental conditions:	Temperature:	17 ± 2 °C
	Humidity:	40 – 70 %
	Air changes:	Approximately 25 – 30 / hour
	Photoperiod:	12 hours light / 12 hours dark

B: Study design and methods

In-life dates: 1996-05-22 to 1996-07-06

Animal assignment and treatment:

Initially, the test substance (approximately 0.1 g) was applied into the conjunctival sac of the left eye of one rabbit by gently pulling the lower lid away from the eyeball. The lids were then gently held together for 1 – 2 seconds after which the animal was released. The other eye remained untreated (control eye). When the eye irritation potential had been fully assessed in the first animal, the test substance was applied into the test eye of the remaining five animals, as described previously. As the initial pain reaction of the first rabbit was moderate and the irritation was less than severe, the eyes of the remaining rabbits were pre-treated with five drops of local anaesthetic (Ophthaine, 0.5 % proparacaine hydrochloride solution) with three minute intervals between each drop.

Both eyes of each rabbit were examined within the twenty-four hours prior to dosing. The examination consisted of a visual assessment with the aid of fluorescein and only rabbits without any apparent eye defects or ocular irritation were used. Immediately after the application of the test substance, an assessment of the initial pain reaction of the rabbit was made using a six-point scale.
The eyes were examined and the Draize scale was used to assess the grade of ocular reaction approximately one hour and 1, 2, 3, 4, 7 and 8 days after application where necessary. In addition, as an aid in the assessment of corneal damage, fluorescein staining was used at all readings from 1 day after application. A modified form of the Kay and Calandra system (Kay and Calandra, 1962) was used to interpret and classify the numerical scores.

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No systemic signs of toxicity were noted during the study. Application into the eye caused moderate initial pain in the first animal dosed; therefore the subsequent five animals were pre-treated with the local anaesthetic OPHTHAINE prior to dosing. The group initial pain reaction was none to moderate (class 0 – 3 on a 0 – 5 scale).

C. BODY WEIGHT

Not reported.

D. NECROPSY

Not reported.

E. EYE OBSERVATIONS

Between ¼ and ½ of the test item was displaced in every animal immediately after dosing.

Corneal effects, consisting of slight to mild opacity affecting up to the entire cornea, were seen in all animals during the first two days, persisting to Day 4 in five rabbits. Slight iritis was seen in all animals during the first two days, persisting to Day 3 in two rabbits. Conjunctival effects consisting of slight to moderate redness, slight to mild chemosis, and slight to severe discharge, were seen in all animals up to Day 4.

Additional observations included mucoid discharge, eye closed, irregular corneal surface, convoluted eyelids, and erythema of the upper and/or lower eyelids, raised corneal opacity, Harderian gland discharge and nictitating membrane partially haemorrhagic.

All signs of irritation had completely regressed in five animals 7 days after application. Slight conjunctival redness was seen in the remaining animal on Day 7; the animal had completely recovered by Day 8.

The individual mean irritation scores (24 – 72-hours) were calculated to be 0.67, 1.00, 1.33, 2.00, 1.00, 2.00 for corneal opacity, 0.33, 0.67, 0.67, 0.67, 1.00, 1.00 for iris lesions, 1.67, 2.00, 2.00, 2.00, 2.00, 2.00 for conjunctival redness and 1.33, 1.33, 1.67, 2.00, 2.00, 2.00 for conjunctival chemosis. The individual scores for each time point, individual mean scores (24 – 72-hours) are presented in the table below.

Table 5.2.5-11 Glyphosate Acid: Eye Irritation to the Rabbit. ([redacted] 1997): Eye irritation – Individual irritation scores

Animal	Scoring [h]	Cornea		Iris	Conjunctiva		
		Opacity	Area		Redness	Chemosis	Discharge
Rabbit 17 (female)	1	0	0	0	1	2	2
	24	**1**	1	**0**	**2**	**2**	3
	48	**1**	1	**1**	**2**	**1**	3
	72	**0**	0	**0**	**1**	**1**	2
	Day 4	0	0	0	1	0	1
	Day 7	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.67**	**---**	**0.33**	**1.67**	**1.33**	**---**
Rabbit 18 (female)	1	0	0	0	2	2	1
	24	**1**	4	**1**	**2**	**2**	3
	48	**1**	4	**1**	**2**	**1**	3
	72	**1**	3	**0**	**2**	**1**	1
	Day 4	1	1	0	1	1	0
	Day 7	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**1.00**	**---**	**0.67**	**2.00**	**1.33**	**---**
Rabbit 19 (female)	1	0	0	0	2	1	1
	24	**2**	4	**1**	**2**	**2**	3
	48	**1**	4	**1**	**2**	**2**	3
	72	**1**	2	**0**	**2**	**1**	1
	Day 4	1	1	0	1	1	0
	Day 7	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**1.33**	**---**	**0.67**	**2.00**	**1.67**	**---**
Rabbit 7 (female)	1	1	1	0	1	1	2
	24	**2**	4	**1**	**2**	**2**	3
	48	**2**	4	**1**	**2**	**2**	3
	72	**2**	4	**0**	**2**	**2**	3
	Day 4	1	3	0	2	1	1

Table 5.2.5-11 Glyphosate Acid: Eye Irritation to the Rabbit. ([redacted] 1997): Eye irritation – Individual irritation scores

	Day 7	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**2.00**	**---**	**0.67**	**2.00**	**2.00**	**---**
Rabbit 8 (female)	1	1	1	0	2	1	2
	24	**1**	2	**1**	**2**	**2**	2
	48	**1**	2	**1**	**2**	**2**	2
	72	**1**	2	**1**	**2**	**2**	2
	Day 4	1	2	0	2	1	1
	Day 7	0	0	0	1	0	0
	Day 8	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**1.00**	**---**	**1.00**	**2.00**	**2.00**	**---**
Rabbit 9 (female)	1	1	1	0	2	1	2
	24	**2**	4	**1**	**2**	**2**	2
	48	**2**	4	**1**	**2**	**2**	2
	72	**2**	3	**1**	**2**	**2**	2
	Day 4	1	3	0	2	1	1
	Day 7	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**2.00**	**---**	**1.00**	**2.00**	**2.00**	**---**

3. Assessment and conclusion

Assessment and conclusion by applicant:

Except the deviation of no treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application and the use of six instead of three animals the study is in concordance with the current OECD TG 405 (2017). This deviation did not compromise the acceptability of the study. Therefore, the study is considered acceptable and the outcome can be reported as valid.

Instillation of glyphosate acid into the rabbit eye elicited moderate to strong response in treated animals. The individual mean scores over 24, 48 and 72-hours for each animal were 0.67, 1.00, 1.33, 2.00, 1.00, 2.00 for corneal opacity, 0.33, 0.67, 0.67, 0.67, 1.00, 1.00 for iris lesions, 1.67, 2.00, 2.00, 2.00, 2.00, 2.00 for conjunctival redness and 1.33, 1.33, 1.67, 2.00, 2.00, 20.0 for conjunctival chemosis.

The effects were reversible within 8 days. Thus, glyphosate acid is considered irritating to rabbit eye.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.5/012
Report author	[redacted]
Report year	1996
Report title	Primary eye irritation study in rabbits
Report No	2981-96
Document No	
Guidelines followed in study	US EPA 81-4 (1984)
Deviations from current test guideline (OECD 405 (2017))	No treatment with systemic analgesic or topical anaesthesia of the animals/animal eyes prior, during or after test substance application. Nine animals treated at the same time, and no tiered testing approach was used. A washout was performed after 30 seconds (in three animals) instead of 24-hours. Animal age is not specified.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

In an eye irritation study, a 0.1 mL volume (equivalent to 0.065 g) of the undiluted glyphosate technical (Batch: 120594, Purity: 98.2 %) was instilled into the right conjunctival sac of nine New Zealand White rabbits. For three animals, the eyes were washed out for one minute with deionised water 30 seconds after treatment with the test item. The eyes of the remaining six animals were washed out at 24-hours after treatment. Following treatment, eye irritation was scored using the Draize scheme at 1, 24, 48, 72-hours, Day 4, 7, 10, 14, 17 and 21. A fluorescein staining was performed at 24-hours and repeated at each observation time point until staining was no longer observed.

Application of glyphosate technical into the rabbit eye resulted in corneal opacity and effects on the conjunctivae, which all resolved after until Day 17, except for two of six eyes (without washing) in which corneal opacity, conjunctival discharge and chemosis persisted until termination of the study. As the washing step was performed too early, the results of these animals will not be shown or discussed any further in the following. The individual mean irritation scores (24 – 72-hours) of eyes that were washed at 24-hours were as follows:

- for corneal opacity: 1.00, 1.00, 2.00, 1.00 1.00, 1.00
- for iris lesions: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00
- for conjunctival redness: 2.67, 3.00, 2.67, 3.00, 2.33, 3.00
- for chemosis of the conjunctiva: 1.67, 2.67, 2.33, 2.00, 2.00, 2.33

Based on the study, glyphosate has the potential to causes serious eye damage under the test conditions chosen.

Materials and methods

A: Materials

1. Test material:

Identification:	Glyphosate Technical (WetCake)
Description:	White powder
Lot/Batch #:	120594
Purity:	98.2 %
Stability of test compound:	Expiry date: 1997-09-08

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Young adult (not further specified)
Sex:	Male and Female
Initial body weight:	Males: 2.600 – 3.150 kg; females: 2.450 – 2.875 kg
Acclimation period:	5 days
Diet/Food:	PMI feeds lab rabbit chow #5321 or #5326, in measured amounts
Water:	Tap water, *ad libitum*
Housing:	Individually in suspended, wire bottom, stainless steel cages
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 30 – 80 % Air changes: 10 – 12 / hour Photoperiod: 12 hours light / dark cycle

B: Study design and methods

In life dates: 1996-07-15 to 1996-08-05

Animal assignment and treatment:

Six male and three female rabbits were prepared for the study. Both eyes of all animals were examined with a fluorescein sodium ophthalmic solution 24-hours before administration.

A dose of 0.1 mL by volume (equivalent to 0.065 g) of glyphosate technical was placed into the right conjunctival sac of one eye of each animal. The eyelids were gently held together for one second and then released to prevent loss of the article. The left eye served as control and remained untreated. After 30 seconds, the eyes of three male animals were washed out for one minute with deionized water.

At 1, 24, 48 and 72-hours and at Day 4, 7, 10, 14, 17 and 21, all eyes were examined for signs of irritation under normal lighting without magnification. In addition, the eyes were examined after 24-hours with a fluorescein sodium ophthalmic solution. This procedure was repeated at every observation time point until fluorescein staining was no longer present. After the 24-hour recording, all eyes were washed out with deionised water for one minute. Effects on cornea, iris, and conjunctivae (redness and chemosis) were scored using the Draize criteria.

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed during the study.

C. EYE OBSERVATIONS

All rabbits that were washed at 24-hours showed signs of corneal opacity, which persisted in two of six animals until the end of the study. None of the animals showed iridial reactions. Conjunctival redness was observed in all animals. It persisted in two of six animals until the end of the study. In addition, conjunctival chemosis and discharge were observed in all animals. However, they recovered within 17 days, except for one of six animals. Fluorescein staining was observed in all animals at 24-hours and was not observed in any animal by Day 21.

A summary of the findings is given in the table below.

Table 5.2.5-12 Primary eye irritation study in rabbits ([redacted] 1996): Eye irritation – Individual irritation scores

Animal No.	Scoring [h]	Cornea		Iris	Conjunctiva		
		Opacity	Area		Redness	Chemosis	Discharge
3412-M (male)	1	0	0	0	2	1	2
	24	**1**	1	**0**	**3**	**2**	0
	48	**1**	1	**0**	**3**	**2**	0
	72	**1**	1	**0**	**2**	**1**	0
	Day 4	0	0	0	2	1	0
	Day 7	0	0	0	1	0	0
	Day 10	0	0	0	0	0	0
	Day 14	0	0	0	0	0	0
	Day 17	0	0	0	0	0	0
	Day 21	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**1.00**	**-**	**0.00**	**2.67**	**1.67**	-
3414-M (male)	1	1	1	0	2	2	2
	24	**1**	4	0	**3**	**3**	2
	48	**1**	4	0	**3**	**3**	3
	72	**1**	4	0	**3**	**2**	2
	Day 4	1	2	0	2	1	1
	Day 7	1	1	0	2	0	1
	Day 10	1	1	0	2	0	1
	Day 14	0	0	0	1	0	0
	Day 17	0	0	0	0	0	0
	Day 21	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**1.00**	**-**	**0.00**	**3.00**	**2.67**	-
3416-M (male)	1	1	2	0	2	2	2
	24	**2**	1	0	**3**	**3**	1
	48	**2**	1	0	**3**	**2**	1
	72	**2**	1	0	**2**	**2**	1

Table 5.2.5-12 Primary eye irritation study in rabbits ([redacted] 1996): Eye irritation – Individual irritation scores

Animal No.	Scoring [h]	Cornea		Iris	Conjunctiva		
		Opacity	Area		Redness	Chemosis	Discharge
	Day 4	2	2	0	2	2	1
	Day 7	2	3	0	2	1	1
	Day 10	2	2	0	2	1	1
	Day 14	1	2	0	1	1	1
	Day 17	1	1	0	1	0	0
	Day 21	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**2.00**	**-**	**0.00**	**2.67**	**2.33**	-
3411-F (female)	1	1	1	0	2	2	2
	24	**1**	4	0	**3**	**2**	1
	48	**1**	4	0	**3**	**2**	1
	72	**1**	4	0	**3**	**2**	1
	Day 4	1	4	0	3	2	1
	Day 7	1	4	0	3	2	0
	Day 10	1	3	0	2	2	0
	Day 14	2	1	0	1	1	0
	Day 17	2	1	0	1	1	0
	Day 21	2	1	0	1	0	0
Individual mean (24, 48, 72 h)		**1.00**	**-**	**0.00**	**3.00**	**2.00**	-
3413-F (female)	1	1	2	0	2	2	2
	24	**1**	4	0	**3**	**2**	2
	48	**1**	4	0	**2**	**2**	2
	72	**1**	4	0	**2**	**2**	2
	Day 4	1	3	0	2	0	0
	Day 7	1	2	0	1	0	0
	Day 10	1	2	0	1	0	0
	Day 14	1	1	0	1	0	0
	Day 17	0	0	0	0	0	0
	Day 21	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**1.00**	**-**	**0.00**	**2.33**	**2.00**	-
3415-F (female)	1	1	2	0	2	2	2
	24	**1**	4	0	**3**	**3**	2
	48	**1**	4	0	**3**	**2**	2
	72	**1**	4	0	**3**	**2**	1
	Day 4	1	2	0	2	1	1
	Day 7	1	2	0	2	0	1
	Day 10	1	2	0	2	0	1
	Day 14	1	3	0	3	2	2
	Day 17	1	4	0	3	2	1
	Day 21	2	1	0	2	1	0
Individual mean (24, 48, 72 h)		**1.00**	**-**	**0.00**	**3.00**	**2.33**	-

3. Assessment and conclusion

Assessment and conclusion by applicant:

Except the deviation of no treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application, the study is in concordance with the current OECD TG 405 (2017). Therefore, the study is considered acceptable and the outcome can be reported as valid.

Instillation of glyphosate into the rabbit eye elicited a moderate response in the treated animal. The individual mean scores at 24, 48 and 72-hours for the animals were 1.00, 1.00, 2.00, 1.00, 1.00, 1.00 for corneal opacity, 0.00 for all animals for iris lesions, 2.67, 3.00, 2.67, 3.00, 2.33, 3.00 for conjunctival redness, and 1.67, 2.67, 2.33, 2.00, 2.00, 2.33 for conjunctival chemosis.

Thus, under test conditions of the study, glyphosate has the potential to cause serious eye damage.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.5/013
Report author	[redacted]
Report year	1995
Report title	HR-001: Primary Eye Irritation study in rabbits
Report No	[redacted] 95-0034
Document No	Not reported
Guidelines followed in study	OECD 405 (1987), US EPA FIFRA Guideline Subdivision F (1984), JMAFF 59 NohSan No. 4200 (1985)
Deviations from current test guideline (OECD 405, 2017)	Number of animals (12), no treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application. Testing of further animals (groups B and C) after irritating effects observed in group A. Irrigation after 30 seconds and 2 minutes in testing groups B and C, no irrigation in group A.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

In an eye irritation study, HR-001 (Batch: T-941209, Purity: 97.56 %) was applied to the eye of 12 female specific pathogen free New Zealand White rabbits to evaluate its primary eye irritating potential. This study was performed according to the method of Draize. Firstly, six animals were assigned to Group A (without eye irrigation after application). Secondly, three animals each were assigned to Group B (with eye irrigation at 30 seconds after application) and Group C (with eye irrigation at 2 minutes after application), since eye irritation was observed in Group A.

- Irritation of cornea: One hour after application, all animals of Group A showed a score of 2. Twenty-four hours after application, one animal of Group A showed a score of 3. At 1 or 24-hours after

application, all animals of Group B and one animal of Group C showed a score of 1. As for the area of cornea involved, Group A showed a score of 4, while Groups B and C showed scores of 2. These opacities disappeared by Day 16 in three of six animals of Group A, and the opacity (score of 1) remained until Day 21 in the other three animals of Group A. In Group B, the opacity disappeared by Day 4, and in Group C, 48 hours after application.

- Irritation of iris: One hour after application, all animals of Group A and C showed scores of 1. In Group B, one animal showed a score of 1 at 1 hour, and one animal showed a score of 1 at 24-hours after application. The irritation of iris disappeared by Day 10 in Group A, 48 hours after application in Group B and 24-hours after application in Group C.

- Irritation of conjunctivae: One hour after application, all groups showed redness scores of 1 and chemosis scores of 2. In addition, some animals in Groups A and B showed chemosis scores of 3. Groups B and C showed discharge scores of 2, and Groups A and C showed discharge scores of 3. Twenty-four or 48 hours after application, scores of 1 for redness of conjunctivae in all animals of Group A, two animals in Group B, and one animal in Group C had changed to scores of 2. These conjunctival irritations gradually began to decrease thereafter and disappeared by Day 16 in Group A and by Day 7 in Groups B and C.

- Corneal vascularisation: On Day 10, slight corneal vascularisation was observed in three animals of Group A. This sign remained until Day 21 after application.

Without irrigation, the individual mean scores over 24, 48 and 72-hours for each animal were as follows:
- for corneal opacity: 2.00, 2.67. 2.00, 2.00, 2.00, 1.67
- for iris lesions: 1.00, 1.00, 1.00, 1.00, 1.00, 0.67
- for conjunctival redness: 2.00 for all animals
- for conjunctival chemosis: 2.00, 1.67, 2.33, 2.33, 2.00, 1.67

The effects were not reversible within 21 days.

Based on the study, glyphosate technical caused serious eye damage under the test conditions chosen.

Materials and methods

A: Materials

1. Test material:

Identification:	HR-001 (glyphosate technical)
Description:	White crystal
Lot/Batch #:	T-941209
Purity:	97.56 %
Water solubility:	12 g/L (25 °C)
Stability of test compound:	Not reported

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White, Kbl:NZW
Source:	[redacted]
Age:	11 weeks
Sex:	Females

Weight at dosing:	2237 – 2570 g	
Acclimation period:	11 days	
Diet/Food:	Pellet Diet GC4 (Oriental Yeast Co., Ltd., Tokyo, Japan), *ad libitum*	
Water:	Water filtered and sterilized, *ad libitum*	
Housing:	Individually in stainless steel cages	
Environmental conditions:	Temperature:	23.9 – 24.0 °C
	Humidity:	52.8 – 57.9 %
	Air changes:	15 times per hour
	Photoperiod:	12 hours light / dark cycle

B: Study design and methods

In life dates: 1995-05-09 – 1995-05-30 (Start of treatment to study completion)

Animal assignment and treatment:
Twelve female specific pathogen free New Zealand rabbits were given a single ocular instillation of 0.1 g of technical glyphosate. The dose was instilled in the conjunctival sac of the left eye of each animal after gently pulling the lower lid away from the eyeball. The lids were then gently held together for about one second in order to prevent loss of test substance. The treated left eyes of animals in Groups B and C were irrigated with lukewarm water 30 seconds (Group B, three animals) or 2 minutes (Group C, three animals) after application. The right eye remained untreated. No irrigation took place in Group A (six animals). All animals were observed for primary eye irritation 1, 24, 48 and 72-hours, 4 and 7 days after application of the test substance, whereas animals of Group A were also observed at 10, 13, 16, 19 and 21 days after instillation. The cornea, iris and conjunctive were examined with a hand slit-lamp during the observation period and findings were scored according to the criteria described in the guideline of MAFF in Japan and the method of Draize. Body weights were measured prior to application, and after the final observation.

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed during the study.

C. BODY WEIGHT

All rabbits showed the expected body weight gain.

D. NECROPSY

No necropsy was performed.

E. EYE OBSERVATIONS

Results of the group without irrigating are summarised in the table below.

Without eye irrigation

- **Irritation of cornea**

One hour after application, all animals showed score 2 (easily discernible translucent area, details of iris slightly obscured). Twenty-four hours after application, one animal showed score 3 (nacreous area, no details of iris visible, size of pupil barely discernible). Concerning the area of cornea involved, Group A animals showed score 4 (greater than three quarters, up to the whole area of the cornea). These opacities disappeared by Day 16 in three of six animals and a score of 1 remained until Day 21 in the other three animals. On Day 10, slight corneal vascularization was observed in three animals; this sign remained until Day 21.

- **Irritation of iris**

One hour after application, all animals showed score 1 (congestion and / or markedly deepened rugae of iris). The irritation disappeared by Day 10.

- **Irritation of conjunctivae**

Redness: One hour after application, all animals showed redness score 1 (definite hyperaemia of some blood vessels). Twenty-four and 48 hours after application, score 2 (redness of conjunctivae) was observed in all animals. The redness disappeared by Day 16.
Chemosis: One hour after application, four animals showed chemosis score 2 (obvious swelling with partial eversion of lids about half closed). In addition, 2 animals showed score 3 (swelling with lids more than half closed). The chemosis disappeared by Day 7.

Discharge: One hour after application all tested animals showed score 3 (discharge with moistening of the lids and hairs just adjacent around to lids). The discharge disappeared by Day 7.

All these conjunctival irritations gradually weakened and disappeared by Day 16.

The individual mean scores over 24, 48 and 72-hours for each animal were: 2.00, 2.67, 2.00, 2.00, 2.00 and 1.67 for corneal opacity; 1.00, 1.00, 1.00, 1.00, 1.00 and 0.67 for iris lesions; 2.00 for all animals for conjunctival redness; and 2.00, 1.67, 2.33, 2.33, 2.00 and 1.67 for conjunctival chemosis. The effects were not reversible within 21 days. Thus, glyphosate technical is considered severely irritating to the eye mucosa of rabbits.

With eye irrigation (30 seconds or 2 minutes after application)
The iridial and conjunctival irritations observed in the irrigation groups (Groups B and C, see tables below, respectively) were almost the same as those in non-irrigating group (Group A), while the corneal irritation was less pronounced when the test substance was irrigated.

Animals in the irrigating groups showed reduced eye irritations and faster recovery as compared with animals of the non-irrigation group. Irrigation 30 seconds or 2 minutes after application was effective for reduction of the induced irritation and for recovery.

Table 5.2.5-13 HR-001: Primary Eye irritation study in rabbits. ([redacted] 1995): Eye irritation without eye irrigation after application – Individual irritation scores

Animal	Scoring [h]	Cornea		Iris	Conjunctiva		
		Opacity	Area		Redness	Chemosis	Discharge
Rabbit 1 (female)	1	2	4	1	1	2	3
	24	**2**	4	**1**	**2**	**2**	3
	48	**2**	4	**1**	**2**	**2**	3

Table 5.2.5-13 HR-001: Primary Eye irritation study in rabbits. ([redacted] 1995): Eye irritation without eye irrigation after application – Individual irritation scores

Animal	Scoring [h]	Cornea		Iris	Conjunctiva		
		Opacity	Area		Redness	Chemosis	Discharge
	72	**2**	4	**1**	**2**	**2**	1
	Day 4	1	3	1	2	1	0
	Day 7	1	2	0	1	0	0
	Day 10	1+	1	0	1	0	0
	Day 13	1+	1	0	1	0	0
	Day 16	1+	1	0	0	0	0
	Day 19	1+	1	0	0	0	0
	Day 21	1+	1	0	0	0	0
Individual mean (24, 48, 72 h)		**2.00**	---	**1.00**	**2.00**	**2.00**	---
	1	2	4	1	1	2	3
	24	**3**	4	**1**	**2**	**2**	3
	48	**3**	4	**1**	**2**	**2**	3
	72	**2**	3	**1**	**2**	**1**	1
	Day 4	2	2	1	2	1	0
Rabbit 2 (female)	Day 7	1	2	0	1	0	0
	Day 10	1+	2	0	1	0	0
	Day 13	1+	2	0	1	0	0
	Day 16	1+	2	0	0	0	0
	Day 19	1+	2	0	0	0	0
	Day 21	1+	2	0	0	0	0
Individual mean (24, 48, 72 h)		**2.67**	---	**1.00**	**2.00**	**1.67**	---
	1	2	4	1	1	3	3
	24	**2**	4	**1**	**2**	**3**	3
	48	**2**	4	**1**	**2**	**2**	3
	72	**2**	4	**1**	**2**	**2**	3
	Day 4	2	3	1	2	1	0
Rabbit 3 (female)	Day 7	1	2	1	1	0	0
	Day 10	1+	2	0	1	0	0
	Day 13	1+	2	0	1	0	0
	Day 16	1+	2	0	0	0	0
	Day 19	1+	1	0	0	0	0
	Day 21	1+	1	0	0	0	0
Individual mean (24, 48, 72 h)		**2.00**	---	**1.00**	**2.00**	**2.33**	---
	1	2	4	1	1	3	3
	24	**2**	4	**1**	**2**	**3**	3
	48	**2**	4	**1**	**2**	**2**	3
	72	**2**	3	**1**	**2**	**2**	1
	Day 4	2	2	1	2	1	1
Rabbit 4 (female)	Day 7	1	2	0	1	0	0
	Day 10	1	2	0	1	0	0
	Day 13	1	2	0	1	0	0
	Day 16	0	0	0	0	0	0
	Day 19	0	0	0	0	0	0
	Day 21	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**2.00**	---	**1.00**	**2.00**	**2.33**	---
	1	2	4	1	1	2	3
Rabbit 5 (female)	**24**	**2**	4	**1**	**2**	**2**	3
	48	**2**	4	**1**	**2**	**2**	3

Table 5.2.5-13 HR-001: Primary Eye irritation study in rabbits. ([redacted] 1995): Eye irritation without eye irrigation after application – Individual irritation scores

Animal	Scoring [h]	Cornea		Iris	Conjunctiva		
		Opacity	Area		Redness	Chemosis	Discharge
	72	**2**	4	**1**	**2**	**2**	1
	Day 4	1	2	1	2	1	0
	Day 7	1	2	0	1	0	0
	Day 10	1	1	0	1	0	0
	Day 13	0	0	0	0	0	0
	Day 16	0	0	0	0	0	0
	Day 19	0	0	0	0	0	0
	Day 21	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**2.00**	---	**1.00**	**2.00**	**2.00**	---
Rabbit 6 (female)	1	2	4	1	1	2	3
	24	**2**	4	**1**	**2**	**2**	3
	48	**2**	2	**1**	**2**	**2**	2
	72	**1**	2	**0**	**2**	**1**	0
	Day 4	1	1	0	1	0	0
	Day 7	0	0	0	0	0	0
	Day 10	0	0	0	0	0	0
	Day 13	0	0	0	0	0	0
	Day 16	0	0	0	0	0	0
	Day 19	0	0	0	0	0	0
	Day 21	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**1.67**	---	**0.67**	**2.00**	**1.67**	---

+ = slight corneal vascularisation

Table 5.2.5-14 HR-001: Primary Eye irritation study in rabbits. ([redacted] 1995): Eye irritation with eye irrigation 30 seconds after application – Individual irritation scores

Animal	Scoring [h]	Cornea		Iris	Conjunctiva		
		Opacity	Area		Redness	Chemosis	Discharge
Rabbit 7 (female)	1	0	0	0	1	3	2
	24	**1**	3	**1**	**1**	**2**	3
	48	**1**	1	**0**	**2**	**2**	1
	72	**1**	1	**0**	**1**	**1**	1
	Day 4	0	0	0	1	0	0
	Day 7	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**1.00**	---	**0.33**	**1.33**	**1.67**	---
Rabbit 8 (female)	1	0	0	0	1	2	2
	24	**1**	2	**0**	**1**	**2**	2
	48	**1**	1	**0**	**1**	**1**	1
	72	**0**	0	**0**	**1**	**0**	0
	Day 4	0	0	0	1	0	0
	Day 7	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.67**	---	**0.00**	**1.00**	**1.00**	---
Rabbit 9 (female)	1	1	2	1	1	2	2
	24	**1**	3	**1**	**2**	**2**	3
	48	**1**	3	**0**	**2**	**1**	1
	72	**1**	2	**0**	**1**	**1**	1
	Day 4	0	0	0	1	0	0

Table 5.2.5-14 HR-001: Primary Eye irritation study in rabbits. ([redacted] 1995): Eye irritation with eye irrigation 30 seconds after application – Individual irritation scores

Animal	Scoring [h]	Cornea		Iris	Conjunctiva		
		Opacity	Area		Redness	Chemosis	Discharge
	Day 7	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**1.00**	**---**	**0.33**	**1.67**	**1.33**	**---**

Table 5.2.5-15 HR-001: Primary Eye irritation study in rabbits. [redacted] 1995): Eye irritation with eye irrigation 2 minutes after application – Individual irritation scores

Animal	Scoring [h]	Cornea		Iris	Conjunctiva		
		Opacity	Area		Redness	Chemosis	Discharge
	1	0	0	1	1	2	3
	24	**1**	2	**0**	**2**	**2**	3
Rabbit 10	**48**	**0**	0	**0**	**2**	**1**	1
(female)	**72**	**0**	0	**0**	**2**	**1**	1
	Day 4	0	0	0	1	0	0
	Day 7	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.33**	---	**0.00**	**2.00**	**1.33**	**---**
	1	0	0	1	1	2	2
	24	**0**	0	**0**	**1**	**2**	2
Rabbit 11	**48**	**0**	0	**0**	**1**	**1**	1
(female)	**72**	**0**	0	**0**	**0**	**0**	0
	Day 4	0	0	0	0	0	0
	Day 7	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.00**	**---**	**0.00**	**0.67**	**1.00**	**---**
	1	0	0	1	1	2	2
	24	**0**	0	**0**	**1**	**1**	2
Rabbit 12	**48**	**0**	0	**0**	**1**	**1**	1
(female)	**72**	**0**	0	**0**	**0**	**0**	0
	Day 4	0	0	0	0	0	0
	Day 7	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.00**	**---**	**0.00**	**0.67**	**0.67**	**---**

3. Assessment and conclusion

Assessment and conclusion by applicant:

Except the deviation of no treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application, the use of 12 instead of 3 animals and no irrigation or after 30 seconds or after 2 minutes instead of 1 hour as recommended for solid test compounds the study follows the current OECD TG 405 (2017). No irrigation is considered to represent a worst-case scenario which did not compromise the acceptability of the study. Therefore, the study is considered acceptable and the outcome can be reported as valid.

Instillation of glyphosate technical into the rabbit eye elicited moderate response in treated animals. The individual mean scores over 24, 48 and 72-hours for each animal were: 2.00, 2.67. 2.00, 2.00, 2.00, 1.67 for corneal opacity; 1.00, 1.00, 1.00, 1.00, 1.00, 0.67 for iris lesions; 2.0 for all animals for conjunctival redness; and 2.00, 1.67, 2.33, 2.33, 2.00, 1.67 for conjunctival chemosis.

The effects were not reversible within 21 days. Thus, under test conditions of the study, glyphosate technical is considered severely irritating to the eye mucosa of rabbits.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.5/014
Report author	[redacted]
Report year	1994
Report title	Glyphosate premix: Acute eye irritation test in the rabbit
Report No	545/41
Document No	Not reported
Guidelines followed in study	US EPA 81-4 (1984), US EPA 798.4500
Deviations from current test guideline (OECD 405, 2017)	Six instead of three animals were used
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive (low purity: 46.1 % glyphosate)
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

In an eye irritation study, six New Zealand White rabbits were treated with glyphosate premix (Batch: 290-JaK-146-4, Purity: 46.1 % glyphosate). One animal was treated first; 0.1 mL of the undiluted test substance was instilled into the right conjunctival sac of the animal. Immediately before treatment, one drop of a local anaesthetic was instilled into both eyes of the animal to reduce the pain. Following treatment, eye irritation was scored using the Draize scheme at 1, 24, 48 and 72-hours. Based on the observations made in the first animal, a further five animals were treated following the same procedure.

Application of glyphosate premix into the rabbit eye resulted in effects on the iris and conjunctivae, which all resolved within 24-hours. The individual mean irritation scores (24 – 72-hours) of the rabbits were as follows:

- for corneal opacity: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00
- for iris lesions: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00
- for conjunctival redness: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00
- for chemosis of the conjunctiva: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00

Based on the study, glyphosate (with a purity of 46.1 %) is not irritating to the eye under the test conditions chosen.

Materials and methods

A: Materials

1. Test material:

Identification:	Glyphosate premix (technical concentrate)
Description:	Pale yellow liquid
Lot/Batch #:	290-JaK-146-4
Purity:	62.2 % as glyphosate isopropylamine salt, 46.1 % as glyphosate
Stability of test compound:	Expiry date: 1995-09-30

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	12 – 20 weeks
Sex:	Male and Females
Body weight at dosing:	2.17 – 2.72 kg
Acclimation period:	At least 5 days
Diet/Food:	Stanrab SQC rabbit diet (Special Diets Services Ltd., Witham, Essex, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in suspended metal cages
Environmental conditions:	Temperature: 18 – 21 °C Humidity: 44 – 57 % Air changes: 15 / hour Photoperiod: 12 hours light / dark cycle

B: Study design and methods

In life dates: 1994-04-19 to 1994-04-23

Animal assignment and treatment:

Both eyes of all six animals (three males and three females) were examined for lesions with fluorescein sodium 24-hours before administration and again immediately before treatment with an ophthalmoscope.

One to two minutes prior to treatment with the test substance, one drop of local anaesthetic was instilled into both eyes of all animals, to minimise pain. A volume of 0.1 mL of glyphosate premix was placed into the conjunctival sac of the right eye of one animal. The eyelids were gently held together for one second, and then released to prevent loss of the article. The left eye served as control and remained untreated. Immediately after instillation of the test material, an assessment of pain was made. Based on the observed responses, five further animals were treated in the same way.

At 1, 24, 48 and 72-hours all eyes were examined for signs of irritation using a standard ophthalmoscope. Effects on cornea, iris, and conjunctivae (redness and chemosis) were scored using the Draize criteria.

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed during the study.

C. EYE OBSERVATIONS

No effects on the cornea were observed in any animal. Effects on the iris were observed in 4/6 animals. Conjunctival redness and chemosis were observed in all animals. In addition, conjunctival discharge was observed in 5/6 animals. All effects on the eyes reversed within 24-hours.
A summary of the findings is given in the table below.

Table 5.2.5-16 Glyphosate premix: Acute eye irritation test in the rabbit. ([redacted] 1994): Eye irritation – Individual irritation scores

Animal No.	Scoring [h]	Cornea		Iris	Conjunctiva		
		Opacity	Area		Redness	Chemosis	Discharge
57 (female)	1	0	0	1	1	1	0
	24	**0**	0	**0**	**0**	**0**	0
	48	**0**	0	**0**	**0**	**0**	0
	72	**0**	0	**0**	**0**	**0**	0
Individual mean (24, 48, 72 h)		**0.00**	**-**	**0.00**	**0.00**	**0.00**	-
12 (female)	1	0	0	0	1	1	1
	24	**0**	0	**0**	**0**	**0**	0
	48	**0**	0	**0**	**0**	**0**	0
	72	**0**	0	**0**	**0**	**0**	0
Individual mean (24, 48, 72 h)		**0.00**	**-**	**0.00**	**0.00**	**0.00**	-
55 (female)	1	0	0	1	1	1	2
	24	**0**	0	**0**	**0**	**0**	0
	48	**0**	0	**0**	**0**	**0**	0
	72	**0**	0	**0**	**0**	**0**	0
Individual mean (24, 48, 72 h)		**0.00**	**-**	**0.00**	**0.00**	**0.00**	-
59 (female)	1	0	0	1	1	1	2
	24	**0**	0	**0**	**0**	**0**	0
	48	**0**	0	**0**	**0**	**0**	0
	72	**0**	0	**0**	**0**	**0**	0
Individual mean (24, 48, 72 h)		**0.00**	**-**	**0.00**	**0.00**	**0.00**	-
65 (male)	1	0	0	1	2	1	2
	24	**0**	0	**0**	**0**	**0**	0
	48	**0**	0	**0**	**0**	**0**	0
	72	**0**	0	**0**	**0**	**0**	0
Individual mean (24, 48, 72 h)		**0.00**	**-**	**0.00**	**0.00**	**0.00**	-

Table 5.2.5-16 Glyphosate premix: Acute eye irritation test in the rabbit. ([redacted] 1994): Eye irritation – Individual irritation scores

Animal No.	Scoring [h]	Cornea		Iris	Conjunctiva		
		Opacity	Area		Redness	Chemosis	Discharge
101 (female)	1	0	0	0	1	1	1
	24	**0**	0	**0**	**0**	**0**	0
	48	**0**	0	**0**	**0**	**0**	0
	72	**0**	0	**0**	**0**	**0**	0
Individual mean (24, 48, 72 h)		**0.00**	**-**	**0.00**	**0.00**	**0.00**	-

3. Assessment and conclusion

Assessment and conclusion by applicant:

Except the deviation that six instead of three animals were used, the study is in concordance with the current OECD TG 405 (2017). Therefore, the study is considered acceptable and the outcome can be reported as valid. Nevertheless, due to the low purity of the test substance (46.1 % as glyphosate), the reliability of the study is considered supplementary, only.

Instillation of Glyphosate premix into the rabbit eye elicited a slight irritation response after 1 hour, which was reversible within 24-hours. The individual mean scores at 24, 48 and 72-hours for the animals were 0.00 for corneal opacity, iris lesions, conjunctival redness and conjunctival chemosis.

Thus, under test conditions of the study, glyphosate is not an irritant to the eye.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.5/015
Report author	[redacted]
Report year	1994
Report title	Glyphosate: Acute eye irritation test in the rabbit
Report No	710/18
Document No	Not reported
Guidelines followed in study	Uncertain
GLP	Uncertain
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	0.76 mg of test substance (glyphosate acid, 95 % purity) into eye of one female albino rabbit (New Zealand White rabbit). Due to the severe eye reactions, test was stopped after 1 hour for humane reasons.
Short description of results:	Scores: Cornea opacity: 4, iris: 1, conjunctival redness: 2, conjunctival chemosis: 2 Further eye observations: Sloughing of the cornea, haemorrhage of the lower conjunctival membrane, blood stained discharge.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	Full study report is not available to the applicant. Only a short summary is provided in the Monograph, B5, 2000

Reasons why the study report is not available for submission	The notifier has not access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance" (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL.
Category study in AIR 5 dossier (L docs)	Category 4b

1. Information on the study

Data point:	CA 5.2.5/015
Report author	[redacted]
Report year	1994
Report title	Glyphosate (Alkaloida, Tiszavasvari): Primary eye irritation study in rabbits
Report No	GHA-93-405/N
Document No	Not reported
Guidelines followed in study	OECD 405 (1981)
Deviations from current test guideline (OECD 405, 2017)	No anaesthetic was given prior, during or after dosing to reduce the pain. Test item was applied to the centre of corneal surface instead of the conjunctivial sac. All animals were tested at the same time instead of using a tiered testing approach. Four animals were used in the test. Body weights, age, housing, and source of the animals were not reported.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

In an eye irritation study, four female albino rabbits were treated with glyphosate (Batch: 36300892, Purity: 99.6 %) by instillation of 0.1 g of the undiluted test substance onto the centre of the cornea of one eye of each animal. The other untreated eye served as control. Following treatment, eye irritation was scored using the Draize scheme at 1, 24, 48, 72, 96 hours and at 7 and 14 days.

Instillation of glyphosate into the rabbit eye resulted in effects on the cornea, iris and conjunctivae in all animals which reversed by Day 14. The individual mean irritation scores (24 – 72-hours) of the rabbits for the cornea, iris, and conjunctivae were as follows:

- for corneal opacity: 2.00, 1.00, 1.33, 1.00
- for iris lesions: 1.00, 1.00, 0.33, 1.00
- for conjunctival redness: 1.00, 1.67, 2.00, 2.00
- for chemosis of the conjunctiva: 2.00, 1.67, 2.00, 3.00

3. Materials and methods

A: Materials

1. Test material:

Identification:	Glyphosate technical
Description:	White or almost white crystalline powder
Lot/Batch #:	36300892
Purity:	99.6 %
Stability of test compound:	Expiry date: 1994-09-01

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rabbit
Strain:	New Zealand
Source:	Not specified
Age:	Not specified
Sex:	Female
Initial body weight:	Not specified
Acclimation period:	5 days
Diet/Food:	Standard rabbit chow with fresh carrots, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Wire box (not further specified)
Environmental conditions:	Temperature: 18 ± 2 °C Humidity: 40 – 70 % Air changes: 10 / hour Photoperiod: 12-hour light / dark cycle

B: Study design and methods

In life dates: 1993-11-29 to 1993-12-12

Animal assignment and treatment:

24-hours prior to treatment, each eye of four female rabbits was examined with fluorescein-Na solution and a medical lamp.

An amount of 0.1 g of the test substance was placed to the centre of the corneal surface of the right eye of each rabbit. The other eye served as control and remained untreated.

At 1, 24, 48, 72, 96 hours, 7 and 14 days post-administration, all eyes were examined for signs of irritation. Effects on cornea, iris, and conjunctivae (redness, chemosis, and discharge) were scored using the Draize criteria.

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed during the study.

C. EYE OBSERVATIONS

After 1 hour, effects on the cornea, iris, and conjunctivae were observed in all four animals. Effects on the iris were resolved by Day 7 while effects on the cornea and conjunctivae were resolved by Day 14.

A summary of the findings is given in the table below.

Table 5.2.5-17 Primary eye irritation study in rabbits ([redacted] 1994): Eye irritation in rabbits – Individual irritation scores

Animal	Scoring [h]	Cornea		Iris	Conjunctiva		
		Opacity	Area		Redness	Chemosis	Discharge
Rabbit 1 (female)	1	2	4	1	1	2	1
	24	**2**	4	**1**	**1**	**2**	3
	48	**2**	4	**1**	**1**	**2**	3
	72	**2**	4	**1**	**1**	**2**	3
	Day 4	2	4	1	1	2	1
	Day 7	1	1	0	1	1	1
	Day 14	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**2.00**	-	**1.00**	**1.00**	**2.00**	-
Rabbit 2 (female)	1	1	4	1	2	2	1
	24	**1**	4	**1**	**2**	**2**	1
	48	**1**	4	**1**	**2**	**2**	1
	72	**1**	4	**1**	**1**	**1**	0
	Day 4	1	4	0	1	1	1
	Day 7	0	0	0	1	1	1
	Day 14	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**1.00**	-	**1.00**	**1.67**	**1.67**	-
Rabbit 3 (female)	1	2	4	1	2	2	2
	24	**2**	4	**1**	**2**	**2**	2
	48	**1**	4	**0**	**2**	**2**	2
	72	**1**	4	**0**	**2**	**2**	2
	Day 4	1	4	0	1	1	1
	Day 7	0	0	0	1	1	1
	Day 14	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**1.33**	-	**0.33**	**2.00**	**2.00**	-
Rabbit 4 (female)	1	2	4	1	2	3	2
	24	**1**	4	**1**	**2**	**3**	3
	48	**1**	4	**1**	**2**	**3**	3
	72	**1**	4	**1**	**2**	**3**	3
	Day 4	1	4	0	1	1	1
	Day 7	1	2	0	1	1	1
	Day 14	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**1.00**	-	**1.00**	**2.00**	**3.00**	-

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD TG 405 (2017) except for minor deviations that are not expected to influence the study outcome such as: No anaesthetic was given prior, during or after dosing to reduce the pain and all animals were tested at the same time instead of using a tiered testing approach.
The test item was applied directly to the centre of corneal surface instead of the conjunctival sac. However, this deviation is regarded as a worst-case scenario. The study is therefore considered valid and

acceptable. The individual mean scores at 24, 48 and 72-hours for the animals were 2.00, 1.00, 1.33, 1.00 for corneal opacity, 1.00, 1.00, 0.33, 1.00 for iris lesions, 1.00, 1.67, 2.00, 2.00 for conjunctival redness, and 2.00, 1.67, 2.00, 3.00 for conjunctival chemosis.
Thus, under test conditions of the study, glyphosate has the potential to cause serious eye damage.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.5/016
Report author	[redacted]
Report year	1991
Report title	Primary eye irritation study with glyphosate technical (FSG 03090 h/05 March 90) in New Zealand White rabbits
Report No	ES.879.EYE
Document No	Not reported
Guidelines followed in study	OECD 405 (1987), US EPA 81-4 (1984)
Deviations from current test guideline (OECD 405, 2017)	No treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during, or after test substance application. Individual scores for eye irritation not included in the report, therefore no mean values could be obtained. A tier testing approach was not used.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

In an eye irritation study, 0.1 g of the undiluted test substance (glyphosate technical, Batch: 60, Purity: 96.8 %) was instilled into the right conjunctival sac of three New Zealand White rabbits (two males and one female). The eyes were rinsed after 24-hours with distilled water to remove the test substance. Animals were observed for 21 days for mortality and signs of eye irritation. Eye irritation was scored using the Draize scheme at 1, 24, 48 and 72-hours and 7, 14 and 21 days after test item instillation.

The female rabbit was found dead on Day 2 post-exposure. Application of glyphosate technical into the rabbit eye resulted in corneal opacity (grade 2) in all animals persisting until death or the end of the study. Furthermore, one male rabbit showed ulceration from Day 14 until end of the study. Redness (grade 1 or 2) of the eye was observed in two animals until Day 7, thereafter one animal recovered while redness of the eye persisted in the other animal until termination of the study. Chemosis (grade 1 or 2) was observed in all animals until death or end of the study. The individual mean irritation scores (24 – 72-hours) of the three rabbits were as follows:

- for corneal opacity: 2.00, 2.00 (score for the third animal not available due to its death after the 48 hour scoring)
- for iris lesions: 0.00, 0.00 (score for the third animal not available due to its death after the 48 hour scoring)

- for conjunctival redness: 1.44 (mean for all three animals due to missing information on individual scores)
- for chemosis of the conjunctiva: 1.11 (mean for all three animals due to missing information on individual scores)

Materials and methods

A: Materials

1. Test material:

Identification:	Glyphosate Technical
Description:	Solid white coloured crystals, odourless
Lot/Batch #:	60
Purity:	96.8 %
Stability of test compound:	More than two years; expiry date 1992-07

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Source:	Bred at the [redacted]
Age:	Approximately 14 weeks
Sex:	Male and Female
Weight at dosing:	1.7 – 2.1 kg
Acclimation period:	At least 4 days
Diet/Food:	Standard "Gold Mohur" brand pelleted rabbit maintenance diet (M/S Lipton India Ltd., Bangalore, India), *ad libitum*
Water:	Deep borewell water passed through activated charcoal filter and exposed to UV light, *ad libitum*
Housing:	Individually in stainless steel / aluminium cages equipped for pelleted feed and drinking water
Environmental conditions:	Temperature: 23 ± 2 °C
	Humidity: 68 ± 6 %
	Air changes: 10 – 15 / hour
	Photoperiod: 12 hours light / dark cycle

B: Study design and methods

In life dates: 1990-09 to 1990-10 (specific dates not reported)

Animal assignment and treatment:

Two male and one female rabbit were used for this test. Both eyes of each animal were examined before administration.

An amount of 0.1 g of finely ground glyphosate technical was placed into the conjunctival sac of the left eye. The eyelids were gently held together for one second and then released to prevent loss of the test

article. The right eye served as control and remained untreated. After 24-hours of exposure, the eye was rinsed with distilled water to remove the test substance.

At 1, 24, 48 and 72-hours and at days 7, 14 and 21 after instillation of the test substance, all eyes were inspected for irritation according to the Draize criteria. Clinical signs on cornea, iris and conjunctivae, as well as chemosis were recorded and investigated for their reversibility. Furthermore, the animals were checked for mortality every day and individual body weights were recorded on the first and last day of the study. A gross pathological examination was performed at death or at study termination.

Results

A. MORTALITY

The female rabbit died on Day 2 after treatment.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed during the study.

C. BODY WEIGHT

Decrease in body weight was observed for all three animals.

D. NECROPSY

Necropsy findings showed opacity of the treated eye in all animals, involving 3/4 or 1/2 of the area, and one male rabbit showed ulceration. Enteritis was seen in the deceased animal (female).

E. EYE OBSERVATIONS

All animals showed signs of opacity (grade 2) from 24-hours after instillation, which persisted either until death or until study termination (Day 21). Furthermore, one male rabbit showed ulceration beginning from Day 14 until end of the study. None of the animals showed iridial reactions. Redness (grade 1 or 2) of the eye persisted until Day 7 in two animals. One animal recovered while redness persisted in the other animal until end of the study in the other animal. Chemosis (grade 1 or 2) was seen in all animals from the beginning until death or end of the study.

A summary of the findings is given in the table below.

Table 5.2.5-18 Primary eye irritation study with glyphosate technical (FSG 03090 h/05 March 90) in New Zealand White rabbits ([redacted] 1991): Eye irritation – Individual irritation scores

Effects	Score	1 h	24 h	48 h	72 h [a]	Mean (24h, 48h, 72h)	Day 7	Day 14	Day 21
Cornea	0	-	-	-	-	Animal 1: 2.00 Animal 2: 2.00 Animal 3: NA [a]	-	-	-
	1	-	-	-	-		-	-	-
	2	3/3	3/3	3/3	2/2		2/2	2/2	2/2
	3	-	-	-	-		-	-	-
	4	-	-	-	-		-	-	-
Iris	0	3/3	3/3	3/3	2/2	Animal 1: 0.00 Animal 2: 0.00	2/2	2/2	2/2
	1	-	-	-	-		-	-	-

Table 5.2.5-18 Primary eye irritation study with glyphosate technical (FSG 03090 h/05 March 90) in New Zealand White rabbits ([redacted] 1991): Eye irritation – Individual irritation scores

	2	-	-	-	-	Animal 3: NA [a]	-	-	
Conjunctiva Redness	0	-	-	-	-	Mean of all two / three animals: 1.44 [a,b]	1/2	1/2	1/2
	1	1/3	1/3	1/3	1/2		1/2	1/2	1/2
	2	2/3	2/3	2/3	1/2		-	-	-
	3	-	-	-	-		-	-	-
Conjunctiva Chemosis	0	-	-	-	-	Mean of all two / three animals: 1.11 [a,b]	-	-	-
	1	2/3	1/3	3/3	2/2		2/2	2/2	2/2
	2	1/3	2/3	-	-		-	-	-
	3	-	-	-	-		-	-	-
	4	-	-	-	-		-	-	-

[a] One animal (female) died on Day 2 post application.
[b] Individual calculation not possible as no individual animal data were provided.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Except the deviation of no treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application, no stepwise approach and the missing possibility for calculation individual mean scores, the study is in concordance with the current OECD TG 405 (2017). The study is considered acceptable and the outcome can be reported as valid. Nevertheless, due to the missing calculation of the individual mean scores, the reliability of the study is considered supplementary, only.

Instillation of glyphosate into the rabbit eye elicited a response in the eye of the treated animals. No individual mean scores at 24, 48 and 72-hours could be calculated. However, due to the lack of reversibility, glyphosate has the potential to causes serious eye damage under the test conditions of the study.

Based on the effects on cornea and conjunctiva, which were not reversible within 21 days, the test substance requires classification as "Serious Eye Damage, Category 1 (H318)" according to the classification criteria laid down in the CLP Regulation (EC No. 1272/2008).

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.5/017
Report author	[redacted]
Report year	1991
Report title	Acute eye irritation study in New Zealand White rabbits treated with the test article glyphosate technico 98 %
Report No	910260
Document No	[redacted]496
Guidelines followed in study	Not reported
GLP	Not reported
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	Application of 100 mg test substance (glyphosate acid, purity 98.0 %)

Short description of results:	Several ocular effects (score 1 and 2) in all animals which were reversible within 3 days
Reasons for why the study is not considered relevant/reliable or not considered as keystudy:	Full study report is not available to the applicant. Only a short summary is provided in the Monograph, B5, 2000.
Reasons why the study report is not available for submission	The notifier has not access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance" (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL.
Category study in AIR 5 dossier (L docs)	Category 4b

1. Information on the study

Data point:	CA 5.2.5/019
Report author	[redacted]
Report year	1990
Report title	Acute eye irritation/corrosion of glyphosate technical in the rabbit
Report No	AGC-900822
Document No	AGC-002
Guidelines followed in study	OECD 405 (1981), B5 Annex to EEC Directive 84/449/EEC
Deviations from current test guideline (OECD 405, 2017)	No treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application. New conclusion drawn based on the current CLP regulation (EC No. 1272/2008). Environmental conditions, acclimation period, and age of animals were not specified. No tiered testing approach was used; instead all three animals were tested together.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes (no certificate attached)
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

In an eye irritation study, 0.1 g of the undiluted test substance (glyphosate technical, Batch: 0190 A, Purity: 98.1 %) was instilled into one conjunctival sac of three New Zealand White rabbits (3 females). Following treatment, eye irritation was scored using the Draize scheme at 1, 24, 48 and 72-hours and daily thereafter until Day 8 of the study period.

Application of glyphosate technical into the rabbit eye resulted in corneal opacity in all animals persisting until Day 7. Furthermore, one rabbit showed effects on the iris until Day 6. Conjunctivae redness was observed in all animals until Day 6, while chemosis reversed 72-hours after treatment. The individual mean irritation scores (24 – 72-hours) of the three rabbits were as follows:

- for corneal opacity: 1.00, 1.00, 1.67
- for iris lesions: 0.00, 0.00, 0.67
- for conjunctival redness: 1.00, 1.00, 1.33

- for chemosis of the conjunctiva: 0.67, 0.67, 1.00

Based on the study, glyphosate technical is irritating to the rabbit eye under the chosen test conditions.

Materials and methods

A: Materials

1. Test material:

Identification:	Glyphosate Technical
Description:	Yellowish (transparent)
Lot/Batch #:	0190 A
Purity:	98.1 %
Stability of test compound:	Provided by the sponsor

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Adult (not specified)
Sex:	Females
Weight at dosing:	2.1 – 3.2 kg
Acclimation period:	Not specified
Diet/Food:	Standard rabbit diet (Redmills, Goresbridge, Co. Kilkenny, Ireland), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individual housing in standard rabbit cages without bedding material. Grid bottom cages used.
Environmental conditions:	Temperature: Not recorded Humidity: Not recorded Air changes: Not specified Photoperiod: 12 hours light / dark cycle

B: Study design and methods

In life dates: 1990-03-30 to 1990-08-22

Animal assignment and treatment:
Three female rabbits were used for this test. Both eyes of each animal were examined before administration.

An amount of 0.1 g of glyphosate technical was placed into the conjunctival sac of one eye. The eyelids were gently held together for one second and then released to prevent loss of the article. The left eye served as control and remained untreated. The animals were restrained during the instillation of the test substance and for a further hour after instillation.

At 1, 24, 48 and 72-hours and daily thereafter until Day 8 post-instillation of the test substance, all eyes were inspected for irritation according to the Draize criteria. Clinical signs on cornea, iris and conjunctivae, as well as chemosis were recorded and investigated for their reversibility. Furthermore, the animals were

observed for lesions and toxic effects.

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed during the study.

C. EYE OBSERVATIONS

All animals showed signs of opacity (up to grade 2) from 1 hour after instillation, which had resolved until Day 8 of the study. One rabbit showed a reaction on the iris beginning 48 hours after treatment and persisting until Day 7. All three rabbits showed some degree of conjunctival redness, which cleared bay Day 7. Chemosis was observed in all animals beginning 1 hour after treatment and returning to normal after 48 hours. All signs of irritation were clear by Day 8.

A summary of the findings is given in the table below.

Table 5.2.5-19 Acute eye irritation/corrosion of glyphosate technical in the rabbit ([redacted] 1990): Eye irritation – Individual irritation scores

Animal	Scoring [h]	Cornea opacity	Iris	Conjunctiva	
				Redness	Chemosis
BL-623 (female)	1	1	0	0	3
	24	**1**	**0**	**1**	**1**
	48	**1**	**0**	**1**	**1**
	72	**1**	**0**	**1**	**0**
	Day 4	1	0	1	0
	Day 5	1	0	1	0
	Day 6	0	0	1	0
	Day 7	0	0	0	0
	Day 8	0	0	0	0
Individual mean (24, 48, 72 h)		**1.00**	**0.00**	**1.00**	**0.67**
BL-628 (female)	1	1	0	0	3
	24	**1**	**0**	**1**	**1**
	48	**1**	**0**	**1**	**1**
	72	**1**	**0**	**1**	**0**
	Day 4	1	0	1	0
	Day 5	1	0	1	0
	Day 6	1	0	1	0
	Day 7	1	0	0	0
	Day 8	0	0	0	0
Individual mean (24, 48, 72 h)		**1.00**	**0.00**	**1.00**	**0.67**
BL-767 (female)	1	1	0	0	3
	24	**1**	**0**	**1**	**2**
	48	**2**	**1**	**1**	**1**
	72	**2**	**1**	**2**	**0**
	Day 4	2	2	2	0
	Day 5	2	2	2	0
	Day 6	1	1	1	0
	Day 7	1	0	0	0
	Day 8	0	0	0	0

Table 5.2.5-19 Acute eye irritation/corrosion of glyphosate technical in the rabbit ([redacted] 1990): Eye irritation – Individual irritation scores

Animal	Scoring [h]	Cornea opacity	Iris	Conjunctiva	
				Redness	Chemosis
Individual mean (24, 48, 72 h)		1.67	0.67	1.33	1.00

3. Assessment and conclusion

Assessment and conclusion by applicant:
Except the deviation of no treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application, no rinsing 1 hour after application and no reporting of environmental conditions, the study is in concordance with the current OECD TG 405 (2017). As the lack of rinsing is considered as worst-case, this deviation is considered to compromise the outcome of the study. Nevertheless, the study is considered acceptable and the outcome can be reported as valid.

Instillation of glyphosate into the rabbit eye elicited slight to moderate response in treated animals. The individual mean scores at 24, 48 and 72-hours for the animal were 1.00, 1.00, 1.67 for corneal opacity, 0.00, 0.00, 0.67 for iris lesions, 1.00, 1.00, 1.33 for conjunctival redness, and 0.67, 0.67, 1.00 for conjunctival chemosis.
Thus, under test conditions of the study, glyphosate is considered as irritating to the eyes.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.5/018
Report author	[redacted]
Report year	1989
Report title	Glyphosate technical: Primary eye irritation test in rabbits
Report No	5886
Document No	243268
Guidelines followed in study	US EPA 81-4
Deviations from current test guideline (OECD 405, 2017)	No treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application. Observation period terminated after 4 days instead of 21 days. Age and weight of animals and number of air changes per hour not specified.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

In an eye irritation study, 0.1 g of the undiluted test substance (glyphosate technical, Batch: 206-Jak-25-1, Purity: 98.6 %) was instilled into the right conjunctival sac of one New Zealand White rabbit. Following treatment, eye irritation was scored using the Draize scheme at 1, 24, 48, 72-hours and 4 days. The study was terminated after 4 days, and due to the degree of eye irritation observed in this initial animal, no further animals were treated.

Application of glyphosate technical into the rabbit eye resulted in corneal opacity, effects on the iris and conjunctivae which all resolved after 72-hours, except for corneal opacity which persisted until the end of the study period. The individual mean irritation scores (24 – 72-hours) of the rabbit were as follows:

- for corneal opacity: 1.00
- for iris lesions: 1.00
- for conjunctival redness: 2.00
- for chemosis of the conjunctiva: 2.00

Based on the study, glyphosate has the potential to causes serious eye damage to the eye under the chosen test conditions.

Materials and methods

A: Materials

1. Test material:

Identification:	Glyphosate Technical (PMG)
Description:	White powder
Lot/Batch #:	206-Jak-25-1
Purity:	98.6 %
Stability of test compound:	Not specified

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Young adult (not further specified)
Sex:	Males and females (nulliparous and non-pregnant)
Weight at dosing:	Not specified
Acclimation period:	6 days
Diet/Food:	Standard rabbit diet (Special Diets Services, Witham, Essex, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in aluminium cages with grid floors beneath which were peat moss filled trays
Environmental conditions:	Temperature: 17 – 19 °C Humidity: 61 % (mean) Air changes: Not specified Photoperiod: 12 hours light / dark cycle

B: Study design and methods

In life dates: 1989-06-26 to 1988-07-01

Animal assignment and treatment:

Three male and three female rabbits were prepared for the study. Both eyes of all animals were examined 24-hours before administration. One rabbit was treated first.

An amount of 0.1 g of glyphosate technical was placed into the right conjunctival sac of one eye. The eyelids were gently held together for one second and then released to prevent loss of the article. The left eye served as control and remained untreated.

At 1, 24, 48, 72-hours and 4 days, the rabbit's eyes were examined for signs of irritation, using a Panoramic Loupe and pen torch. Effects on cornea, iris and conjunctivae (redness and chemosis) were scored using the Draize criteria. Due to severe irritating effects, the remaining five animals were not treated.

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed during the study.

C. EYE OBSERVATIONS

The rabbit showed signs corneal opacity, covering half the eye, up to 48 hours after treatment. Effects on the iris were observed between 24-hours and 72-hours. In addition, conjunctival redness and chemosis were observed between 1 hour and 72-hours after treatment. A slight to moderate discharge was seen up to 72-hours. The observations resolved after 72-hours, except for corneal opacity which persisted until termination of the study (Day 4). Because of the degree of response, no further animals were treated.

A summary of the findings is given in the table below.

Table 5.2.5-20 Glyphosate technical: Primary eye irritation test in rabbits. ([redacted], 1989): Eye irritation – Individual irritation scores

Animal No.	Scoring [h]	Cornea		Iris	Conjunctiva		
		Opacity	Area		Redness	Chemosis	Discharge
1 (male)	1	D [a]	0	0	2	2	1
	24	**D** [a]	0	**1**	**2**	**3**	2
	48	**1**	2	**1**	**2**	**2**	1
	72	**1**	2	**1**	**2**	**1**	1
	Day 4	**1**	2	**0**	**0**	**0**	0
Individual mean (24, 48, 72 h)		**1.00** [b]	**-**	**1.00**	**2.00**	**2.00**	-

[a] Dullness of cornea over whole area
[b] Mean of 48 and 72 as no value was given for 24 h

3. Assessment and conclusion

Assessment and conclusion by applicant:

Except the deviation of no treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application, the study is in concordance with the current OECD TG 405 (2017). This deviation is considered not to compromise the outcome of the study. Therefore, the study is considered acceptable and the outcome can be reported as valid.

Instillation of glyphosate into the rabbit eye elicited a strong response in the treated animal. The individual mean scores at 24 and 72-hours for the animals were 1.00 for corneal opacity and iris lesions, and 2.00 for conjunctival redness and conjunctival chemosis.

Thus, under test conditions of the study, glyphosate is considered to causes serious eye damage.

Assessment and conclusion by RMS:

2. Information on the study

Data point:	CA 5.2.5/019
Report author	[redacted]
Report year	1989
Report title	Primary eye irritation with glyphosate technical (isopropylamine salt 62 % in water equivalent to 46 % of N-phosphonomethylglycine acid) in the rabbit (rinsed / unrinsed)
Report No	238083
Document No	[redacted]423
Guidelines followed in study	Not reported
GLP	Not reported
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	The eyes of three males and three females were treated with 0.1 mL test substance (glyphosate salt, purity 62 %). The eyes of the females were rinsed while the eyes of the males remained unrinsed.
Short description of results:	Redness of the conjunctivae observed 24-hours after treatment.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	Full study report is not available to the applicant. Only a short summary is provided in the Monograph, B5, 2000, *Category 4b*
Reasons why the study report is not available for submission	The notifier has not access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance" (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL.
Category study in AIR 5 dossier (L docs)	Category 4b

1. Information on the study

Data point:	CA 5.2.5/020
Report author	[redacted]
Report year	1988
Report title	Primary Eye Irritation Study of Glyphosate Batch/Lot/NBR No. XLI-

	55 in New Zealand White Rabbits
Report No	88.2053.009
Document No	Not reported
Guidelines followed in study	US EPA 81-4 (1984)
Deviations from current test guideline (OECD 405, 2017)	No treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application. Six animals were dosed at once instead of three animals in a step wise approach as required by the current OECD guideline 405 (2017). Irrigation took place after 24-hours instead of after one hour.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

In an eye irritation study, 0.1 g of the undiluted glyphosate (Batch: XLI-55, Purity: 97.76 %) was instilled into the conjunctival sac of one eye of six young adult New Zealand rabbits whereas the second eye served as control. Animals were observed for 21 days. Eye irritation was scored using the Draize scheme 1, 24, 48 and 72-hours and 7, 14 and 21 days after test substance instillation.

Under the conditions of this study, glyphosate produced corneal opacity and conjunctival irritation with blistering in all rabbits after test material instillation. Three rabbits exhibited pannus on the cornea, one rabbit had prominent vascularization of the conjunctiva, and another animal had a blood-like discharge. One rabbit was found dead 20 days after dose administration. However, this death was not considered treatment related. Corneal opacity persisted through study day 21 (termination) in three of five (3/5) animals. Of the remaining two rabbits, one exhibited slight conjunctival discharge at study termination and the other rabbit's treated eye appeared normal 14 days after dose administration. The individual mean irritation scores (24 – 72-hours) of the six rabbits were as follows:

- for corneal opacity: 2.67, 1.67, 2.00, 1.00, 2.33, 2.67
- for iris lesions: 0.00, 0.00, 1.00, 0.00, 0.00, 0.00
- for conjunctival redness: 2.00, 2.00, 2.00, 2.00, 2.00, 2.00
- for conjunctival chemosis: 2.00, 3.33, 3.33, 2.67, 2.00, 2.00

Based on the study, glyphosate TC was seriously damaging to the eyes under the chosen test conditions.

Materials and methods

A: Materials

1. Test material:

Identification:	Glyphosate
Description:	White powder
Lot/Batch #:	XLI-55
Purity:	97.76 %
Stability of test compound:	Stored at room temperature

2. **Vehicle and/or positive control:** None

3. **Test animals:**

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Young adult
Sex:	Males and females
Weight at dosing:	Between 2 – 3 kg
Acclimation period:	At least five days
Diet/Food:	NIH 09 Rabbit Ration certified feed (Zeigler Brothers, Gardners, PA, US), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in wire mesh cages
Environmental conditions:	Temperature: 20 – 23.9 °C Humidity: 40 – 60 % Air changes: Not specified Photocycle: 12 hours light / dark cycle

B: STUDY DESIGN AND METHODS

In life dates: 1988-04-11 to 1988-05-02

Animal assignment and treatment:

The test was conducted using six young adult New Zealand albino rabbits. The test substance (0.1 g) was instilled into one eye of each rabbit. The lower eyelid was pulled gently away from the eyeball to form a cup (conjunctival sac) and the test substance inserted therein. The lids were then held together for one second and released. Following scoring at 24-hours after dose administration, any residual material was rinsed from the eye with physiological saline. Treated and untreated eyes were examined at 1, 24, 48 and 72-hours and 7, 14 and 21 days after test substance instillation. The cornea, iris, and conjunctiva were scored separately according to the Draize system. The animals were observed twice daily for mortality at least five hours apart. Body weights were determined on study day 1 prior to dose administration and at death. At study termination, surviving animals were sacrificed.

Results

A. MORTALITY

One rabbit was found dead 20 days after dose administration. Prior to death, this animal exhibited anorexia, and gross necropsy revealed a clear gel-like substance in the large intestine. These findings are consistent with mucoid enteropathy, a condition occasionally noted in stock laboratory rabbits. Therefore, the death was considered spontaneous and unrelated to treatment.

B. CLINICAL OBSERVATIONS

Not reported.

C. BODY WEIGHT

Not reported.

D. NECROPSY

Not reported.

E. EYE OBSERVATIONS

At one hour after test substance instillation, all animals exhibited conjunctival irritation (redness, swelling, blistering and discharge). Corneal opacity was noted one hour after test substance instillation in four of six animals. Corneal opacity and conjunctival irritation were noted in all rabbits at the 24, 48 and 72-hour and 7 day examinations. Three rabbits exhibited pannus on the cornea; two eyes (iris) had sluggish reactions to light; one rabbit had prominent vascularisation of the conjunctival, and another animal had a blood-like discharge. Corneal opacity persisted through study termination (Day 21) in three of five rabbits. Of the remaining two rabbits, one exhibited slight conjunctival discharge at study termination and the other rabbit's treated eye appeared normal 14 days after dose administration.

The individual mean irritation scores (24 – 72-hours) were calculated to be 2.67, 1.67, 2.00, 1.00, 2.33 and 2.67 for corneal opacity, 0.00, 0.00, 1.00, 0.00, 0.00, 0.00 for iris lesions, 2.00 for all animals for conjunctival redness, and 2.00, 3.33, 3.33, 2.67, 2.00, 2.00 for conjunctival chemosis. The individual scores for each time point, individual mean and group mean scores (24 – 72-hours) are presented in the table below.

Table 5.2.5-21 Primary Eye Irritation Study of Glyphosate Batch/Lot/NBR No. XLI-55 in New Zealand White Rabbits ([redacted] 1988c): Eye irritation – Individual irritation scores

Animal No.	Scoring[a] [h]	Cornea		Iris	Conjunctivae		
		Opacity	Area		Redness	Chemosis	Discharge
Rabbit 1 (88 – 1181)	1	2	1	0	2	2 [b]	2
	24	**2**	3	**0**	**2**	**2 [b]**	2
	48	**3**	1	**0**	**2**	**2 [b]**	2 [c]
	72	**3 [d]**	1	**0**	**2**	**2 [b]**	2
	7 days	3 [d]	1	0	2	1	0
	14 days	2	1	0	1	0	0
	21 days	2	1	0	0	0	0
Individual mean 24-72 h		**2.67**	**---**	**0.00**	**2.00**	**2.00**	**---**
Rabbit 2 (88 – 1182)	1	2	1	0	2	2 [b]	3
	24	**2**	2	**0**	**2**	**4 [b]**	3
	48	**2**	2	**0**	**2**	**4 [b]**	2
	72	**1**	1	**0**	**2**	**2 [b]**	1
	7 days	2	1	0	1	1	0
	14 days	2	1	0	0	0	1
	21 days	0	0	0	0	0	1
Individual mean 24-72 h		**1.67**	**---**	**0.00**	**2.00**	**3.33**	**---**
Rabbit 3 (88 – 1183)	1	2	1	0	2	2 [b,e]	2
	24	**2**	2	**1**	**2**	**4 [b,e]**	2
	48	**2**	1	**1**	**2**	**3 [b,e]**	2
	72	**2**	1	**1**	**2**	**3 [b,e]**	2
	7 days	3 [d]	2	1	3	2 [b]	2
	14 days	2	1	1	1	0	0
	21 days	2	1	0	0	0	0
Individual mean 24-72 h		**2.00**	**---**	**1.00**	**2.00**	**3.33**	**---**
Rabbit 4 (88 – 1258)	1	0	0	0	2	3 [b]	3
	24	**1**	4	**0**	**2**	**4 [b]**	3

Table 5.2.5-21 Primary Eye Irritation Study of Glyphosate Batch/Lot/NBR No. XLI-55 in New Zealand White Rabbits ([redacted] 1988c): Eye irritation – Individual irritation scores

Animal No.	Scoring[a] [h]	Cornea		Iris	Conjunctivae		
		Opacity	Area		Redness	Chemosis	Discharge
	48	**1**	3	**0**	**2**	**2** [b]	2
	72	**1**	3	**0**	**2**	**2** [b]	2
	7 days	3	1	0	2	2	1
	14 days	0	0	0	0	0	0
	21 days	0	0	0	0	0	0
Individual mean 24-72 h		**1.00**	**---**	**0.00**	**2.00**	**2.67**	**---**
Rabbit 5 (88 – 1260)	1	0	0	0	2	2 [b]	2
	24	**2**	2	**0**	**2**	**2** [b]	2
	48	**2**	2	**0**	**2**	**2** [b]	2
	72	**3**	1	**0**	**2**	**2** [b]	1
	7 days	4 [d]	1	1	2	2	1
	14 days	3 [d]	1	1	2	1	0
	21 days	3 [d]	1	0	1	0	0
Individual mean 24-72 h		**2.33**	**---**	**0.00**	**2.00**	**2.00**	**---**
Rabbit 6 (88 – 1261)	1	2	1	0	2	2 [b]	3
	24	**2**	2	**0**	**2**	**2** [b]	2
	48	**3**	1	**0**	**2**	**2** [b]	2
	72	**3**	1	**0**	**2**	**2** [b]	0
	7 days	2	1	0	1	1	0
	14 days	2	1	0	0	0	0
	21 days	- [f]	-	-	-	-	-
Individual mean 24-72 h		**2.67**	**---**	**0.00**	**2.00**	**2.00**	**---**
Group mean 24 – 72 h		**2.06**	**---**	**0.17**	**2.00**	**2.56**	**---**

[a] Scores for treated eyes; untreated eyes appeared normal at all times.
[b] Mucus membrane of the eyelid appeared blistered.
[c] Blood-like discharge noted.
[d] Pannus on the cornea.
[e] Prominent vascularisation of the conjunctiva.
[f] Animal found dead 20 days after dose administration.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Except the deviation of no treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application and some minor deviations the study is in concordance with the current OECD TG 405 (2017). These deviations did not compromise the acceptability of the study. Therefore, the study is considered acceptable and the outcome can be reported as valid.

Instillation of glyphosate into the rabbit eye elicited strong response in treated animals. The individual mean scores over 24, 48 and 72-hours for each animal were calculated to be 2.67, 1.67, 2.00, 1.00, 2.33 and 2.67 for corneal opacity, 0.00, 0.00, 1.00, 0.00, 0.00, 0.00 for iris lesions, 2.0 for all animals for conjunctival redness and 2.00, 3.33, 3.33, 2.67, 2.00, 2.00 for conjunctival chemosis. Some effects were not reversible within 21 days.

Thus, glyphosate is considered to have the potential to seriously damage the eye.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.5/021
Report author	[redacted]
Report year	1987
Report title	Primary eye irritation study of MON 8722 in New Zealand White rabbits
Report No	9307A
Document No	Not reported
Guidelines followed in study	US EPA 81-4 (1982)
Deviations from current test guideline (OECD 405, 2017)	No anaesthetic was given prior, during or after dosing to reduce the pain. All animals were tested at the same time instead of using a tiered testing approach. Sex of the animals was not specified. Mean values had to be recalculated based on the current CLP regulation (EC No. 1272/2008).
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

In an eye irritation study, six New Zealand White rabbits were treated with glyphosate sodium salt (MON 8722) by instillation of 0.1 g of the undiluted test substance into the conjunctival sac of one eye of each animal. The eyes were rinsed 24-hours after treatment with physiological saline. Following treatment, eye irritation was scored using the Draize scheme at 1, 24, 48 and 72-hours. Furthermore, the animals were observed for mortality and systemic toxicity daily. At the end of the study period, all animals were killed by intracardiac injection of sodium pentorbarbital.

Instillation of MON 8722 into the rabbit eye resulted in effects on the conjunctivae in all animals, which resolved within 48 hours. The individual mean irritation scores (24 – 72-hours) of the rabbits were as follows:

- for corneal opacity: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00
- for iris lesions: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00
- for conjunctival redness: 0.33, 0.00, 0.00, 0.00, 0.00, 0.00
- for chemosis of the conjunctiva: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00

Based on the study, glyphosate sodium salt is not irritating to the eye under the test conditions chosen.

Materials and methods

A. Materials

1. Test material:

Identification: Glyphosate sodium salt (MON 8722)
Description: White powder
Lot/Batch #: XLG-256

Purity:	70.7 %
Stability of test compound:	Not specified

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Young adult (not further specified)
Sex:	Not specified
Initial body weight:	1.8 – 2.3 kg
Acclimation period:	At least 5 days
Diet/Food:	NIH 09 rabbit ration, certified feed, Zeigler Brothers, Inc., Gardners, PA, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in wire-mesh cages
Environmental conditions:	Temperature: 20 – 23 °C Humidity: 40 – 60 % Air changes: Not specified Photoperiod: 12 hours light / dark cycle

B: Study design and methods

In life dates: 1986-11-05 to 1986-11-08

Animal assignment and treatment:

Both eyes of all six animals were examined for lesions with fluorescein sodium and an ultraviolet lamp 24-hours before administration.

An amount of 0.1 g of the test substance was placed into the conjunctival sac of one eye of each animal. The eyelids were gently held together for one second and then released to prevent loss of the article. The left eye served as control and remained untreated. Twenty-four hours after the treatment and after scoring, the eyes were rinsed with physiological saline to remove any remaining test material.

At 1, 24, 48 and 72-hours all eyes were examined for signs of irritation. Effects on cornea, iris, and conjunctivae (redness, chemosis, and discharge) were scored using the Draize criteria. In addition, all animals were observed for mortality and systemic toxicity, daily. The body weights were measured immediately prior to dosing on study day 1. All animals were euthanized by intracardiac injection of sodium pentobarbital at the end of the study period.

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed during the study.

C. EYE OBSERVATIONS

No effects on the cornea an on the iris were observed in any animal. Conjunctival redness, chemosis, and discharge were seen in all animals after 1 hour, however these observations resolved within 48 hours. In addition, blistering of the mucus membrane appeared blistered at 1 hour only. All effects were reversible.

A summary of the findings is given in the table below.

Table 5.2.5-22 Primary eye irritation study of MON 8722 in New Zealand White rabbits ([redacted] 1987b): Eye irritation – Individual irritation scores

Animal No.	Scoring [h]	Cornea		Iris	Conjunctiva		
		Opacity	Area		Redness	Chemosis	Discharge
86-3355	1	0	0	0	2	2 [a]	1
	24	**0**	0	**0**	**1**	**0**	0
	48	**0**	0	**0**	**0**	**0**	0
	72	**0**	0	**0**	**0**	**0**	0
Individual mean (24, 48, 72 h)		**0.00**	**-**	**0.00**	**0.33**	**0.00**	-
86-3358	1	0	0	0	2	2 [a]	1
	24	**0**	0	**0**	**0**	**0**	0
	48	**0**	0	**0**	**0**	**0**	0
	72	**0**	0	**0**	**0**	**0**	0
Individual mean (24, 48, 72 h)		**0.00**	**-**	**0.00**	**0.00**	**0.00**	-
86-3359	1	0	0	0	2	2 [a]	2
	24	**0**	0	**0**	**0**	**0**	0
	48	**0**	0	**0**	**0**	**0**	0
	72	**0**	0	**0**	**0**	**0**	0
Individual mean (24, 48, 72 h)		**0.00**	**-**	**0.00**	**0.00**	**0.00**	-
86-3365	1	0	0	0	2	1 [a]	1
	24	**0**	0	**0**	**0**	**0**	0
	48	**0**	0	**0**	**0**	**0**	0
	72	**0**	0	**0**	**0**	**0**	0
Individual mean (24, 48, 72 h)		**0.00**	**-**	**0.00**	**0.00**	**0.00**	-
86-3366	1	0	0	0	2	1 [a]	1
	24	**0**	0	**0**	**0**	**0**	0
	48	**0**	0	**0**	**0**	**0**	0
	72	**0**	0	**0**	**0**	**0**	0
Individual mean (24, 48, 72 h)		**0.00**	**-**	**0.00**	**0.00**	**0.00**	-
86-3367	1	0	0	0	1	1 [a]	1
	24	**0**	0	**0**	**0**	**0**	0
	48	**0**	0	**0**	**0**	**0**	0
	72	**0**	0	**0**	**0**	**0**	0
Individual mean (24, 48, 72 h)		**0.00**	**-**	**0.00**	**0.00**	**0.00**	-

[a] Mucus membrane appeared blistered.

3. Assessment and conclusion

Assessment and conclusion by applicant:
Except the deviation of no treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application, the study is in concordance with the current OECD TG 405 (2017). This deviation is considered not to compromise the outcome of the study. Therefore, the study is considered acceptable and the outcome can be reported as valid.
Instillation of glyphosate into the rabbit eye elicited slight response in treated animals. The individual mean scores at 24, 48 and 72-hours for all animals were 0.00 for corneal opacity, iris lesions, and conjunctival chemosis and 0.33, 0.00, 0.00, 0.00, 0.00, 0.00 for conjunctival redness.
Thus, under test conditions of the study, glyphosate sodium salt is considered as not irritating to the eyes.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.5/022
Report author	[redacted]
Report year	1987
Report title	Primary eye irritation study of MON-8750 in New Zealand White rabbits
Report No	[redacted]-86-431/9308A
Document No	Not reported
Guidelines followed in study	US EPA 81-4 (1982)
Deviations from current test guideline (OECD 405 (2017))	No anaesthetic was given prior, during or after dosing to reduce the pain. All animals were tested at the same time instead of using a tiered testing approach. Sex and age of the animals, and number of air changes were not specified.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

In an eye irritation study, six New Zealand White rabbits were treated with glyphosate (MON 8750, Batch: XLG-255, Purity: 90.8 %) by instillation of 0.1 g of the undiluted test substance into the conjunctival sac of one eye of each animal. The eyes were rinsed 24-hours after treatment with physiological saline. Following treatment, eye irritation was scored using the Draize scheme at 1, 24, 48 and 72-hours. Furthermore, the animals were observed for mortality and systemic toxicity daily. At the end of the study period, all animals were killed by intracardiac injection of sodium pentorbarbital.

Instillation of MON 8750 into the rabbit eye resulted in effects on the conjunctivae, which all resolved within 72-hours. The individual mean irritation scores (24 – 72-hours) of the rabbits were as follows:

- for corneal opacity: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00
- for iris lesions: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00

- for conjunctival redness: 0.33, 0.67, 0.33, 0.33, 0.67, 0.33
- for chemosis of the conjunctiva: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00

Based on the study, glyphosate is not irritating to the eye under the chosen test conditions.

Materials and methods

A: Materials

1. Test material:

Identification:	Glyphosate (MON 8750)
Description:	White powder
Lot/Batch #:	XLG-255
Purity:	90.8 %
Stability of test compound:	Not specified

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Young adult (not further specified)
Sex:	Not specified
Initial body weight:	1.8 – 2.3 kg
Acclimation period:	At least 5 days
Diet/Food:	NIH 09 Rabbit Ration, certified feed (Zeigler Brothers, Inc., Gardners, PA, US), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in wire-mesh cages
Environmental conditions:	Temperature: 20 – 23 °C Humidity: 40 – 60 % Air changes: Not specified Photoperiod: 12 hours light / dark cycle

B: Study design and methods

In life dates: 1986-11-05 to 1986-11-05

Animal assignment and treatment:

Both eyes of all six animals were examined for lesions with fluorescein sodium and an ultraviolet lamp 24-hours before administration.

An amount of 0.1 g of the test substance was placed into the conjunctival sac of one eye of each animal. The eyelids were gently held together for one second and then released to prevent loss of the article. The left eye served as control and remained untreated. Twenty-four hours after the treatment and after scoring, the eyes were rinsed with physiological saline to remove any remaining test material.

At 1, 24, 48 and 72-hours all eyes were examined for signs of irritation. Effects on cornea, iris, and conjunctivae (redness, chemosis, and discharge) were scored using the Draize criteria. In addition, all animals were observed for mortality and systemic toxicity daily. The body weights were measured immediately prior to dosing on study day 1. All animals were euthanized by intracardiac injection of sodium pentobarbital at the end of the study period.

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed during the study.

C. EYE OBSERVATIONS

No effects on the cornea an on the iris were observed in any animal. Conjunctival redness, chemosis and discharge were seen in all animals after 1 hour, however these observations resolved within 72-hours. In addition, blistering of the mucus membrane appeared blistered at 1 hour only. All effects were reversible.

A summary of the findings is given in the table below.

Table 5.2.5-23 Primary eye irritation study of MON-8750 in New Zealand White rabbits ([redacted] 1987a): Eye irritation – Individual irritation scores

Animal No.	Scoring [h]	Cornea		Iris	Conjunctiva		
		Opacity	Area		Redness	Chemosis	Discharge
86-3368	1	0	0	0	1	1 [a]	1
	24	**0**	0	**0**	**1**	**0**	0
	48	**0**	0	**0**	**0**	**0**	0
	72	**0**	0	**0**	**0**	**0**	0
Individual mean (24, 48, 72 h)		**0.00**	-	**0.00**	**0.33**	**0.00**	-
86-3372	1	0	0	0	2	2 [a]	2
	24	**0**	0	**0**	**1**	**0**	1
	48	**0**	0	**0**	**1**	**0**	0
	72	**0**	0	**0**	**0**	**0**	0
Individual mean (24, 48, 72 h)		**0.00**	-	**0.00**	**0.67**	**0.00**	-
86-3372	1	0	0	0	2	2 [a]	1
	24	**0**	0	**0**	**1**	**0**	0
	48	**0**	0	**0**	**0**	**0**	0
	72	**0**	0	**0**	**0**	**0**	0
Individual mean (24, 48, 72 h)		**0.00**	-	**0.00**	**0.33**	**0.00**	-
86-3374	1	0	0	0	2	1 [a]	1
	24	**0**	0	**0**	**1**	**0**	0
	48	**0**	0	**0**	**0**	**0**	0
	72	**0**	0	**0**	**0**	**0**	0
Individual mean (24, 48, 72 h)		**0.00**	-	**0.00**	**0.33**	**0.00**	-
86-3375	1	0	0	0	2	1 [a]	2
	24	**0**	0	**0**	**1**	**0**	0
	48	**0**	0	**0**	**1**	**0**	0

Table 5.2.5-23 Primary eye irritation study of MON-8750 in New Zealand White rabbits ([redacted] 1987a): Eye irritation – Individual irritation scores

Animal No.	Scoring [h]	Cornea		Iris	Conjunctiva		
		Opacity	Area		Redness	Chemosis	Discharge
	72	**0**	0	**0**	**0**	**0**	0
Individual mean (24, 48, 72 h)		**0.00**	**-**	**0.00**	**0.67**	**0.00**	-
86-3376	1	0	0	0	2	2 [a]	2
	24	**0**	0	**0**	**1**	**0**	0
	48	**0**	0	**0**	**0**	**0**	0
	72	**0**	0	**0**	**0**	**0**	0
Individual mean (24, 48, 72 h)		**0.00**	**-**	**0.00**	**0.33**	**0.00**	-

[a] Mucus membrane appeared blistered.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Except the deviation of no treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application, the study is in concordance with the current OECD TG 405 (2017). This deviation is considered not to compromise the outcome of the study. Therefore, the study is considered acceptable and the outcome can be reported as valid.

Instillation of glyphosate into the rabbit eye elicited slight response in treated animals. The individual mean scores at 24, 48 and 72-hours for the animal were 0.00 for corneal opacity, iris lesions and conjunctival chemosis, and 0.33, 0.67, 0.33, 0.33, 0.67, 0.33 for conjunctival redness.

Thus, under test conditions of the study, glyphosate is considered as not irritating to the eyes.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.5/023
Report author	[redacted]
Report year	1983
Report title	Mucous membrane irritation test on rabbits with glyphosate (tech) of excel industries Ltd.
Report No	Not reported
Document No	Not reported
Guidelines followed in study	None
Deviations from current test guideline (OECD 405, 2017)	No anaesthetic was given prior, during or after dosing to reduce the pain. All animals were tested at the same time instead of using a tiered testing approach. Environmental conditions, housing, animal age, acclimation period, and dates of the study not specified. Eyes were washed out after 30 seconds instead of 1 hour. Irritation was not evaluated at 1 hour.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities[1,2]	No

Acceptability/Reliability:	Invalid
Category study in AIR 5 dossier (L docs)	Category 3b

2. Full summary

In an eye irritation study, six albino rabbits were treated with glyphosate (Batch: 8.7.83, Purity: 95 %) by instillation of 0.1 g of the undiluted test substance into the conjunctival sac of one eye of each animal. The other untreated eye served as control. The eyes of all animals were rinsed 30 seconds after treatment with water. Following treatment, eye irritation was scored using the Draize scheme at 24, 48, 72, 96 hours and at 7 and 15 days.

Instillation of glyphosate into the rabbit eye resulted in effects on the cornea, iris and conjunctivae in all animals which reversed latest by 96 hours. Though appropriate table explanations were missing in the study report, mean irritation scores (24 – 72-hours) have been derived using the standard Draize categories as follows:

- for corneal opacity: 0.33, 0.33, 0.33, 0.33, 0.00, 0.33
- for iris lesions: 0.33, 0.33, 0.33, 0.33, 0.33, 0.33
- for conjunctival redness: 1.00, 1.00, 0.67, 1.00, 0.67, 1.00
- for chemosis of the conjunctiva: 2.00, 1.33, 1.33, 1.00, 0.67, 1.00

Materials and methods

A: Materials

1. Test material:

Identification:	Glyphosate (Tech)
Description:	White amorphous powder
Lot/Batch #:	R&D sample, sample 8.7.83
Purity:	95 %
Stability of test compound:	Not specified

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rabbit
Strain:	NWS
Source:	[redacted]
Age:	Not specified
Sex:	Male and female
Initial body weight:	1.5 – 2.5 kg
Acclimation period:	Not specified
Diet/Food:	Lucerne grass, carrots, germinated grams with wheatbran, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually (not further specified)

Environmental conditions:	Temperature:	Not specified
	Humidity:	Not specified
	Air changes:	Not specified
	Photoperiod:	Not specified

B: Study design and methods

In life dates: Not specified

Animal assignment and treatment:

An amount of 0.1 g of the test substance was placed into the conjunctival sac of one eye of each animal (three males and three females). The other eye served as control and remained untreated. The eyes of all animals were rinsed with water 30 seconds after instillation of the test substance.

At 24, 48, 72, 96 hours, 7 and 15 days post-administration, all eyes were examined for signs of irritation. Effects on cornea, iris and conjunctivae (redness, chemosis and discharge) were scored using the Draize criteria.

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed during the study.

C. EYE OBSERVATIONS

After 24-hours, effects on the cornea were observed in five of six animals, and effects on iris and conjunctivae were observed in all animals. Effects on the cornea had resolved after 48 hours while effects on the iris and conjunctivae persisted until 72-hours. After 96 hours, no effects were observed in any animal. Though appropriate table explanations were missing in the study report, the table headings in the report have been presented in the table below according to the standard Draize categories (see table footnote). Mean values are derived from the parameters as presented here.

A summary of the findings is given in the table below.

Table 5.2.5-24 Mucous membrane irritation test on rabbits with glyphosate ([redacted] 1983): Eye irritation in rabbits – Individual irritation scores

Animal	Scoring [h]	Cornea [a]		Iris	Conjunctiva [b]		
		Opacity	Area		Redness	Chemosis	Discharge
Rabbit 1	**24**	**1**	1	**1**	**2**	**3**	2
	48	**0**	0	**0**	**1**	**2**	2
	72	**0**	0	**0**	**0**	**1**	1
	96	0	0	0	0	0	0
	Day 7	0	0	0	0	0	0
	Day 15	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.33**	-	**0.33**	**1.00**	**2.00**	-
Rabbit 2	**24**	**1**	1	**1**	**2**	**3**	2

Table 5.2.5-24 Mucous membrane irritation test on rabbits with glyphosate ([redacted] 1983): Eye irritation in rabbits – Individual irritation scores

Animal	Scoring [h]	Cornea [a]		Iris	Conjunctiva [b]		
		Opacity	Area		Redness	Chemosis	Discharge
	48	**0**	0	**0**	**1**	**1**	2
	72	**0**	0	**0**	**0**	**0**	0
	96	0	0	0	0	0	0
	Day 7	0	0	0	0	0	0
	Day 15	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.33**	-	**0.33**	**1.00**	**1.33**	-
Rabbit 3	**24**	**1**	0	**1**	**1**	**3**	2
	48	**0**	0	**0**	**1**	**1**	1
	72	**0**	0	**0**	**0**	**0**	0
	96	0	0	0	0	0	0
	Day 7	0	0	0	0	0	0
	Day 15	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.33**	-	**0.33**	**0.67**	**1.33**	-
Rabbit 4	**24**	**1**	1	**1**	**2**	**2**	2
	48	**0**	0	**0**	**1**	**1**	2
	72	**0**	0	**0**	**0**	**0**	0
	96	0	0	0	0	0	0
	Day 7	0	0	0	0	0	0
	Day 15	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.33**	-	**0.33**	**1.00**	**1.00**	-
Rabbit 5	**24**	**0**	0	**1**	**1**	**1**	2
	48	**0**	0	**0**	**1**	**1**	1
	72	**0**	0	**0**	**0**	**0**	0
	96	0	0	0	0	0	0
	Day 7	0	0	0	0	0	0
	Day 15	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.00**	-	**0.33**	**0.67**	**0.67**	-
Rabbit 6	**24**	**1**	1	**1**	**2**	**2**	3
	48	**0**	0	**0**	**1**	**1**	1
	72	**0**	0	**0**	**0**	**0**	0
	96	0	0	0	0	0	0
	Day 7	0	0	0	0	0	0
	Day 15	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.33**	-	**0.33**	**1.00**	**1.00**	-

[a] Cornea scores labelled A and B in the report. In the Draize scale for scoring ocular lesions, A is for opacity and B is for the area of cornea involved. Therefore, the column headings of "Opacity" and "Area" are used in this table for data in the report labelled A and B, respectively.

[b] Conjunctivae scores are labelled A, B, and C in the report. In the Draize scale for scoring ocular lesions, A is for redness, B is for chemosis, and C is for discharge. Therefore, the column headings of "Redness", "Chemosis", and "Discharge" are used in this table for data in the report labelled A, B, and C, respectively.

3. Assessment and conclusion

Assessment and conclusion by applicant:
Except the deviation of no treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application, removal of the test substance after 30 seconds and the missing possibitlity for calculation of individual mean scores, the study is in concordance with the current OECD TG 405 (2017).
Due to the removal of the test substance after 30 seconds and as therefore, the irritating potential of glyphosate could not be estimated, the study is considered not acceptable.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.2.5/026
Report author	[redacted]
Report year	1981
Report title	Primary eye irritation of MON 0139 to rabbits
Report No	800260
Document No	[redacted]-80-261
Guidelines followed in study	None
Deviations from current test guideline (OECD 405, 2017)	No anaesthetic was given prior, during, or after dosing to reduce the pain. All animals were tested at the same time instead of using a tiered testing approach. Environmental conditions, housing, and diet were not specified. Test material not specified (purity, batch and stability) and eyes were washed out 20 seconds (three animals) or not rinsed (six animals) instead of 1 hour after treatment.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

In an eye irritation study, nine New Zealand White rabbits were treated with glyphosate (MON 0139, Batch: SSRT-11012, Purity: not specified) by instillation of 0.1 mL of the undiluted test substance into the conjunctival sac of the right eye of each animal. The eyes of three rabbits were rinsed 20 seconds after treatment with physiological saline. The eyes of the remaining six animals remained unwashed. Following treatment, eye irritation was scored using the Draize scheme at 24, 48 and 72-hours. A drop of fluorescein ophthalmic solution was placed onto the cornea, held there for 20 seconds and washed out with physiological saline.

Instillation of MON 0139 into the rabbit eye did not result in any effects on the cornea, iris or conjunctivae in any animal. The individual mean irritation scores (24 – 72-hours) of the rabbits (unwashed eyes) were as follows:

- for corneal opacity: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00
- for iris lesions: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00
- for conjunctival redness: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00
- for chemosis of the conjunctiva: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00
- for conjunctival discharge: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00

Based on the study, glyphosate was not irritating to the eye under the test conditions chosen.

Materials and methods

A: Materials

1. Test material:

Identification:	Glyphosate (MON 0139), IPA salt
Description:	Amber liquid
Lot/Batch #:	SSRT-11012
Purity:	Not specified
Stability of test compound:	Not specified

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Young adult
Sex:	Males and females
Initial body weight:	2.03 – 2.85 kg
Acclimation period:	At least 5 days
Diet/Food:	Not specified, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually (not further specified)
Environmental conditions:	Temperature: Not specified
	Humidity: Not specified
	Air changes: Not specified
	Photoperiod: Not specified

B: Study design and methods

In life dates: 1980-08-26 to 1980-08-29

Animal assignment and treatment:
Both eyes of all nine animals were examined for lesions with fluorescein sodium prior to administration of the test substance.

A volume of 0.1 mL of the test substance was placed into the conjunctival sac of the right eye of each animal. The eyelids were gently held together for one second and then released to prevent loss of the article. The left eye served as control and remained untreated. The eyes of three animals were rinsed with

physiological saline 20 seconds after instillation of the test substance. The eyes of the remaining six animals were not washed.

At 24, 48 and 72-hours all eyes were examined for signs of irritation. Therefore, a drop of fluorescein sodium ophthalmic solution was placed onto the cornea for 10 seconds before it was washed out with physiological saline. Effects on cornea, iris and conjunctivae (redness, chemosis and discharge) were scored using the Draize criteria.

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed during the study.

C. EYE OBSERVATIONS

No effects on the cornea, iris or conjunctivae were observed in any animal at any time point.
A summary of the findings is given in the table below.

Table 5.2.5-25 Primary eye irritation of MON 0139 to rabbits ([redacted] 1981): Eye irritation – Individual irritation scores

Animal	Scoring [h]	Cornea opacity	Iris	Conjunctiva		
				Redness	Chemosis	Discharge
Unwashed eyes						
Rabbit 1 (male)	**24**	0	0	0	0	0
	48	0	0	0	0	0
	72	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.00**	**0.00**	**0.00**	**0.00**	**-**
Rabbit 2 (male)	**24**	0	0	0	0	0
	48	0	0	0	0	0
	72	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.00**	**0.00**	**0.00**	**0.00**	**-**
Rabbit 3 (male)	**24**	0	0	0	0	0
	48	0	0	0	0	0
	72	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.00**	**0.00**	**0.00**	**0.00**	**-**
Rabbit 4 (female)	**24**	0	0	0	0	0
	48	0	0	0	0	0
	72	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.00**	**0.00**	**0.00**	**0.00**	**-**
Rabbit 5 (female)	**24**	0	0	0	0	0
	48	0	0	0	0	0
	72	0	0	0	0	0

Table 5.2.5-25 Primary eye irritation of MON 0139 to rabbits (1981): Eye irritation – Individual irritation scores

Individual mean (24, 48, 72 h)		**0.00**	**0.00**	**0.00**	**0.00**	**-**
Rabbit 6 (female)	**24**	0	0	0	0	0
	48	0	0	0	0	0
	72	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.00**	**0.00**	**0.00**	**0.00**	**-**
Washed eyes						
Rabbit 7 (male)	**24**	0	0	0	0	0
	48	0	0	0	0	0
	72	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.00**	**0.00**	**0.00**	**0.00**	**-**
Rabbit 8 (female)	**24**	0	0	0	0	0
	48	0	0	0	0	0
	72	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.00**	**0.00**	**0.00**	**0.00**	**-**
Rabbit 9 (female)	**24**	0	0	0	0	0
	48	0	0	0	0	0
	72	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.00**	**0.00**	**0.00**	**0.00**	**-**

3. Assessment and conclusion

Assessment and conclusion by applicant:
Except the deviation of no treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application and immediate or no rinsing of the eye, the study is in concordance with the current OECD TG 405 (2017). As the lack of rinsing is considered as worst-case, this deviation is not considered to compromise the negative outcome of the study. Therefore, the study is considered acceptable and the outcome can be reported as valid.

Instillation of glyphosate into the rabbit eye elicited no response in treated animals. The individual mean scores at 24, 48 and 72-hours for all animals were 0.00 for corneal opacity, iris lesions, conjunctival redness and conjunctival chemosis.
Thus, under test conditions of the study, glyphosate is considered as non-irritating to the eyes.

Assessment and conclusion by RMS:

CA 5.2.6 Skin sensitisation

A variety of skin sensitisation tests are available for glyphosate tested as acid or IPA/sodium salt. Two LLNA assays, eight Buehler tests as well as 12 GPMT tests were performed with glyphosate. Neither glyphosate acid nor the salts have shown sensitizing effects in guinea pigs or mice. Glyphosate is considered to be not a skin sensitiser and therefore no classification for skin sensitisation is warranted.

Table 5.2.6-1: Studies on skin sensitisation with glyphosate

Annex Point	Study	Study type	Substance(s)	Reference list- related category§	Result
CA 5.2.6/001	[redacted] 2011	*in vivo*: CBA/J Rj mouse, ♀ (LLNA)	Glyphosate technical (Batch: 569753 (BX20070911), Purity: 96.3 % w/w)	Valid, Category 2a	Not sensitising
CA 5.2.6/002	[redacted] 2010	*in vivo*: Dunkin Hartley guinea pig, ♀ (Magnusson & Kligman Maximisation Test)	Glyphosate TC (Batch: 2009051501, Purity: 96.4 %)	Valid, Category 2a	Not sensitising
CA 5.2.6/003	[redacted] 2010	*in vivo*: Dunkin Hartley guinea pig, ♂ (Magnusson & Kligman Maximisation Test)	Glyphosate TC (Batch: 20090506, Purity: 97.3 %)	Valid#, Category 2a	Not sensitising
CA 5.2.6/004	[redacted] 2009	*in vivo*: Albino Dunkin Hartley, CRL:(AH)BR, SPF guinea pig, ♂ (Magnusson & Kligman Maximisation Test)	Glyphosate technical (Batch: GI-1045, Purity: 96.66 %)	Valid, Category 2a	Not sensitising
CA 5.2.6/005	[redacted] 2009	*in vivo*: Dunkin Hartley guinea pig, ♂ (Magnusson & Kligman Maximisation Test)	Glyphosate TC (Batch: 20080801, Purity: 98.8 %)	Valid#, Category 2a	Not sensitising
CA 5.2.6/006	[redacted], 2009	*in vivo*: Hartley-Albino guinea pigs, ♂/♀ (Buehler Test)	Glyphosate Tech Grade Mixed Batch (Batch: 080704-1 thru 5, Purity: 96.4 %)	Valid, Category 2a	Not sensitising
CA 5.2.6/007	[redacted], 2008	*in vivo*: Hartley guinea pigs, ♂ (Buehler Test)	Glyphosate technical (Batch: 20070606, Purity: 98.05 %)	Valid, Category 2a	Not sensitising
CA 5.2.6/008	[redacted] 2007	*in vivo*: Albino Dunkin Hartley, CRL:(HA)BR, SPF guinea pig, ♀ (Magnusson & Kligman Maximisation Test)	Glyphosate technical (NUP 05068) (Batch: 200609062, Purity: 95.1 %)	Valid, Category 2a	Not sensitising
CA 5.2.6/009	[redacted] 2007	*in vivo*: CBA/Ca/Ola/Hsd mouse, ♀ (LLNA)	Glyphosate technical material (Batch: 0507, Purity: 96.1 %)	Valid, Category 2a	Not sensitising
CA 5.2.6/010	[redacted] 2006	*in vivo*: Albino Dunkin Hartley guinea pig, ♀ (Magnusson & Kligman Maximisation Test)	Glyphosate Technical (Batch: H05H016A, Purity: 95.7 %)	Valid, Category 2a	Not sensitising
CA 5.2.6/011	[redacted] 2005	*in vivo*: Hartley albino guinea pig, ♂ / ♀ (Buehler Test)	Glyphosate acid technical (Batch: 040205, Purity: 97.23 %)	Valid, Category 2a	Not sensitising

Table 5.2.6-1: Studies on skin sensitisation with glyphosate

CA 5.2.6/012	[redacted], 1996	*in vivo*: Albino Cr1 (HA) BR guinea pig, ♀ (Buehler Test)	Glyphosate acid (Batch: P24, Purity: 95.6 %)	Valid, Category 2a	Not sensitising
CA 5.2.6/013	[redacted] 1995	*in vivo*: Hartley, Crj:Hartley guinea pig, ♀(Magnusson & Kligman Maximisation Test)	HR-001 (Batch: T-941209; Purity: 97.56 %)	Valid, Category 2a	Not sensitising
CA 5.2.6/014	[redacted], 1994	*in vivo*: Dunkin Hartley guinea pig, ♀ (Magnusson & Kligman Maximisation Test)	Glyphosate	Valid, Category 4a	Not sensitising
CA 5.2.6/015	[redacted], 1994	*in vivo*: Albino Dunkin-Hartley guinea pig, ♀ (Magnusson & Kligman Maximisation Test)	Glyphosate premix (Batch: 290-Jak-146-4; Purity: 62.2 % as glyphosate isopropylamine salt; 46.1 % as glyphosate)	Valid, Category 2a	Not sensitising
CA 5.2.6/016	[redacted], 1994	*in vivo*: Albino guinea pig, ♂ (Modified Buehler Test)	Glyphosate (Batch: 36300892; Purity: 97.2 %)	Valid, Category 2a	Not sensitising
CA 5.2.6/017	[redacted] 1993	*in vivo*: English guinea pig, ♂ / ♀ (Maximisation Test)	Glyphosate technical (Purity: 95 %)	Valid, Category 4a	Not sensitising
CA 5.2.6/018	[redacted] 1992	*in vivo*: Albino Dunkin-Hartley guinea pig, ♂ / ♀ (Modified Buehler Test)	MON 8722 (glyphosate, sodium salt) (Batch: RUD-9108-3241-F)	Valid, Category 2a	Not sensitising
CA 5.2.6/019	[redacted] 1991	Albino Dunkin-Hartley guinea pig, (Magnusson & Kligman Maximisation Test)	Glyphosate technical	Valid, Category 2a	Not sensitising
CA 5.2.6/020	[redacted] 1989	Albino Dunkin-Hartley guinea pig, ♀ (Magnusson & Kligman Maximisation Test)	Glyphosate technical (Batch: 206-Jak-25-1, Purity: 98.6 %)	Valid, Category 2a	Not sensitising
CA 5.2.6/021	[redacted] 1988	*in vivo*: Albino Dunkin-Hartley guinea pig, ♂ / ♀ (Modified Buehler Test)	MON 8750 (Batch: XLH-274; Purity: 86.2 % glyphosate acid)	Valid, Category 2a	Not sensitising
CA 5.2.6/022	[redacted] 1983	*in vivo*: Albino Hartley guinea pig, ♂ / ♀ (Buehler Test)	Glyphosate (Batch: NBP 1782608, Purity: 99.7 %)	Valid, Category 2a	Not sensitising

[#] performed at [redacted]

§: The category describes the acceptability/reliability of the study within the AIR 5 submission. This category does not describe hazard classification. (for details please refer to the Doc ID: 110054-B-GRG_Jun_2020)

1. Information on the study

Data point:	CA 5.2.6/001
Report author	[redacted]
Report year	2011
Report title	Glyphosate technical: Local lymph node assay in the mouse
Report No	10/218-037E
Document No	Not reported
Guidelines followed in study	OECD 429 (2010)
Deviations from current test guideline (OECD 429, 2010)	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

A sample of Glyphosate Technical (Batch: 569753; Purity: 96.3 %) was assessed for its skin sensitisation potential using the mouse Local Lymph Node Assay.

The test item solutions were applied on the dorsal surface of ears of young adult, female CBA/J Rj mice (25 µL/ear) for three consecutive days (Days 1, 2, and 3). On Day 6, five hours prior to termination animals were intravenously injected via the tail vein with tritiated methyl thymidine (^{3}HTdR). Cell proliferation in the local lymph nodes was measured by incorporation of ^{3}HTdR and the values obtained were used to calculate stimulation indices (SI).

Groups of 4 mice received 50, 25 or 10 % w/v glyphosate technical in propylene glycol (PG), PG alone (negative controls) or 25 % α-Hexylcinnamaldehyde in PG (positive controls).

No mortality, systemic toxicity or local irritation was observed during the study. No treatment related effects were observed on animal body weights in any treated groups.

Stimulation index values of the test item were 1.0, 1.0 and 1.2 at treatment concentrations of 50, 25 and 10 % (w/v), respectively.

A significant lymphoproliferative response (stimulation index value of 12.2) was noted for α-Hexylcinnamaldehyde in this experiment, confirming the validity of the protocol used for this study.

Therefore, based on the results, Glyphosate Technical is not considered a skin sensitiser in the Local Lymph Node Assay.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate Technical
Description:	Dry white powder
Lot/Batch number:	569753
Purity:	96.3 % w/w
CAS#:	Not reported
Stability of test compound:	Stable under storage conditions (2 - 8 °C); expiry date: 2011-08-31
2. Vehicle and/or positive control:	Propylene glycol (vehicle) / α-Hexylcinnamaldehyde (HCA) (positive control)
3. Test animals:	
Species:	Mice
Strain:	CBA/J Rj
Sex:	Female, nulliparous, non-pregnant
Age:	9 - 10 weeks
Weight at dosing:	20.1 - 21.6 g
Source:	[redacted]
Housing:	Group caging in Type II polypropylene/polycarbonate cages with Lignocel® Hygienic Animal Bedding and glass tunneltubes available to animals during the study
Acclimatisation period:	13 days
Diet:	ssniff® SM R/M-Z+H "Autoclavable complete feed for rats and mice – breeding and maintenance" produced by ssniff Spezialdiäten GmbH, 59494 Soest, Germany, *ad libitum*
Water:	Tap water, *ad libitum*
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 30 - 70 % Air changes: 15 – 20/hour Photocycle: 12 hours light/dark cycle

B: Study design and methods

In-life dates: 2010-10-20 to 2010-10-26

A sample of Glyphosate Technical (96.3 % w/w Glyphosate technical) was assessed for its skin sensitisation potential using the mouse Local Lymph Node Assay, following dermal exposure when administered topically to young adult, female CBA/J Rj mice.

Animal assignment and treatment:

Vehicle selection: During the preliminary compatibility test the solubility of the test item was examined. The test item was insoluble in all solvent but formed an achievable gel-like formulation in propylene glycol (PG) and DMSO. The formulation in PG seemed more stable, therefore it was selected as vehicle for the test. The achievable maximum concentration was 50 (w/v) %.

Dose selection rationale: A Preliminary Irritation/Toxicity Test was performed on CBA/J Rj mice using two doses, at test item concentrations of 50 and 25 % (w/v), respectively. This preliminary experiment was conducted in a similar experimental manner to the main study, but it was terminated on Day 6 without radioactive proliferation assay.

During the Preliminary Irritation/Toxicity Test no mortality, systemic toxicity or local irritation were observed. No treatment related effect on body weights was observed. The observations recorded in this preliminary test suggest that the formulations, the application of the material and the local effects on the animal are acceptable for a valid LLNA.

Based on the results of the preliminary experiments the following dose levels were selected for the main assay: 0 (negative control), 10, 25, and 50 % (w/v) Glyphosate Technical, and positive control (25 % HCA). Each group comprised four mice.

Table 5.2.6-1 Glyphosate technical: Local lymph node assay in the mouse ([redacted], 2011): Animal assignment to the treatment groups

Treatment group	Test item concentration (w/v) %	Number of animals per group
Negative control (PG*)	---	4
Glyphosate Technical	50	4
Glyphosate Technical	25	4
Glyphosate Technical	10	4
Positive control (25 % HCA** in PG*)	---	4

*: propylene glycol, vehicle

**: α-Hexylcinnamaldehyde

Treatment and observations:

Each animal was topically dosed once a day for three consecutive days (Days 1, 2, and 3) on the dorsal surface of each ear with 25 µL of the appropriate formulation, applied using a pipette. There was no treatment on Days 4 and 5.

Topical application:

All animals were observed at least once daily (Days 1 - 6) for any clinical signs, including local irritation and systemic toxicity. Individual body weights were recorded on Day 1 (beginning of the assay) and at Day 6 (prior to ^{3}HTdR injection).

Proliferation assay:

On Day 6 each mouse was intravenously injected via the tail vein with 250 µL of sterile PBS (phosphate buffered saline) containing approximately 20 µCi of ^{3}HTdR using a gauge 25G1" hypodermic needle with 1 mL sterile syringe.

Five hours after intravenous injection, the mice were sacrificed by CO_2 asphyxiation. The draining auricular lymph nodes were excised by making a small incision on the skin between the jaw and sternum, pulling the skin gently back towards the ears and exposing the lymph nodes. The nodes were then removed using forceps and the carcasses discarded. The nodes of mice from each test group was pooled and collected in separate Petri dishes containing a small amount (1 - 2 mL) of PBS to keep the nodes wet before processing.

A single cell suspension (SCS) of pooled lymph node cells (LNCs) were prepared and collected in disposable tubes by gentle mechanical disaggregating of the lymph nodes through a cell strainer using the plunger of a disposable syringe. The cell strainer was washed with PBS (up to 10 mL). Pooled LNCs were pelleted with a relative centrifugal force (RCF) of 190 x g (approximately) for 10 minutes at 4 °C. After centrifugation supernatants were discarded. Pellets were gently resuspended, and 10 mL of PBS was added to the tubes. The washing step was repeated twice. This procedure was repeated for each group of pooled lymph nodes.

After the final washing step, the suspensions were centrifuged, and the supernatants were removed leaving a small volume (<0.5 mL) of supernatant above each pellet. Each pellet was gently agitated before suspending the LCNs in 3 mL of 5 % TCA (trichloroacetic acid) for precipitation of macromolecules. After incubation with 5 % TCA at 2-8 °C overnight (approximately 18 hours) precipitate was recovered by centrifugation at 190 x g for 10 minutes at 4 °C), and supernatants were removed, and pellets were resuspended in 1 mL of 5 % TCA solution and dispersed using an ultrasonic water bath. Each precipitate was transferred to a suitable sized scintillation vial with 10 mL of scintillation liquid and thoroughly mixed. The vials were loaded into a β-scintillation counter and ^{3}HTdR incorporation was measured for up to 10 minutes per sample.

The β-counter expressed the ^{3}HTdR incorporation as the number of radioactive disintegrations per minute (DPM). Similarly, background radiation levels were also measured in two 1 mL aliquots of 5 % TCA.

Clinical observations: During the study (Day 1 to Day 6) all animals were observed at least once daily for any clinical signs, including local irritation and systemic toxicity.

Bodyweights: The bodyweight of each animal was recorded prior to dosing on Day 1 and at Say 6 prior to injection of ^{3}H-methyl thymidine.

Statistics / Data evaluation: In the absence of any positive results, the statistical analysis of the data was not performed.

DPM was measured for each pooled group of nodes. The measured DPM values were corrected with the background DPM value (“DPM”). The results were expressed as “DPN” (DPM divided by the number of lymph nodes) following the industry standard for data presentation.

A stimulation index of 3 or greater is the criteria for defining a positive result.

The test item is regarded as a sensitiser if both of the following criteria are fulfilled:

- That exposure to at least one concentration resulted in an incorporation of ^{3}HTdR at least 3-fold or greater than recorded in control mice, as indicated by the stimulation index.
- The data are compatible with a conventional dose response, although allowance must be made (especially at high topical concentrations) for either local toxicity or immunological suppression.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortality was observed in any of the treatment groups during the main study, including the positive control group.

B. CLINICAL OBSERVATIONS

No signs of systemic toxicity were observed in any of the treatment groups during the main study, including the positive control group.

C. BODY WEIGHT

No treatment related effects were observed on body weight.

D. NECROPSY

It was not reported whether a necropsy was performed.

E. SKIN REACTIONS

No cutaneous reactions were observed at the site of the treatment in any treatment groups.

Proliferation assay: Appearance of the lymph nodes was normal in the negative control group and in the test item treated groups. Larger than normal lymph nodes were observed in the positive control group.

A significant lymphoproliferative response (stimulation index value of 12.2) was noted for HCA demonstrating the appropriate performance of the assay.

Table 5.2.6-2 Glyphosate technical: Local lymph node assay in the mouse ([redacted], 2011): Radiolabel incorporation into lymph-nodes of mice treated with glyphosate technical

Concentration (%w/v)	Disintegrations per minute (DPM/group)	Group DPM	Number of lymph nodes assayed	DPN	Stimulation Index Values (SI)
Background (5 (w/v) % TCA)	34	-	NA	-	-
Negative control PG	715	681	8	85.1	1.0
Glyphosate Technical 50 % in PG	717	683	8	85.4	1.0
Glyphosate Technical 25 % in PG	712	678	8	84.8	1.0
Glyphosate Technical 10 % in PG	828	794	8	99.3	1.2
Positive control 25 % HCA	8336	8302	8	1037.8	12.2

*: Propylene glycol, vehicle; **: α-Hexylcinnamaldehyde; TCA: trichloroacetic acid; N/A = not applicable

III. CONCLUSIONS

In conclusion, under the conditions of the present assay, Glyphosate Technical tested in a suitable vehicle, was shown to have no skin sensitisation potential (non-sensitiser) in the Local Lymph Node Assay.

3. Assessment and conclusion

Assessment and conclusion by applicant: Assessment and conclusion by applicant:
The GLP study is in concordance with the current OECD 429 (2010). Therefore, the study is considered acceptable / reliable and the outcome can be reported as valid.
After treatment of female mice with glyphosate (up to 45 %) in a local lymph node assay, stimulation index determined was less than 3-fold. Therefore, based on the results, glyphosate is not considered a skin sensitiser in the Local Lymph Node Assay.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.6/002
Report author	[redacted]
Report year	2010
Report title	Examination Of Glyphosate TC In The Skin Sensitisation Test In Guinea Pigs According To Magnusson And Kligman (Maximisation Test)
Report No	24879
Document No	NOT REPORTED
Guidelines followed in study	OECD 406 (1992); Commission Directive 96/54/EC B.6 (1996), OPPTS 870.2600 (1998)
Deviations from current test guideline (OECD 406, 1992)	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Glyphosate TC (Batch: 2009051501, Purity: 96.4 %) was tested for its sensitising effect on the skin of the guinea pig in the Maximisation Test. The test-substance concentrations for the main test were selected based on the results of the pre-test.

The intradermal induction was performed with a 0.01 % dilution of the test item in physiological saline and an emulsion of Freund's Complete Adjuvant (FCA)/physiological saline. The epidermal induction was conducted for 48 hours under occlusion with the test item at 50 % one week after the intradermal induction.

Two weeks after induction the animals were challenged by epidermal application of the test item at 25 % under occlusive dressing. The study was performed using a control group consisting of five animals, one test group consisting of ten animals, and a positive control group consisting of 20 animals.

The animals did not show any signs of systemic toxicity and showed an expected body weight development. Intracutaneous induction stage did not reveal any skin reactions. None of the vehicle control or test animals exhibited a positive skin reaction (defined as scores of ≥ 1) after the challenge treatment.

Animals treated with the positive control benzocaine in 40 % ethanolic 0.9 % NaCl solution exhibited a sensitising reaction in all animals in form of a discrete or patchy erythema (grade 1).

Based on the results, glyphosate TC has no sensitising effect on the skin of the guinea pig in the Maximisation test.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate TC
Description:	White solid powder
Lot/Batch #:	2009051501
Purity:	96.4 %
Stability of test compound:	At room temperature in the dark stable; expiry date: 2011-05-15

2. Vehicle and/or positive control: Purified water (*aqua ad iniectabilia*)

3. Test animals:

Species:	Guinea pig
Strain:	Dunkin Hartley
Source:	[redacted]
Age:	32 days
Sex:	Female
Weight at dosing:	312 - 355 g; positive control group: 249 - 317 g
Acclimation period:	At least 5 days
Diet/Food:	ssniff Ms-H V2333 (ssniff Spezialdiäten GmbH, Soest, Germany), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	In pairs in Makrolon cages (MZK 80/25) with granulated textured wood bedding
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 55 ± 15 % Air changes: Not reported Photocycle: 12 hours light/dark cycle

B: Study design and methods

In life dates: 2009-10-15 to 2009-11-28

Animal assignment and treatment:

Glyphosate TC was tested for its sensitising effect on the skin of the young adult female Dunkin Hartley guinea pig using the Maximisation test according to Magnusson and Kligman. The test substance concentrations for the main study were selected based on the results of the pre-testing performed with eight animals: six for the topical administration and two for the intracutaneous administration.

In the preliminary test, six concentrations of Glyphosate TC were tested by intracutaneous injection: 0.01, 0.1, 0.5, 1, 5, or l0 % suspensions in purified water. A concentration of 0.01 % revealed a discrete or patchy erythema, concentrations of 0.1 to 5 % revealed a moderate and confluent erythema, and the concentration of 10 % revealed an intense erythema and swelling 24 to 72-hours after administration, respectively.

Six concentrations of Glyphosate TC were tested by topical application: 0.5, 1, 5, 10, 25, and 50 % suspensions in purified water. No skin reactions were observed up to a concentration of 25 %. The concentration of 50 % revealed a discrete or patchy erythema 24 to 72-hours after start of exposure.

The concentrations selected for the main test were 0.01 % for the intracutaneous induction, 50 % for the topical induction, and 25 % for the challenge.

The main study was performed in ten test animals, five negative control animals, and 20 positive control animals (see table below).

Table 5.2.6-3: Examination of Glyphosate TC in the Skin Sensitisation Test in Guinea Pigs According to Magnusson and Kligman (Maximisation Test) ([redacted]): Animal assignment to the treatment groups

Treatment group	Number of animals
Pretest	
Intradermal	2
Epidermal	6
Main Study	
Negative Control Group (purified water)	5
Test Group	10
Positive Control Group (benzocaine)	20

The induction phase consisted of an intradermal injection at Day 0 and an epidermal application on Day 7. On Day 0 the test substance was injected (0.1 mL/site) into the clipped dorsal skin of the shoulder region at a concentration of 0.01 % in purified water, together with injections of Freund´s Complete Adjuvant in physiological saline, or test item in a 1:1 (v/v) mixture of Freund´s Complete Adjuvant and physiological saline.

On Day 6 the skin was shaved and coated with 0.5 mL sodium laurylsulfate 10 % in vaseline in order to induce a local irritation. On Day 7 the test substance was topically applied at a concentration of 50 % in purified water to the clipped and shaved skin of the shoulder region using the patch technique. The patch was left occluded in place for 48 hours.

The challenge was conducted on Day 21 by an occlusive patch at a concentration of 25 % in purified water which was applied to the shaved and depilated left flank of each animal for 24-hours. The right flank of each animal was treated in the same way with the vehicle alone. Twenty-four (24) and 48 hours after removal of the dressing skin reactions were scored according the Magnusson and Kligman grading scale (see table below).

Magnusson Kligman grading scale

Score	Reaction
0	No visible change
1	Discrete or patchy erythema
2	Moderate and confluent erythema
3	Intense erythema and swelling

Body weights were determined at the first day of treatment of the main study and at termination. Mortality and clinical signs were recorded daily during the study period. No necropsy was performed.

The animals of the positive control group were treated with a 2 % benzocaine solution (dissolved in 40 % ethanolic 0.9 % NaCl solution) intracutaneously in the first induction phase and with a 5 % solution topically in the second induction phase and at challenge. The positive control was run in a separate study during May, 2009.

II. RESULTS AND DISCUSSION

A. MORTALITY

No deaths occurred.

B. CLINICAL OBSERVATIONS

No signs of systemic toxicity were observed.

C. BODY WEIGHT

All animals showed the expected gain in body weight.

D. NECROPSY

No necropsy was performed.

E. SKIN REACTIONS

Intracutaneous induction did not reveal any skin reactions.

No skin reactions were observed 24 or 48 hours after the challenge treatment with glyphosate TC in the control or test group.

Animals treated with the positive control benzocaine in 40 % ethanolic 0.9 % NaCl solution exhibited a sensitising reaction in all animals in form of a discrete or patchy erythema (grade 1). Accordingly, the sensitivity and reliability of the experimental technique could be demonstrated.

Table 5.2.6-4: Examination of Glyphosate TC in the Skin Sensitisation Test in Guinea Pigs According to Magnusson and Kligman (Maximisation Test) ([redacted]): Summary of skin reactions after challenge

Treatment group	Incidence*	
	24-hours	48 hours
Test group	0/10	0/10
Negative control group	0/5	0/5
Positive control group	20/20	20/20

*: number of animals with findings / number of animals tested

III. CONCLUSIONS

Under the present test conditions, Glyphosate TC was found to be not sensitising to guinea pigs in a test model according to Magnusson and Kligman.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The GLP study is in concordance with the current OECD 406 (1992). Therefore, the study is considered acceptable and the outcome can be reported as valid.

After a challenge treatment no skin reactions in any treated or control guinea pigs were observed 48 and 72-hours after the start of the challenge. Therefore, based on the results, glyphosate has no sensitising effect on the skin of the guinea pig in the Maximisation test.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.6/003
Report author	[redacted]
Report year	2010
Report title	Examination of Glyphosate TC in Skin Sensitisation Test in Guinea Pigs according to Magnusson and Kligman (Maximisation Test)
Report No	24607
Document No	N/A
Guidelines followed in study	OECD 406 (1992); US EPA OPPTS 870.2600 (1998); EC method B. 6. Skin Sensitisation (96/54/EC)
Deviations from current test guideline (OECD 406, 1992)	Temperature of 22 °C ± 3 °C was outside of the temperature set of 20 °C (± 3 °C). This deviation did not affect the study outcome.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The purpose of this study was to determine the potential of Glyphosate TC (Batch: 20090506, Purity: 97.3 %) to produce skin sensitisation reactions in guinea pigs in a test model according to Magnusson and Kligman.

A 0.5 % (0.1 mL) concentration of Glyphosate TC in purified water chosen for the 1st (intracutaneous) induction stage revealed a discrete or patchy erythema in all ten animals 24 and 48 hours after administration. Two (2) mL of a 50 % concentration of Glyphosate TC in purified water /animal chosen for the 2nd (topical) induction stage was non-irritating to the shaved skin in the preliminary experiment. Hence, in the main study the skin was coated with 0.5 mL sodium laurylsulfate (10 %) on the day before stage 2 induction in order to induce a local irritation.

The challenge with 2 mL of a 25 % concentration of Glyphosate TC in purified water /animal revealed no skin irritation in any animal and, thus, the test item had no sensitising properties. The vehicle control revealed no skin reactions.

Animals of the same strain treated with the positive control benzocaine in 40 % ethanolic 0.9 % NaCl solution exhibited a sensitising reaction in all animals in form of a discrete or patchy erythema (grade 1).

The animals gained the expected weight within the test period. Behaviour of the animals remained unchanged. No necropsy was performed.

Therefore, based on the results, glyphosate has no sensitising effect on the skin of the guinea pig in the Maximisation test.

I. MATERIALS AND METHODS

A. Materials

1. Test material:

Identification:	Glyphosate TC
Description:	White solid powder
Lot/Batch #:	20090506
Purity:	97.3 % (Certificate of Analysis)
Stability of test compound:	No data given in the report Expiry date: May 2011

2. Vehicle and/or positive control:

Purified water (*aqua ad iniectabilia)*/ Benzocaine

3. Test animals:

Species:	Guinea pig
Strain:	Dunkin-Hartley
Source:	[redacted]
Age:	32 days
Sex:	Male
Weight at dosing:	299 - 364 g (excluding positive control group) Positive control group: 319 - 346 g
Acclimation period:	At least 5 days
Diet/Food:	Commercial diet, ssniffB MS-H V2233 (ssniff Spezialdiäten GmbH) served as food. The food was offered, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	The animals were kept in pairs in MAKROLON cages (MZK 80/25). Granulated textured wood (Granulat A2, J. BRANDENBURG, 49424 Goldenstedt, Germany) was used as bedding material in the cages. The cages were changed and cleaned twice a week
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 55 ± 15 % Air changes: Not reported Photocycle: 12 hours light/dark cycle

B: Study design and methods

In life dates: 2009-10-26 to 2010-01-30

Animal assignment and treatment:

The purpose of this study was to determine the potential of Glyphosate TC to provoke skin sensitisation reactions in guinea pigs. The test substance concentrations for the main study were selected based on the results of the pre-testing performed with eight animals: six animals for the topical administration and two animals for the intracutaneous administration. Six concentrations of Glyphosate TC were tested by intracutaneous injection: 0.01, 0.1, 0.5, 1, 5, or 10 % suspensions in aqua ad iniectabilia: Concentrations up to 0.1 % did not reveal any skin reactions. Concentrations of 0.5 or 1 % revealed a discrete or patchy erythema, a concentration of 5 % a moderate and confluent erythema and a concentration of 10 % revealed an intense erythema and swelling 24 to 72-hours after administration, respectively. Six concentrations of Glyphosate TC were tested by topical application: 0.5, 1, 5, 10, 25, and 50 % suspensions in purified water.

No skin reaction was observed at any concentration.

The concentrations selected for the main test were 0.5 % concentration for the 1st (intracutaneous) induction stage, a 50 % concentration for the 2nd (topical) induction stage and a 25 % concentration for the challenge. Possible sensitising properties of the test item were evaluated by administration of the test item to the shoulder region, first by intracutaneous application (stage 1) and 7 days later by topical administration (stage 2, exposure time: 48 hours).

The skin reaction results of the first induction exposure were evaluated at 24 and 48 hours, of the second induction at 48 and 72-hours after beginning of exposure.

In a challenge test (stage 3) the test item was again applied topically but to the flank region (exposure time: 24-hours). This area was then examined for reactions which might indicate sensitising properties of the test item.

Days 23 and 24: 21 hours after removing the filter paper the challenge area was cleaned and cleared of hair if necessary three hours later (at 48 hours from the start of challenge application) the skin reaction was observed and recorded. 24-hours after this observation a second observation (72-hours) was performed and recorded.

Table 5.2.6-5 Examination of Glyphosate TC in Skin Sensitisation Test in Guinea Pigs according to Magnusson and Kligman (Maximisation Test) ([redacted] 2010): Animal assignment to the treatment groups

Treatment group	Number of animals
Main Study	
Test Group	10
Negative Control Group (purified water)	5
Positive Control Group (benzocaine)	20

The skin reactions were graded according to Magnussen & Kligman.

Score	**Erythema**
0	No visible change
1	discrete or patchy erythema
2	moderate and confluent erythema
3	intense erythema and swelling

Mortality and clinical signs were recorded daily during the observation period. Body weight was determined at start of study and at study termination.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

Behaviour of the animals remained unchanged. Given the negative response in all treated animals further testing was not considered necessary in order to reduce animal experiments for animal welfare reasons.

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance.

D. NECROPSY

No necropsies were performed.

E. SKIN REACTIONS

No oedema or erythema were observed in test animals following challenge with technical glyphosate. The rate of sensitisation in the test substance treatment group was therefore 0 %.

The vehicle control revealed no skin reactions.

Animals of the same strain treated with benzocaine in 40 % ethanolic 0.9 % NaCl solution exhibited a sensitising reaction in all animals in form of a discrete or patchy erythema (grade 1).

Table 5.2.6-6 Examination of Glyphosate TC in Skin Sensitisation Test in Guinea Pigs according to Magnusson and Kligman (Maximisation Test) ([redacted] 2010): Summary of skin reactions after challenge

Mean skin reaction scores	Intradermal induction (shoulder)		Topical induction (shoulder)		Challenge (flank)			
					48 h		72 h	
	24 h	48 h	48 h	72 h	left	right	left	right
Negative Control Group	0	0	1	1	0	0	0	0
Test Group	1	1	1	1	0	0	0	0
Positive Control Group	1	1	2	2	1	0	1	0

III. CONCLUSIONS

Under the present test conditions Glyphosate TC revealed no sensitising properties in guinea pigs in a test model according to Magnusson and Kligman.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The GLP study is in concordance with the current OECD 406 (1992). Therefore, the study is considered acceptable and the outcome can be reported as valid.
After challenge treatment with glyphosate, no skin reactions in guinea pigs were observed after 24 and 48 hours. Therefore, based on the results, glyphosate has no sensitising effect on the skin of the guinea pig in the Maximisation test.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.6/004
Report author	[redacted]
Report year	2009
Report title	Glyphosate Technical: Contact Hypersensitivity in albino guinea pigs – Maximization-Test
Report No	C22908
Document No	Not reported
Guidelines followed in study	OECD 406 (1992); Commission Regulation (EC) No 440/2008 (2008); method B.6
Deviations from current test guideline (OECD 406, 1992)	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Glyphosate Technical (Batch: GI-1045, Purity: 96.66 %) was tested for its sensitising effect on the skin of 15 male Dunkin Hartley guinea pigs (ten test and five control) in the Maximisation Test. For the intradermal induction animals were injected with a 10 % dilution of the test material in purified water and in an emulsion of Freund´s Complete Adjuvant in physiological saline.

After one week, the epidermal induction phase of the study was conducted under occlusive conditions. Animals were exposed for 48 hours to a 50 % dilution of the test item in purified water. Control animals were intradermally induced with purified water and Freund´s Complete Adjuvant in physiological saline and epidermally induced with purified water under occlusive conditions.

Two weeks after epidermal induction, the control and test animals were challenged by epidermal application of the test item at 15 % in purified water and purified water alone under occlusive dressing. Cutaneous reactions were evaluated at 24 and 48 hours after removal of the dressing.

No mortality occurred during the study, no necropsies were performed. No clinical signs of systemic toxicity were observed in the animals. No positive/skin reactions (0/10 animals in the test group, 0/5 in the control group) were observed in the animals 24 and 48 hours after challenge. There was no effect on body weight gain.

Therefore, based on the results, Glyphosate Technical has no sensitising effect on the skin of the guinea pig in the Maximisation test.

I. MATERIALS AND METHODS

A: Materials

Identification:	Glyphosate Technical
Description:	Solid
Lot/Batch #:	GI-1045
Purity:	96.66 % w/w
Stability of test compound:	Expiry date: 2010-07 Test item dilution: Stable in purified water for 2 days
2. Vehicle and/or positive control:	Purified water
3. Test animals:	
Species:	Guinea pig
Strain:	Albino Dunkin Hartley, CRL:(HA)BR, SPF
Source:	[redacted]
Age:	4 – 6 weeks (at pre-test / at beginning of acclimatization period)
Sex:	Male
Weight at dosing:	348 – 358 g (pre-test) 335 – 365 g (beginning of acclimation period)
Acclimation period:	Approximately 2 weeks for main study animals; no acclimation for pretest animals
Diet/Food:	Pellet standard Provimi Kliba 3418 guinea pig breeding / maintenance diet, containing Vitamin C (Provimi Kliba AG, 4303 Kaiseraugst, Switzerland), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in Makrolon type-4 cages with standard softwood bedding ("Lignocel", Schill AG, 4132 Muttenz, Switzerland)
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 30 - 70 % Air changes: 10 - 15/hour Photocycle: 12 hours light/dark cycle

B: Study design and methods

In life dates: 2009-01-14 to 2009-02-27

Animal assignment and treatment:
Glyphosate Technical was tested for its sensitising effect on the skin of guinea pig in the Maximization-Test according to Magnusson-Kligman. Fifteen (ten test and five control) animals were employed for the main test, and three animals for the intradermal and epidermal pretest (see table below for animal group assignments).

Table 5.2.6-7: Glyphosate Technical: Contact Hypersensitivity in albino guinea pigs – Maximization-Test ([redacted] 2009): Animal assignment to the treatment groups

Treatment group	Number of animals per group
Pretest	
Intradermal	1
Epidermal	2
Main Study	

Table 5.2.6-7: Glyphosate Technical: Contact Hypersensitivity in albino guinea pigs – Maximization-Test ([redacted], 2009): Animal assignment to the treatment groups

Negative Control Group	5
Test Group	10

The concentrations of test substance for the main test were selected based on the results of a pre-test (during the acclimatization period of the main animals). Four intradermal injections (0.1 mL/site of a 1:1 (v/v) mixture of Freund's Complete Adjuvant (FCA)/physiological saline were made into the shaved neck of one guinea pig; six days later intradermal injections were made in the same guinea pig at concentrations of 5 %, 10 %, and 15 % of the test item in purified water; 15 % was the maximum feasible concentration due to its ability to pass through the intradermal injection needle. Dermal reactions were assessed 24-hours later. Based on the results, the test item concentration selected for the main study was 10 %.

To determine the concentration for the epidermal induction and challenge of the main study, four intradermal injections were made as described above in two guinea pigs. Six days later four patches of filter paper (3 x 3 cm) were saturated with the test item at 10 %, 15 %, 25 %, and 50 % in purified water in a volume of 0.2 mL and applied onto the clipped and shaved flanks of the same guinea pigs; 50 % was the maximum feasible concentration of the test item. Dermal reactions were assessed 24 and 48 hours after patch removal. Based on the results, the concentrations selected for the epidermal induction and challenge were 50 % and 15 %, respectively.

For the main study, the test item and vehicle were weighed, and a weight/weight dilution was prepared. Homogeneity of the test item preparation was ensured using a magnetic stirrer and/or spatula. The preparations were made immediately prior to each dosing. Homogeneity of the test item preparation was maintained during treatment using a magnetic stirrer when possible.

The intradermal induction of sensitisation in the test group was performed in the scapular region with a 10 % dilution of the test item in purified water and in an emulsion of FCA/physiological saline. The epidermal induction of sensitisation was conducted for 48 hours under occlusion with the test item at 50 % in purified in 0.3 mL water one week after the intradermal induction. The animals of the control group were intradermally induced with purified water and FCA/physiological saline and epidermally induced with purified water under occlusion.

Two weeks after epidermal induction the control and test animals were challenged by epidermal application of 0.2 mL test item at 15 % in purified water and 0.2 mL purified water alone under occlusive dressing for 24-hours. Cutaneous reactions were evaluated at 24 and 48 hours after removal of the dressing (according to the criteria laid down in test guidelines).

Body weights were determined at delivery/acclimatization start, at the end of the pretest, at test day 1 (day of treatment), and at the termination of the study. Mortality was checked daily. Clinical signs of toxicity were recorded daily beginning at the time of delivery and ending at study termination.

A positive control (reliability check) with a known sensitiser was conducted in a separate study from 2008-10-08 to 2008-11-14. The positive controls with α-hexylcinnamaldehyde at 3 % in PEG 300 showed that the chosen guinea pig strain was able to detect sensitising compounds under the laboratory conditions chosen.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no treatment related deaths during the course of the study, hence no necropsies were performed. One pre-test animal was found in bad conditions before the start of pre-test and during the acclimatization period of the main test animals. This animal was sacrificed for ethical reasons and replaced by a new animal.

B. CLINICAL OBSERVATIONS

No signs of systemic toxicity were observed in the animals.

C. BODY WEIGHT

The body weight of the animals was within the range commonly recorded for animals of this strain and age. One animal lost visible amount of body weight (31 %) before the start of the intradermal pre-test. It was killed for ethical reasons and replaced by another animal.

D. NECROPSY

No necropsies were performed.

E. SKIN REACTIONS

Skin Effects in the Intradermal Induction (Test Day 1)

The expected and common findings were observed in the control and test group after the different applications using FCA intradermally. These findings consisted of erythema, oedema, necrotizing dermatitis, encrustation, and exfoliation of encrustation.

Skin Effects in the Epidermal Induction (Test Day 8)

Control group – No erythematous or oedematous reaction was observed in the animals treated with purified water only.

Test group – Discrete/patchy erythema was observed in eight out of ten test animals at the 24-hour observation and persisted in seven animals up to the 48-hour reading after treatment with the test item at 50 % in purified water.

Skin Effects in the Challenge Procedure

Control group and Test group – No positive/skin reactions were observed in the animals when treated with either purified water only or when treated with the test item at 15 % in purified water.

The incidence of skin reactions with the positive control, α-hexylcinnamaldehyde, was 100 %, at 24 and 48 hours, demonstrating the reliability of the study.

Table 5.2.6-8: Glyphosate Technical: Contact Hypersensitivity in albino guinea pigs – Maximization-Test ([redacted], 2009): Summary of skin reactions after challenge

Treatment group		Incidence*	
		24-hours	48 hours
Test group	15 % Glyphosate Technical in purified water (right flank)	0/10	0/10
	Purified water (left flank)	0/10	0/10
Negative control group	15 % Glyphosate Technical in purified water (right flank)	0/5	0/5
	Purified water (left flank)	0/5	0/5

*: number of animals with findings / number of animals tested

III. CONCLUSIONS

Based on the above mentioned findings in the Magnusson & Kligman Test in guinea pigs and in accordance to Commission Directive 2001/59/EC, Glyphosate Technical does not have to be classified and labelled as a skin sensitiser.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The GLP study is in concordance with the current OECD 406 (1992). Therefore, the study is considered acceptable and the outcome can be reported as valid.
After challenge treatment with glyphosate, no skin reactions in any treated or control guinea pigs were observed 24 and 48 hours. Therefore, based on the results, glyphosate has no sensitising effect on the skin of the guinea pig in the Maximisation test.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.6/005
Report author	[redacted]
Report year	2009
Report title	Examination of Glyphosate TC in Skin Sensitisation Test in Guinea Pigs according to Magnusson and Kligman (Maximisation Test)
Report No	23915
Document No	Not reported
Guidelines followed in study	OECD 406 (1992); US EPA OPPTS 870.2600 (1998); EC method B.6. (Skin Sensitisation)
Deviations from current test guideline (OECD 406, 1992)	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The potential of Glyphosate TC (Batch: 20080801, Purity: 98.8 %) to produce skin sensitisation reactions in guinea pigs was investigated in a test model according to Magnusson and Kligman.

A 0.5 % suspension of glyphosate in purified water chosen for the 1st (intracutaneous) induction stage revealed a discrete or patchy erythema 24 and 48 hours after administration.

Two (2) mL of a 50 % suspension of Glyphosate TC in purified water/animal was chosen for the 2nd (topical) induction stage and was not irritating to the shaved skin in the preliminary experiment. Hence, in the main study the skin was coated with sodium laurylsulfate on the day before the 2nd induction in order to induce a local irritation.

The challenge with 2 mL of a 50 % suspension of Glyphosate TC in purified water/animal revealed no skin irritation in any animal and, thus, the test item had no sensitising properties.

The vehicle control revealed no skin reactions.

Animals of the same strain treated with the positive control benzocaine in 40 % ethanolic 0.9 % NaCl solution exhibited a sensitising reaction in all animals in form of a discrete or patchy erythema (grade 1).

No mortality and no clinical signs were observed. The body weight gains of the animals treated with Glyphosate TC was within the range of the vehicle control at study termination. The significant difference in body weight between test item-treated and control animals at start of the study is regarded to be without any biological relevance. No necropsy was performed.

Therefore, based on the results, Glyphosate TC has no sensitising effect on the skin of the guinea pig in the Maximisation test.

I. MATERIALS AND METHODS

A. Materials

1. Test material:

Identification:	Glyphosate TC
Description:	White solid powder
Lot/Batch #:	20080801
Purity:	98.8 %
Stability of test compound:	No data given in the report, expiry date: 2010-08-01

2. Vehicle and/or positive control:

Vehicle:	Purified water (*Aqua ad iniectabilia*)
Positive control:	Benzocaine

3. Test animals:

Species:	Guinea pig
Strain:	Dunkin-Hartley
Source:	[redacted]
Age:	32 days
Sex:	Male
Weight at dosing:	313 – 358 g (excluding positive control group); positive control group: 271 - 331 g
Acclimation period:	At least 5 days
Diet/Food:	Commercial diet, ssniffB MS-H V2233 (ssniff Spezialdiäten GmbH, 59494 Soest, Germany), *ad libitum.*
Water:	Tap water, *ad libitum*
Housing:	In pairs, in MAKROLON cages (MZK 80/25). Granulated textured wood (Granulat A2, J. Brandenburg, 49424 Goldenstedt, Germany) was used as bedding material in the cages. The cages were changed and cleaned twice a week.
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 55 ± 15 % Air changes: Not reported Photocycle: 12 hours light/dark cycle

B: Study design and methods

In life dates: 2009-02-04 to 2009-03-28

Animal assignment and treatment:

The purpose of this study was to determine the potential of Glyphosate TC to provoke skin sensitisation reactions in guinea pigs.

A preliminary study was conducted to determine the appropriate dose level of the test item following intracutaneous and topical administration. For the intracutaneous administration, two animals were injected (scapular region, shaved and depilated to remove hair) with 0.1 mL of 0.01, 0.1, 0.5, 1, 5, or 10 % suspensions of the test item in purified water (three concentrations in each animal); 10 % was the maximum technically feasible concentration that could be administered by intradermal injection. No skin reactions were observed with the 0.01 % concentration at 24, 48, and 72-hours after administration. Discrete or patchy erythema was observed at a concentration of 0.1 % at 48 and 72-hours, and at 0.5 % at 24 to 72-hours after administration. Concentrations of 1 and 5 % revealed a moderate and confluent erythema 24 to 72-hours after administration. A concentration of 10 % revealed an intense erythema and swelling 24 to 72-hours after administration. Therefore, the concentration of 0.5 % for the intracutaneous induction of the main study.

For the topical dose selection, 2 mL of test preparation in concentrations of 0.5, 1, 10, 25, or 50 % test item in purified water was applied to the test area of three animals with shaved skin and three animals with depilated skin (two concentrations per animal); the sites were covered with an occlusive dressing for 24 or 42 hours and the application sites were assessed immediately, 42, and 48 hours (depilated) or immediately and 24-hours (non-depilated) after removal of the patch. There were no skin reactions up to the highest concentration of 50 %; therefore, 50 % was selected as the concentration for the induction and challenge of the main study.

Induction: For the main study, on Day 0 three pairs of intradermal injections of 0.1 mL were given in the shoulder region which was cleared of hair: Freund's Complete Adjuvant (FCA) diluted 1:1 with 0.9 % sodium chloride, 0.5 % test item in purified water, and 0.5 % test item in a 1:1 mixture with FCA/physiological saline. On Day 6, 0.5 mL sodium laurylsulfate 0 % in vaseline was applied to the shaved skin to induce a local irritation since Glyphosate TC was non irritating in the preliminary study. On Day 7, 50 % test item in purified water was applied to the shaved skin in the shoulder region using the patch test technique as described above for 48 hours. Skin reactions of the first induction exposure were evaluated at 24 and 48 hours, and of the second induction at 48 and 72-hours after beginning of exposure.

Challenge: On Day 21, 50 % test item in purified water was applied to the shaved skin (flank) using the patch test technique as described above for 24-hours. Skin reactions were recorded 48 and 72-hours after the start of the challenge application.

The vehicle control group were treated in the same way as the test group but received purified water instead of the test item for the induction phases. The animals were challenged with the test item in the same manner as the test group.

The positive control group were treated as described above, but with a 2 % (w/v) benzocaine in 40 % ethanolic 0.9 % sodium chloride solution intracutaneously, and with a 4 % (w/v) benzocaine in 40 % ethanolic 0.9 % sodium chloride solution topically in the induction and challenge phases. The positive control was performed in a separate study during November to December, 2008.

Animals were observed for mortality and clinical signs daily. Body weights were determined at the start and at study termination.

Table 5.2.6-9: Examination of Glyphosate TC in Skin Sensitisation Test in Guinea Pigs according to Magnusson and Kligman (Maximisation Test) ([redacted], 2009): Animal assignment to the treatment groups

Group	Number of Animals Per Group
Preliminary Study	
Intracutaneous	2
Topical	6
Main Study	
Test Group	10
Negative Control Group	5
Positive Control Group (benzocaine)	20

The skin reaction to the challenge was scored according to the following criteria:

Score	Erythema (Magnusson and Kligman scale)
0	No erythema
1	discrete or patchy erythema
2	moderate and confluent erythema
3	intense erythema and swelling

Mortality and clinical signs were recorded daily during the observation period. Body weight was determined at start of study and at study termination.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

Behaviour of the animals remained unchanged. Given the negative response in all treated animals further testing was not considered necessary in order to reduce animal experiments for animal welfare reasons.

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance.

D. NECROPSY

No necropsies were performed.

E. SKIN REACTIONS

Skin Effects in the Intradermal Induction (Test Day 1)

The expected and common findings were observed in the control and test group. These findings consisted of discrete or patchy erythema 24 and 48 hours after administration.

Skin Effects in the Epidermal Induction (Test Day 8)

There were findings of discrete or patchy erythema observed in the control and test groups, which had been treated with sodium laurylsulfate on the day before the second induction to induce a local irritation.

Skin Effects in the Challenge Procedure

No positive/skin reactions were observed in the animals when treated with either purified water only or when treated with the test item at 50 % in purified water. The rate of sensitisation in the test substance treatment group was therefore 0 %.

The positive control benzocaine in 40 % ethanolic 0.9 % NaCl solution exhibited a sensitising reaction in all animals in form of a discrete or patchy erythema (grade 1).

Table 5.2.6-10: Examination of Glyphosate TC in Skin Sensitisation Test in Guinea Pigs according to Magnusson and Kligman (Maximisation Test) ([redacted], 2009): Mean skin reaction scores

Treatment groups	Intradermal induction (shoulder)		Topical induction (shoulder)		Challenge (flank) 48 h		72 h	
	24 h	48 h	48 h	72 h	left	right	left	right
Negative Control Group	0	0	1	1	0	0	0	0
Test Group	1	1	1	1	0	0	0	0
Positive Control Group	1	1	1	1	1	0	1	0

III. CONCLUSIONS

Under the present test conditions Glyphosate TC revealed no sensitising properties in guinea pigs in a test model according to Magnusson and Kligman.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The GLP study is in concordance with the current OECD 406 (1992). Therefore, the study is considered acceptable and the outcome can be reported as valid.
After challenge treatment with glyphosate, no skin reactions in guinea pigs were observed after 24 and 48 hours. Therefore, based on the results, glyphosate has no sensitising effect on the skin of the guinea pig in the Maximisation test.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.6/006
Report author	[redacted]
Report year	2009
Report title	Glyphosate – Skin Sensitization Study in Guinea Pigs. Buehler Test
Report No	12174-08
Document No	Not reported
Guidelines followed in study	US EPA OPPTS 870.2600, equivalent to OECD 406
Deviations from current test guideline (OECD 406, 1992)	Evaluation of skin reactions 24 and 48 hours instead of 30 and 54 hours after challenge; humidity was in the range of 25-98 % instead of 30-70 %; It was not reported whether clinical signs of toxicity were recorded during the study.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

A skin sensitisation study was conducted on 15 male and 15 female short-haired albino guinea pigs to determine if test substance Glyphosate Tech Grade Mixed 5-Batch (Batch: 080704-1 thru 5, Purity: 96.4 %) produced a sensitising reaction.

Males and females were assigned to each of two groups, designated Groups I (5/sex) and II (10/sex). Group I animals remained untreated during the induction phase of the study and served as a naive control group. Group II animals, the test group, were treated with 400 mg of the test substance moistened with 2 mL of deionized water. The animals were treated once weekly for three weeks, for a total of three treatments. After a two-week rest period, all animals (Groups I and II) were challenged at a virgin test site with an application of 400 mg of test substance moistened with 2 mL of deionized water.

The test substance produced neither irritation in the test animals (Group II) nor the naive control animals (Group I) after the challenge treatment.

Therefore, based on the results, Glyphosate Tech Grade Mixed 5-Batch has no sensitising effect on the skin of the guinea pig in the Buehler Test.

I. MATERIALS AND METHODS

A. Materials

1. Test material:

Identification:	Glyphosate Tech Grade Mixed 5-Batch
Description:	White powder
Lot/Batch #:	080704-1 thru 5
Purity:	96.4 % (Certificate of Analysis)
Stability of test compound:	No data given in the report

2. Vehicle and/or positive control:	Deionised water/ alpha-Hexylcinnamaldehyde
3. Test animals:	
Species:	Guinea Pig
Strain:	Hartley-Albino
Source:	[redacted]
Age:	Approx. 4 weeks
Sex:	Male and female (nulliparous and non-pregnant)
Weight at dosing:	Male: 359-414 g; female: 341-387 g
Acclimation period:	5 days
Diet/Food:	PMI Feeds, Inc.™ Guinea Pig Diet #5025; available *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individual housing in suspended, wire bottom, stainless steel cages
Environmental conditions:	Temperature: 19 ± 2 °C Humidity: 25 - 98 % Air changes: 10 - 12/hour Photocycle: 12 hours light/dark cycle

B: Study design and methods

In life dates: 2008-11-03 to 2009-01-02

Animal assignment and treatment:

Irritation screening was done in two male and two female animals with 400 mg of test substance moistened with deionized water, and 75 %, 50 %, and 25 % w/v concentrations of the test substance in deionized water. Based on the results of the irritation screening, Group II animals (the test group) were treated once weekly for three weeks with 400 mg of test substance moistened with 2 mL of deionized water. After a two-week rest period, all animals (Test and control) were each challenged at a virgin test site with an application of 400 mg of test substance moistened with 2 mL of deionized water. Observations for skin reactions at each test site were made approximately 24 hours after each treatment. In addition, observations for skin reactions were made approximately 48 hours after the first induction treatment and 48 hours after the challenge treatment. An average score for each time period was obtained by adding all of the scores for each time period and dividing by the number of test sites scored for that time period. The test substance is considered a sensitiser if the mean irritation scores, the total number of animals with scores, and/or the total number of scores for the virgin test site in the test group after the challenge treatment are appreciably greater than those for the naive challenge group. The average skin reaction score of this study was 0.0.

Table 5.2.6-11 Glyphosate – Skin Sensitisation Study in Guinea Pigs. Buehler Test ([redacted], 2009): Animal assignment to the treatment groups

Treatment group	Number of animals
Main Study	
Test Group	10/sex (20)
Negative Control Group	5/sex (10)

The skin reaction to the challenge was scored according to the following criteria:

Score **Erythema**

0	No reaction
0.5	Very faint, usually nonconfluent
1	Faint, usually confluent
2	Moderate
3	Strong, with or without oedema

Mortality was monitored during the observation period. Body weight was determined at start of study (Day 0) and at study termination (Day 31).

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

No clinical observations were reported.

C. BODY WEIGHT

Body weight was recorded and individual body weight on Day 0 and Day 31 (study termination) was provided, but an analysis of the data was not performed.

D. NECROPSY

Performance of necropsy was not reported.

E. SKIN REACTIONS

The test substance, Glyphosate, produced neither irritation in the test animals (Group II) nor the naive control animals (Group I) after the challenge treatment, and therefore did not elicit a sensitising reaction in guinea pigs.

A positive control was not performed within the scope of current study. However, a reliability check of the performing laboratory (with alpha-Hexylcinnamaldehyde according to the Buehler Method in guinea pigs (In life: 2008-06-05 to 2008-07-05) was provided. A mean score of 1.2 for the test group after challenge treatment, when compared with naive control group mean score of 0.1, confirmed the sensitivity of guinea pigs to the positive control material.

Table 5.2.6-12 Glyphosate – Skin Sensitisation Study in Guinea Pigs. Buehler Test ([redacted], 2009): Summary of skin reactions after challenge

Treatment group	Incidence*	
	24 hours	48 hours
Test group	0/20	0/20
Negative control group	0/10	0/10

*: number of animals with findings / number of animals tested

III. CONCLUSIONS

The test substance, Glyphosate, produced neither irritation in the test animals (Group II) nor the naive control animals (Group I) after the challenge treatment, and therefore did not elicit a sensitising reaction in guinea pigs.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The GLP study is in concordance with US EPA OPPTS 870.2600 guidelines, equivalent to the current OECD 406 (1992). Therefore, the study is considered acceptable and the outcome can be reported as valid.
After challenge treatment with glyphosate, no skin reactions in guinea pigs were observed after 24 and 48 hours. Therefore, based on the results, glyphosate has no sensitising effect on the skin of the guinea pig in the Buehler Test.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.6/007
Report author	[redacted]
Report year	2008
Report title	Skin Sensitisation Test for Glyphosate Technical in Guinea Pigs. Buehler Test
Report No	[redacted]-3996.318.431.07
Document No	Not reported
Guidelines followed in study	OECD 406
Deviations from current test guideline (OECD 406, 1992)	A positive control group was not used; a reliability check using historical control data from the study performing laboratory was not provided. The vehicle DMSO is not the preferred one recommended by the guideline. The deviations did not affect the outcome of the study.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The skin sensitisation test with Glyphosate Technical (Batch: 20070606, Purity: 98.05 %) in guinea pigs (*Cavia porcellus*) was carried out according to the Buehler Test Method, in order to evaluate its skin sensitisation potential.

A pilot study was conducted in order to determine the suitable doses of test substance for induction and challenge applications. The induction and challenge doses chosen were 0.5 g of test item (equivalent to 1 mL, of a 50 % w/v test solution). One test solution for each application was made using DMSO as vehicle

to increase the contact area and to allow transdermal absorption. After each application cotton lint patches were held in contact with the skin for an approximated 6-hour exposure period. Twenty treatment animals were exposed to the test item in inductions and challenge applications. Ten control animals were exposed to the vehicle on inductions and to the test item on challenge application.

Skin reactions were evaluated approximately 30 and 54 hours after each application by clinical examination (inductions) and according to the Magnusson & Kligman's grading scale (pilot study and challenge).

Neither compound-related clinical signs nor behavioural alterations were observed during inductions. No animal from control group was positive for the test item after challenge application.

One animal from treatment group was positive for the test item after challenge application.

Therefore, based on the results, Glyphosate Technical has no sensitising effect on the skin of the guinea pig in the Buehler Test.

I. MATERIALS AND METHODS

A. Materials

1. Test material:

Identification:	Glyphosate Technical
Description:	White powder
Lot/Batch #:	20070606
Purity:	98.05 %
Stability of test compound:	No data given in the report

2. Vehicle and/or positive control: DMSO

3. Test animals:

Species:	Guinea Pig
Strain:	Hartley
Source:	[redacted]
Age:	Eight to nine weeks old
Sex:	Male
Weight at dosing:	444 - 556 g
Acclimation period:	7 days
Diet/Food:	Pelleted commercial diet - "Nuvilab Cobaias 6001"
Water:	Tap water (enriched with ascorbic acid (300 mg/L), *ad libitum*
Housing:	Polypropylene cages (88 x 55 x 28 cm) with autoclaved wood shavings, five animals per cage during the experimental phase
Environmental conditions:	Temperature: 18 - 23 °C Humidity: 30 - 70 % Air changes: 10 - 15/hour Photocycle: 12 hours light/dark cycle

B: Study design and methods

In life dates: 2008-06-12 to 2008-07-12

Animal assignment and treatment:
The skin sensitising potential of glyphosate was assessed using the Buehler Test method. The test consists of a pre-test to identify an appropriate induction and challenge concentrations of the test substance, and the Maximization test itself.

Table 5.2.6-13: Skin Sensitisation Test for Glyphosate Technical in Guinea Pigs. Buehler Test ([redacted], 2008): Animal assignment to the treatment groups

Treatment group	Number of animals
Pre-Test Study	
Pre-test Group	2
Main Study	
Test Group	20
Negative Control Group	10

The skin of the animals (left flank for inductions and right flank for challenge) was mechanically and closely clipped free of hair using an electric razor at each application day. Skin was observed for lesions after clipping the fur. According to the Buehler's application method, animals were exposed to cotton lint patches with an approximated 6 cm² surface area. To allow transdermal absorption and to increase the contact surface, the test item was applied in solution using DMSO as vehicle, according to the solubility of the test item.

Based on the preliminary study, animals of the test group of the main study were exposed to patches loaded with 1 mL of 50 % (w/v) test solutions in DMSO (equivalent 0.5 g of the test item) for the induction and challenge applications. Control animals were exposed to 1 mL of vehicle for induction and to 1 mL of the test solution during challenge. Since control animals were not exposed to the test item on inductions, a hypersensitive state could not be induced in these animals, which then constituted a negative control in order to allow the differentiation between skin irritation and skin sensitisation at challenge. Patches were held in contact with the skin by an occlusive dressing during an approximated 6-hour exposure period in each application, after which patches were carefully removed from the skin and any residue cleaned up using DMSO.

Three applications were carried out during induction phase with a seven-day interval between inductions and after a fourteen-day interval between third induction and challenge application was conducted.

Animals were clinically examined approximately 30 and 54 hours after each application. Skin reactions were evaluated in agreement with Magnusson & Kligman's grading scale after challenge application.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

Neither compound-related clinical signs nor behavioural alterations were observed during inductions.

C. BODY WEIGHT

Individual body weights were recorded but an analysis of the data was not performed.

D. NECROPSY

No necropsy was performed.

E. SKIN REACTIONS

No animal from control group was positive for the test item after challenge application. One animal from treatment group was positive for the test item after challenge application.

Table 5.2.6-14: Skin Sensitisation Test for Glyphosate Technical in Guinea Pigs. Buehler Test (, 2008): Summary of skin reactions after challenge

Treatment group	Incidence*	
	30 hours	54 hours
Test group	1/20	1/20
Negative control group	0/10	0/10

*: number of animals with findings / number of animals tested

III. CONCLUSIONS

The epidermal application of Glyphosate Technical using DMSO as vehicle did not cause skin sensitisation in guinea pigs, according to the Buehler Test Method.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The GLP study is in concordance with OECD 406 guideline. Therefore, the study is considered acceptable and the outcome can be reported as valid.
After challenge treatment with glyphosate (50.9%), no skin reactions in guinea pigs were observed after 24 and 48 hours. Therefore, based on the results, glyphosate has no sensitising effect on the skin of the guinea pig in the Buehler Test.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.6/008
Report author	
Report year	2007
Report title	Glyphosate Technical (NUP 05068): Contact Hypersensitivity in Albino Guinea Pigs, Maximisation Test
Report No	B02316
Document No	Not reported
Guidelines followed in study	OECD 406 (1992); Commission Directive 96/54/EC B.6 (1996), JMAFF guideline 2-1-6 (2005)
Deviations from current test guideline (OECD 406, 1992)	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid

Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

For the determination of potential sensitising properties of glyphosate technical (NUP 05068) (Batch: 200609062, Purity: 95.1 %) a Maximisation Test was conducted using female guinea pig. For the main test, test-substance concentrations were selected based on the results of the pre-test. The intradermal induction was performed with a 3 % dilution of the test item in PEG 300 and an emulsion of Freund's Complete Adjuvant (FCA)/physiological saline. The epidermal induction was conducted for 48 hours under occlusion with the test item at 50 % in PEG 300 one week after the intradermal induction. Two weeks after induction the animals were challenged by epidermal application of the test item at 25 % under occlusive dressing. The study was performed using one control group consisting of five females, and one test group consisting of ten females.

None of the animals exhibited a positive skin reaction (defined as scores of ≥ 1) after the challenge treatment. The animals did not show any signs of systemic toxicity, and except for one animal, showed an expected body weight development. Alpha-hexyl¬cinnamaldehyde was used as positive control substance in a separately conducted study of the same laboratory. Skin reaction of 100 % demonstrate the sensitivity and reliability of the experimental technique.

Therefore, based on the results, glyphosate technical has no sensitising effect on the skin of the guinea pig in the Maximisation test.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate technical (NUP 05068)
Description:	Solid
Lot/Batch #:	200609062
Purity:	95.1 %
Stability of test compound:	Stable under storage conditions (20 ± 5 °C), light protected; expiry date: 2008-09-14
2. Vehicle and/or positive control:	Polyethylene glycol 300 (PEG 300)

3. Test animals:

Species:	Guinea pig
Strain:	Albino Dunkin Hartley, CRL:(HA)BR, SPF
Source:	[redacted]
Age:	5 – 6 weeks
Sex:	Female (nulliparous and non-pregnant)
Weight at dosing:	Pre-test: 362 – 372 g; main test: 337 – 381 g
Acclimation period:	Main test: at least 10 days
Diet/Food:	Pelleted standard Provimi Kliba 3418 guinea pig breeding / maintenance diet, containing Vitamin C (Provimi Kliba AG, CH-Kaiseraugust), *ad libitum*
Water:	Tap water, *ad libitum*

Housing:	Individually in Makrolon type 4 cages with standard softwood bedding	
Environmental conditions:	Temperature:	22 ± 3 °C
	Humidity:	30 - 70 %
	Air changes:	10 - 15/hour
	Photocycle:	12 hours light/dark cycle

B: Study design and methods

In life dates: 2007-01-10 to 2007-02-15

Animal assignment and treatment:
Glyphosate technical (NUP 05068) was tested for its sensitising effect on the skin of young female Dunkin Hartley guinea pig using the Maximisation test according to Magnusson and Kligman. The test substance concentrations for the main study were selected based on the results of the pre-testing performed with three animals. The main study was performed in ten test animals and five control animals.

Table 5.2.6-15: Glyphosate Technical (NUP 05068): Contact Hypersensitivity in Albino Guinea Pigs, Maximisation Test ([redacted], 2007): Animal assignment to the treatment groups

Treatment group	Number of animals
Pretest	
Intradermal Pretest	1
Epidermal Pretest	2
Main Study	
Negative Control Group	5
Test Group	10

In the pretest, four intradermal injections (0.1 mL/site) of a 1:1 (v/v) mixture of Freund's Complete Adjuvant/physiological saline were made into the neck of one guinea pig. Five days later, intradermal injections (0.1 mL/site) were made at concentrations of 3, 5, and 10 % test substance in PEG 300. Dermal reactions were assessed at 24-hours. Based on the skin reactions and the ability to apply the test substance, 3 % was the concentration selected for the main study.

Also in the pretest, two guinea pigs received the intradermal injections of Freund's Complete Adjuvant/physiological saline as described above. Five days later, the test substance was applied to shaved flanks of the animals in concentrations of 10, 15, 25, and 50 % in PEG 300 in 0.2 mL or 0.2 g; 50 % was the highest feasible concentration. The patches were covered by an occlusive dressing for 24-hours. Skin reactions were assessed 24-hours after patch removal. Based on the skin reactions, 50 % and 25 % were selected as the induction and challenge concentrations, respectively.

The induction phase consisted of an intradermal injection on Day 1 and an epidermal application on Day 8. On Day 1 the test substance was injected (0.1 mL/site) into the clipped dorsal skin from the scapular region at a concentration of 3 % either in PEG 300 or in a 1:1 (v/v) mixture of Freund´s Complete Adjuvant and physiological saline. On Day 8 the test substance was topically applied at a concentration of 50 % in PEG 300 (0.3 mL of test item preparation) to the clipped and shaved skin of the scapular area and covered with an occlusive dressing, which was left in place for 48 hours. The reaction sites were assessed 24 and 48 hours after removal of the bandage.

The challenge was conducted on Day 22 by an occlusive patch containing 0.2 mL of the test material at a concentration of 25 % in PEG 300, the highest non-irritating concentration, that was applied to the clipped and shaved left flank of each animal for 24-hours. The clipped and shaved right flank of each animal was

treated in the same way with the vehicle only (PEG 300). Twenty-four (24) and 48 hours after removal of the dressing skin reactions were scored according the Magnusson and Kligman grading scale.

Body weights were determined at the first day of treatment of the main study and at termination. Mortality and clinical signs were recorded daily during the study period.

A positive control (reliability check) with a known sensitiser was not included in this study. However, a separate study was performed from June to August 2006 in the laboratory. The positive controls with alpha-hexylcinnamaldehyde (3 % in PEG 300) showed that the chosen guinea pig strain was able to detect sensitising compounds under the laboratory conditions chosen.

Evaluation criteria for classification as a potential skin sensitiser: at the 24-hour and/or 48-hour reading, 30 % or more of the test animals exhibit a positive response (scores ≥ 1) in the absence of similar results in the vehicle control group.

II. RESULTS AND DISCUSSION

A. MORTALITY

No deaths occurred.

B. CLINICAL OBSERVATIONS

No signs of systemic toxicity were observed.

C. BODY WEIGHT

All animals showed the expected gain in body weight with the exception of one of the pre-test animals that did not gain body weight between the day of epidermal application and the day of sacrifice one week later.

D. NECROPSY

No necropsy was performed.

E. SKIN REACTIONS

After induction with the test item at 50 % in PEG 300, discrete/patchy erythema were observed in nine out of the ten animals at the 24- and/or 48-hour reading. No erythematous or oedematous reaction was observed in the animals treated with PEG 300 only.

No skin reactions were observed 24 or 48 h after removal of the challenge treatment with glyphosate technical (NUP 05068) in the control or in the test group.

Table 5.2.6-16: Glyphosate Technical (NUP 05068): Contact Hypersensitivity in Albino Guinea Pigs, Maximisation Test ([redacted], 2007): Summary of skin reactions after challenge

Treatment group	Incidence*	
	24-hours	48 hours
Test group	0/10	0/10
Negative control group	0/5	0/5

*: number of animals with findings / number of animals tested

III. CONCLUSIONS

According to the findings in an adjuvant sensitisation test (M&K-test) in guinea pigs, glyphosate technical (NUP 05068) is not classified for skin sensitisation based on the EU classification criteria.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The GLP study is in concordance with the current OECD 406 (1992). Therefore, the study is considered acceptable and the outcome can be reported as valid.
After epidermal induction with 50 % glyphosate technical and a challenge treatment with 25 % no skin reactions in any treated or control guinea pigs were observed 48 and 72-hours after the start of the challenge. Therefore, based on the results, glyphosate has no sensitising effect on the skin of the guinea pig in the Maximisation test.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.6/009
Report author	[redacted]
Report year	2007
Report title	Glyphosate Technical Material: Skin Sensitisation (Local Lymph Node Assay In The Mouse)
Report No	GM8048-REG
Document No	Not reported
Guidelines followed in study	OECD 429 (2002): OPPTS 870.2600 (2003): 2004/73/EC B.42 (2004)
Deviations from current test guideline (OECD 429, 2010)	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AfR 5 dossier (L docs)	Category 2a

2. Full summary

A sample of Glyphosate Technical Material (Batch: 0507, Purity: 96.1 %) was assessed for its skin sensitisation potential using the mouse Local Lymph Node Assay. The assay determines the level of lymphocyte proliferation in the lymph nodes draining the site of chemical application by measuring the amount of radiolabelled thymidine incorporated into the dividing cells. The test substance was applied as 10, 25 or 45 % w/v preparations in dimethyl sulphoxide. Dose levels were selected according to solubility and acute oral toxicity data (no further data were provided for the dose selection).

On three consecutive days, groups of four female CBA/Ca/Ola/Hsd mice were daily administered 25 µL of a 10, 25 or 45 % w/v preparation of the test substance in dimethyl sulphoxide to the dorsal surface of each ear. On Day 6, approximately five hours before sacrifice, all animals were injected with approximately 250 µL of phosphate buffered saline (PBS) containing 20 µCi of a 2.0 Ci/mmol specific activity ^{3}H-methyl

thymidine. After sampling the draining auricular lymph nodes, a single cell suspension was prepared, and ^{3}H-methyl thymidine is measured by β-scintillation counting.

The application of the test substance at concentrations of 10, 25, and 45 % w/v in dimethyl sulphoxide resulted in an isotope incorporation which was less than 3-fold at all concentrations.

In the positive control study, the application of hexylcinnamaldehyde at concentrations of 5 %, 10 %, and 25 % w/v in acetone in olive oil (4:1) resulted in a greater than 3-fold increase in isotope incorporation at the 25 % w/v concentration. Therefore, hexylcinnamaldehyde was shown to be a skin sensitiser, confirming the validity of the protocol used for the study.

Therefore, based on the results, Glyphosate Technical Material is not considered a skin sensitiser in the Local Lymph Node Assay.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate Technical Material
Description:	White solid
Lot/Batch number:	0507
Purity:	96.1 % (Certificate of analysis)
Stability of test compound:	Stable under storage conditions – ambient temperature in the dark

2. Vehicle and/or positive control: Dimethyl sulphoxide and / Hexylcinnamaldehyde (vehicle: acetone in olive oil

3. Test animals:

Species:	Mouse
Strain:	CBA/Ca/Ola/Hsd
Sex:	Female
Age:	8 - 12 weeks
Weight at dosing:	16.5 - 20.8 g
Source:	[redacted]
Housing:	Maximum four per cage, in cages suitable for animals of this strain and weight range. Environmental enrichment provided included tents, bases and nestlets.
Acclimatisation period:	At least 5 days
Diet:	Diet (RM1), supplied by Special Diets Services Limited, Witham, Essex, UK, *ad libitum*
Water:	Mains water supplied by an automatic system, *ad libitum*
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 30 - 70 % Air changes: A minimum of 15 changes/hour Photocycle: 12 hours light/dark cycle

B: Study design and methods

In-life dates: 2007-01-10 to 2007-01-16

Animal assignment and treatment: A sample of glyphosate technical material was assessed for its skin sensitisation potential using the mouse Local Lymph Node Assay. The assay determines the level of T lymphocyte proliferation in the lymph nodes draining the site of chemical application by measuring the amount of radiolabelled thymidine incorporated into the dividing cells. The test substance was applied as 10, 25 or 45 % w/v preparations in dimethyl sulphoxide. Groups of four female CBA/Ca/Ola/Hsd mice

were used for this study.

Table 5.2.6-17 Glyphosate Acid: Glyphosate Technical Material: Skin Sensitisation (Local Lymph Node Assay in the Mouse) ([redacted], 2007): Animal assignment to the treatment groups

Treatment group	Number of animals per group
Concentration of test substance (% w/v)	
0 (vehicle only)	4
10	4
25	4
45	4
Concentration of hexylcinnamaldehyde (% w/v)	
0 (vehicle only)	4
5	4
10	4
25	4

Dose selection rationale: Approximately 25 µL of a 10, 25, or 45 % w/v preparation of the test substance was used in this study as 45 % w/v was the limit of solubility.

Treatment preparation and administration: Approximately 25 µL of a 10, 25, or 45 % w/v preparation of the test substance in dimethyl sulphoxide was applied, using a variable volume micro-pipette, to the dorsal surface of each ear. A vehicle control group was similarly treated using dimethyl sulphoxide alone. The procedure was repeated daily for three consecutive days. Three days after the third application, all the animals were injected, via the tail vein, with approximately 250 µL of phosphate buffered saline (PBS) containing 20 µCi of a 2.0 Ci/mmol specific activity ^{3}H-methyl thymidine. Approximately 5 hours later, the animals were humanely killed by inhalation of halothane vapour followed by cervical dislocation. The draining auricular lymph nodes were removed from each animal and, together with the nodes from the other animals in the group, were placed in a container of PBS.

A single cell suspension was prepared by mechanical disaggregation of lymph nodes through a 200-mesh stainless steel gauze. The cell suspensions were then washed three times by centrifugation with approximately 10 mL of PBS. Approximately 3 mL of 5 % w/v trichloroacetic acid (TCA) was added and, after overnight precipitation at 4 °C, the samples were pelleted by centrifugation and the supernatant was discarded. The cells were then resuspended in approximately 1 mL of TCA.

The lymph node suspensions were transferred to scintillation vials and 10 mL of scintillant (Optiphase) was added prior to β-scintillation counting using a Packard Tri-Carb 3100TR Liquid Scintillation Counter.

Clinical observations: Animals were checked at least once daily for signs of systemic toxicity.

Bodyweights: The bodyweight of each animal was recorded prior to dosing on Day 1 and prior to injection of ^{3}H-methyl thymidine on Day 6.

Positive control: The reliability of the test system was assessed in a positive control study using the same method with a known sensitiser (hexylcinnamaldehyde) applied as 5 %, 10 % or 25 % w/v preparations in acetone in olive oil.

Statistics / Data Evaluation: The results are expressed as a disintegrations per minute (dpm) value per lymph node for each group. The activity of each test group is then divided by the activity of the vehicle control group to give a test:control ratio known as the stimulation index (SI), for each concentration.

The criterion for a positive response is that one or more concentrations of the test substance should elicit a 3-fold or greater increase in isotope incorporation relative to the vehicle control group. The assay is able to

identify those materials that elicit responses in standard guinea pig tests for skin sensitisation. Consequently, a test substance which does not fulfil the above criterion is designated as unlikely to be a skin sensitiser.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortalities were reported.

B. CLINICAL OBSERVATIONS

Clinical observations were made but none were reported.

C. BODY WEIGHT

Body weights were measured but no significant changes were reported.

D. NECROPSY

It was not reported whether a necropsy was performed.

E. SKIN REACTIONS

Group Mean Counts per Minute: The application of the test substance at concentrations of 10, 25 and 45 % w/v in dimethyl sulphoxide resulted in an isotope incorporation which was less than 3-fold at all concentrations. Consequently, the test substance is considered not to be a skin sensitiser under the conditions of the test.

Table 5.2.6-18 Glyphosate Acid: Glyphosate Technical Material: Skin Sensitisation (Local Lymph Node Assay in the Mouse) ([redacted], 2007): Radiolabel incorporation into lymph-nodes of mice treated with glyphosate technical material

Concentration of NOA446510 (%w/v)	Number of lymph nodes assayed	Disintegrations per minute (dpm)	dpm per lymph node	Test control ratio (SI)
0 (vehicle only)	8	3912	489	N/A
10	8	2394	299	0.6
25	8	3292	412	0.8
45	8	4067	508	1.0

N/A = not applicable

In the positive control study, the application of hexylcinnamaldehyde at concentrations of 5 %, 10 % and 25 % w/v in acetone in olive oil (4:1) resulted in a greater than 3-fold increase in isotope incorporation at the 25 % w/v concentration. Therefore, hexylcinnamaldehyde was shown to be a skin sensitiser, confirming the validity of the protocol used for the study.

Table 5.2.6-19 Glyphosate Acid: Glyphosate Technical Material: Skin Sensitisation (Local Lymph Node Assay in the Mouse) ([redacted], 2007): Radiolabel incorporation into lymph-nodes of mice treated with the positive control substance (hexylcinnamaldehyde)

Concentration of hexylcinnamaldehyde (%w/v)	Number of lymph nodes assayed	Disintegrations per minute (dpm)	dpm per lymph node	Test control ratio (SI)
0 (vehicle only)	8	5939	742	N/A
5	8	10111	1264	1.7
10	8	13747	1718	2.3
25	8	38015	4752	6.4

III. CONCLUSIONS

Glyphosate technical material is considered not to be a skin sensitiser under the conditions of the test.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The GLP study is in concordance with the current OECD 429 (2010). Therefore, the study is considered acceptable / reliable and the outcome can be reported as valid.
After treatment of female mice with glyphosate (up to 45 %) in a local lymph node assay, stimulation index determined was less than 3-fold. Therefore, based on the results, glyphosate is not considered a skin sensitiser in the Local Lymph Node Assay.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.6/010
Report author	[redacted]
Report year	2006
Report title	Glyphosate Technical: Skin Sensitisation in the Guinea Pig – Magnusson and Kligman Maximisation method
Report No	SMK-PH-05/0218
Document No	Not reported
Guidelines followed in study	OECD 406 (1992); 96/54/EC B.6 (1996); 12 NohSan No. 8147, Guideline No. 2-1-6 (2010)
Deviations from current test guideline (OECD 406, 1992)	It was not reported whether clinical signs of toxicity were made during the study. A positive control group was not conducted during the study; but for the reliability check limited historical control data was provided. No data on the diet used was reported. Room temperature 19 °C – 25 °C instead of 17 °C – 23 °C. The deviations did not affect the outcome of the study.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid

Category study in AIR 5 dossier (L docs) Category 2a

2. Full summary

The potential of Glyphosate Technical (Batch: H05H016A, Purity: 95.7 %) to produce skin sensitisation reactions in guinea pigs was investigated in a test model according to Magnusson and Kligman. Twenty test and ten control animals were used for the main study. The test-substance concentrations for the main test were selected based on the results of preliminary sighting test as follows: intradermal induction: 0.195 % (v/v) in isotonic sodium chloride solution; topical induction: 60 % (w/w) in distilled water; challenge: 60 % (w/w) and 30 % (v/v) in distilled water. Approximately 24-hours after induction as well as 24 and 48 hours after challenge, the animals were scored for skin reactions. Body weight was determined.

Two animals were found dead on Day 3 and on Day 5. No skin reactions were noted after induction and after challenge in the control group and the treatment group.

The positive response observed in the positive control validation study with alpha-Hexylcinnamaldehyde validates the test system used in the study.

Therefore, based on the results, Glyphosate Technical has no sensitising effect on the skin of the guinea pig in the Maximisation test.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate Technical
Description:	White powder
Lot/Batch #:	H05H016A
Purity:	95.7 % (Certificate of analysis)
Stability of test compound:	No data available Expiry date: 2008-03-25

2. Vehicle and/or positive control: Distilled water

3. Test animals:

Species:	Guinea Pigs
Strain:	Albino Dunkin Hartley
Source:	[redacted]
Age:	Not specified
Sex:	Female (nulliparous and non-pregnant)
Weight at dosing:	295 - 370 g
Acclimation period:	5 days
Diet/Food:	Not specified. The diet, drinking water and bedding were routinely analysed and were considered not to contain any contaminant that could reasonably be expected to affect the purpose or integrity of the study.
Water:	Tap water, ad libitum

Housing:	Groups of two or three animals in makrolon cages furnished with woodflakes	
Environmental conditions:	Temperature:	19 to 25 °C
	Humidity:	30 - 70 %
	Air changes:	At least 10 changes/hour
	Photocycle:	12 hours light/dark cycle

B: Study design and methods

In life dates: 05 September 2005 to 13 October 2005

Animal assignment and treatment:

The method used for assessing the sensitising properties of the test material was based on the Guinea Pig Maximisation Test of Magnusson B & Kligman A M, (1969) J. Invest. Dermatol. 52: 268 - 276.

A preliminary study was conducted to determine the appropriate dose level of the test item following intradermal and topical administration. For the intradermal induction dose selection, injections (0.1 mL/injection site) were made on the clipped shoulder of two guinea pigs, at concentrations of 25 % (w/w), 12.5 % (v/v), 6.25 % (v/v), 3.125 % (v/v), 1.56 % (v/v), and 0.78 % (v/v) in isotonic sodium chloride solution. A macroscopic evaluation of the injection sites was conducted approximately 24-hours after injection to determine whether these concentrations caused necrosis. Due to necrosis, two additional concentrations of 0.39 % (v/v) and 0.195 % (v/v) were also investigated.

For the topical induction dose selection, two guinea pigs were treated with preparations of the test material (60 % (w/w), 30 % (v/v), 15 % (v/v), and 7.5 % (v/v) in distilled water). Applications were made to the clipped flanks under occlusive dressings for an exposure period of 24-hours. The degree of erythema and oedema was evaluated approximately 24-hours after dressing removal. The highest concentration producing only mild to moderate dermal irritation was selected for the topical induction stage of the main study.

For the dose selection for topical challenge, four preparations of the test material (60 % (w/w), 30 % (v/v), 15 % (v/v), and 7.5 % (v/v) in distilled water) were applied to the clipped flanks of three guinea pigs under occlusive dressings for an exposure period of 24-hours. These guinea pigs did not form part of the main study but had been treated identically to the control animals of the main study, up to Day 14. The degree of erythema and oedema was evaluated approximately 24-hours after dressing removal. The highest non-irritant concentration of the test material and one lower concentration were selected for the topical challenge stage of the main study.

A group of thirty guinea pigs was used for the main study, twenty test and ten control

Table 5.2.6-20 Glyphosate Technical: Skin Sensitisation in the Guinea Pig – Magnusson and Kligman Maximisation method ([redacted] 2006): Animal assignment to the treatment groups

Treatment group	Number of animals
Main Study	
Test Group	20
Negative Control Group	10

Two phases were involved in the main study; (a) an induction of a response and (b) a challenge of that response. Induction of the test animals: A row of three injections (0.1 mL each) was made on each side of the spine, consisting of a) Freund's Complete Adjuvant plus isotonic sodium chloride in the ratio 1:1 b) a

0.195 % (v/v) formulation of the test material in isotonic sodium chloride c) a 0.195 % (v/v) formulation of the test material in a 1:1 preparation of Freund's Complete Adjuvant plus isotonic sodium chloride.

On Day 6, the scapular region of all test and control animals was shaved and sodium lauryl sulphate (10 % in petroleum jelly) was spread evenly over the area to create local irritation. On Day 7 the same area on the shoulder region used previously for intradermal injections was treated with a topical application of the test material formulation (60 % (w/w) in distilled water) under occlusive dressing for 48 hours. The intradermal induction on the control animals was performed using an identical procedure without the test material. Injection b) was therefore the vehicle alone, injection c) was a 50 % formulation of the vehicle in a 1:1 preparation of Freund's Complete Adjuvant plus isotonic sodium chloride. Similarly, the topical induction procedure was identical to that used for the test animals except that the test material was omitted.

For the challenge phase, test material formulation at the maximum non- irritant concentration (60 % (w/w) in distilled water) was applied to one side of the shorn flank of each animal under an occlusive dressing. To ensure that the maximum non- irritant concentration was used at challenge, the test material at a concentration of 30 % (v/v) in distilled water was similarly applied under an occlusive dressing to the opposite skin site on the shorn flank. After 24-hours, the dressing was carefully removed and discarded. The topical challenge sites were cleaned if required. Prior to the 24-hour observation the flanks were clipped to remove regrown hair.

Approximately 24 and 48 hours after challenge dressing removal, the degree of erythema and oedema was quantified. Any other reactions were also recorded.

The skin reaction to the challenge was scored according to the following criteria:

Scales for Evaluation of Skin Reactions

Score	**Erythema**
0	No visible modification
1	Slight or patches of erythema
2	Moderate confluent erythema
3	Intense erythema and swelling
Score	**Oedema**
0	No visible modification
1	Slight oedema
2	Moderate oedema
3	Severe oedema

II. RESULTS AND DISCUSSION

A. MORTALITY

One test group animal was found dead on Day 3 and one other test group animal was found dead on Day 5. The cause of death was not determined but was considered not to be treatment related. The absence of these animals was considered not to affect the purpose or integrity of the study.

B. CLINICAL OBSERVATIONS

Clinical observations were not reported.

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance.

D. NECROPSY

No necropsy was performed.

E. SKIN REACTIONS

No skin reactions were noted in treated animals after the topical induction phase or topical challenge phase (see table below).

The historical positive control alpha-hexylcinnamaldehyde was reported to exhibit a sensitising reaction in all animals after 24-hours.

Table 5.2.6-21 Glyphosate Technical: Skin Sensitisation in the Guinea Pig – Magnusson and Kligman Maximisation method ([redacted], 2006): Summary of skin reactions after challenge

Treatment group	Incidence*			
	30 % **		60 %	
	24 h	48 h	24 h	48 h
Test Group	0/20	0/20	0/20	0/20
Negative Control Group	0/10	0/10	0/10	0/10

*: Number of animals with findings / number of animals tested
**: Test substance concentration during challenge phase

III. CONCLUSIONS

The test material produced a 0 % (0/18) sensitisation rate and was considered as non-sensitiser to guinea pig skin under the conditions of the test.

3. Assessment and conclusion

Assessment and conclusion by applicant: I
The GLP study is in concordance with the current OECD 406 (1992). Therefore, the study is considered acceptable and the outcome can be reported as valid.
After challenge treatment with glyphosate (30 and 60 %), no skin reactions in guinea pigs were observed after 24 and 48 hours. Therefore, based on the results, glyphosate has no sensitising effect on the skin of the guinea pig in the Maximisation test.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.6/011
Report author	[redacted]
Report year	2005
Report title	Glyphosate acid technical – Dermal Sensitization in Guinea Pigs (Buehler Method)
Report No	15279
Document No	Not reported
Guidelines followed in study	OECD 406 (1992); US EPA OPPTS 870.2600 (2003); JMAFF 59 NohSan No. 4200 (1985)
Deviations from current test guideline (OECD 406, 1992)	Yes, humidity was not provided. It was not reported whether clinical signs of toxicity were recorded during the study.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

A dermal sensitisation test was conducted with guinea pigs to determine the potential for Glyphosate Acid Technical (Batch: 040205, Purity: 97.23 %) to produce sensitisation after repeated topical applications.

A 70 % w/w mixture of the test substance in distilled water was topically applied to twenty healthy test guinea pigs, once each week for a three-week induction period. Twenty-seven days after the first induction dose, a challenge dose of the test substance at its highest non-irritating concentration (HNIC, determined in the preliminary irritation screen to be a 70 % w/w mixture in distilled water) was applied to a naive site on each guinea pig. A naive control group (ten animals) was maintained under the same environmental conditions and treated with the test substance at challenge only. Approximately 24 and 48 hours after each induction and challenge dose, the animals were scored for erythema.

The positive response observed in the historical positive control validation study with alpha-Hexylcinnamaldehyde Technical (HCA) validates the test system used in this study.

Therefore, based on the results, Glyphosate Acid Technical has no sensitising effect on the skin of the guinea pig in the Buehler Test.

I. MATERIALS AND METHODS

A. Materials

1. Test material:

Identification:	Glyphosate Acid Technical
Description:	White crystalline powder
Lot/Batch #:	040205
Purity:	97.23 % (Certificate of analysis)
Water solubility	12 g/L

pH	2.5 in 1 % solution
Stability of test compound:	No data given in the report
2. Vehicle and/or positive control:	Distilled water/ alpha-Hexylcinnamaldehyde Technical
3. Test animals:	
Species:	Guinea pig
Strain:	Hartley albino
Source:	[redacted]
Age:	Young adult
Sex:	Male and Female
Weight at dosing:	327-391 g
Acclimation period:	5 or 38 days
Diet/Food:	Pelleted Purina Guinea Pig Chow #5025
Water:	Tap water, *ad libitum*
Housing:	The animals were group housed in suspended stainless steel caging with mesh floors or plastic perforated bottom caging
Environmental conditions:	Temperature: 18-22 °C Humidity: Not reported Air changes: Not reported Photocycle: 12 hours light/dark cycle

B: Study design and methods

In life dates: 2004-05-03 to 2004-06-03

Animal assignment and treatment:

A dermal sensitisation test was conducted with guinea pigs to determine the potential for Glyphosate Acid Technical to produce sensitisation after repeated topical applications. The study consists of a preliminary test to identify an appropriate induction and challenge concentrations of the test substance, and main test.

Table 5.2.6-22: Glyphosate acid technical – Dermal Sensitisation in Guinea Pigs (Buehler Method) ([redacted], 2005): Animal assignment to the treatment groups

Treatment group	Number of animals
Pre-Test Study	
Pre-test Group	4
Main Study	
Test Group	20
Negative Control Group	10

On the day before application of the test substance, the fur of the animals was removed by clipping the dorsal area and flanks.

A 70 % w/w mixture of the test substance in distilled water was topically applied to twenty healthy test guinea pigs, once each week for a three-week induction period animal using an occlusive25 mm Hill Top Chamber. The chambers were secured in place and wrapped with non-allergenic Durapore adhesive tape to avoid dislocation of the chambers and to minimize loss of the test substance. After the 6-hour exposure

period, the chambers were removed, and the test sites were gently cleansed of any residual test substance. Approximately 24 and 48 hours after each induction application, readings of local reactions (erythema) according to the scoring system was performed.

Twenty-seven days after the first induction dose, a challenge dose of the test substance at its highest non-irritating concentration (determined in a preliminary irritation test with 4 animals to be a 70 % w/w mixture in distilled water) was applied to a naive site on each guinea pig. A naive control group (ten animals) was maintained under the same environmental conditions and treated with the test substance at challenge only. Approximately 24 and 48 hours after each induction and challenge dose, the animals were scored for erythema.

The skin reaction to the challenge was scored according to the following criteria:

Scoring System

Score	**Erythema**
0	No reaction
0.5	Very faint erythema, usually non-confluent
1	Faint erythema, usually confluent
2	Moderate erythema
3	Severe erythema with or without oedema

Individual body weights of the animals were recorded prior to initiation and again on the day after challenge.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

Clinical observations were not reported.

C. BODY WEIGHT

Body weight was recorded but an analysis of the data was not performed.

D. NECROPSY

Performance of necropsy was not reported.

E. SKIN REACTIONS

<u>Induction Phase:</u>
Induction treatment with 70 % w/w mixture of the test substance in distilled water caused very faint erythema (0.5) at most test sites.

<u>Challenge Phase:</u>
After challenge with 70 % w/w mixture of the test substance in distilled water very faint erythema (0.5) was observed at six of twenty test sites 24-hours following the challenge application. Similar irritation persisted at one affected site through 48 hours.

The positive response observed in the historical positive control validation study with HCA validates the test system used in the study. After induction very faint to faint erythema (0.5- 1) was noted for all positive control sites during the induction phase. After challenge seven of ten positive control animals exhibited signs of a sensitisation response (faint erythema) 24-hours after challenge. Similar indications persisted at three of these sites through 48 hours.

Table 5.2.6-23: Glyphosate acid technical – Dermal Sensitisation in Guinea Pigs (Buehler Method) ([redacted] 2005f): Sensitisation response indices

	Incidence of Positive Response		Severity	
	24 h	48 h	24 h	48 h
Test Group	0/20	0/20	0.15	0.03
Negative Control Group	0/10	0/10	0.10	0.00

III. CONCLUSIONS

Based on these findings and on the evaluation system used, Glyphosate Acid Technical is not considered to be a contact sensitiser.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The GLP study is in concordance with OECD 406 guidelines. Therefore, the study is considered acceptable and the outcome can be reported as valid.
After challenge treatment with glyphosate, no skin reactions in guinea pigs were observed after 24 and 48 hours. Therefore, based on the results, glyphosate has no sensitising effect on the skin of the guinea pig in the Buehler Test.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.6/012
Report author	[redacted]
Report year	1996
Report title	Glyphosate Acid: Skin Sensitisation To The Guinea Pig
Report No	[redacted]/P/4699
Document No	Not reported
Guidelines followed in study	OECD 406 (1992); 92/69/EC B.6 (1992); US EPA Guidelines Section 81-6
Deviations from current test guideline (OECD 406, 1992)	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

In a dermal sensitisation study with glyphosate acid (Batch: P24, Purity: 95.6 % w/w a.i.) young adult, female, Albino Cr1 (HA) BR guinea pigs were tested using the method of Magnusson and Kligman (1970). The study involved the treatment of guinea pigs using two procedures: the potential induction of an immune response and a challenge of that response. The sensitisation response of the animals was determined 24 and 48 hours after challenge by assessing the degree of erythema. The dose levels selected for the induction and challenge stages of the study were determined by a sighting study in the guinea pig.

In the main study, a 0.1 % w/v preparation in deionised water was used for the intradermal injections and a 75 % w/v preparation of glyphosate acid in deionised water was used for the topical application. For challenge concentrations of 75 and 30 % (w/v) preparation of glyphosate acid in deionised water were used.

Challenge of previously-induced guinea pigs with a 75 % w/v preparation of glyphosate acid in deionised water elicited a response characteristic of an irritant.

Challenge of previously-induced guinea pigs with a 30 % w/v preparation of glyphosate acid in deionised water did not elicit a skin sensitisation response.

A historical positive control study using hexylcinnamaldehyde demonstrated the sensitivity of the test system.

Therefore, based on the results, glyphosate acid has no sensitising effect on the skin of the guinea pig in the Maximisation test.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate acid (technical)
Description:	White solid
Lot/Batch number:	P24
Purity:	As given in report 95.6 % a.i
Stability of test compound:	Not reported

2. Vehicle and/or positive control: Deionised water and / hexylcinnamaaldehyde (HCA)

3. Test animals:

Species:	Guinea pig
Strain:	Albino Cr1 (HA) BR
Age:	Young adult
Sex:	Female
Weight at dosing:	250 - 317 g
Source:	[redacted]
Housing:	Individually in suspended cages in racks suitable for animals of this strain and the weight range expected during the course of the study.
Acclimatisation period:	At least 6 days
Diet:	RGP), supplied by Labsure, Manea, Cambridgeshire, UK, *ad libitum*
Water:	Mains water, *ad libitum*
Environmental conditions:	Temperature: 17 ± 2 °C Humidity: 40 - 70 % Air changes: Approximately 25 changes/hour Photocycle: 12 hours light/dark cycle

B: Study design and methods

In-life dates: 1995-04-25 to 1995-05-19

Animal assignment and treatment: In a dermal sensitisation study with glyphosate acid (95.6 % w/w a.i.) young adult, female, Albino Cr1 (HA) BR guinea pigs were tested using the method of Magnusson and Kligman (1970). The study involved the treatment of guinea pigs using two procedures: the potential induction of an immune response and a challenge of that response.

Table 5.2.6-24 Glyphosate Acid: Skin Sensitisation to the Guinea Pig ([redacted] 1996): Animal assignment to the treatment groups

Treatment group	Number of animals
Main Study	
Test group – treated with test substance at both the induction and challenge	20
Negative control group – treated with test substance only at the challenge	10

Induction: An area approximately 5 x 5 cm on the scapular region of each animal was clipped free of hair and a row of three injections (0.05 - 0.1 mL each) was made on each side of the mid-line. The injections were:

i) Top: Freund's Complete Adjuvant plus deionised water in the ratio 1:1;

ii) Middle: a 0.1 % w/v preparation of the test substance in deionised water;

iii) Bottom: a 0.1 % w/v preparation of the test substance in a 1:1 preparation of Freund's Complete Adjuvant plus deionised water.

Control animals were treated the same as the test animals, except that they were treated with deionised water in place of the test substance.

One day prior to topical induction, the application site was clipped and 0.5 mL of a 10 % w/v preparation of sodium lauryl sulphate in paraffin wax was applied in order to provoke a mild inflammatory response.

One week after intradermal injection, the scapular area was treated with a topical application of the test substance as a 75 % w/v preparation in deionised water. This preparation (0.2 - 0.3 mL) was applied on filter paper (approximate size 4 cm x 2 cm) which was held in place by a piece of surgical tape. The tape was covered by a strip of adhesive bandage (approximate size 20 – 30 cm x 5 cm) and secured by a piece of self-adhesive PVC tape. This occlusive dressing was kept in place for approximately 2 days.

Deionised water only was applied to the filter paper for control animals.

The application sites were checked approximately 1 day after removal of the dressings.

Challenge: Two weeks after the topical inductions, an area, approximately 15 cm x 5 cm, on both flanks of all the test and control animals, was clipped free of hair. An occlusive dressing was prepared which consisted of two pieces of filter paper (approximate size 1 cm x 1.5-2.0 cm) stitched to a piece of rubber sheeting (approximate size 12 cm x 5 cm).

A 75 % w/v preparation of the test substance in deionised water (0.05 - 0.1 mL) was applied to one of the pieces of filter paper and a 30 % w/v preparation in deionised water (0.05 - 0.1 mL) was applied to the

second piece of filter paper. The dressing was placed on the shorn flank of the guinea pig so that the 75 % w/v preparation was on the left and the 30 % w/v preparation was on the right. It was then covered with a strip of adhesive bandage (approximate size 25-40 cm x 7.5 cm) which was secured by a self-adhesive PVC tape.

After approximately 1 day, the dressings were carefully removed. Skin sites were examined approximately 1 and 2 days after removal of the dressings and any erythematous reactions were quantified and recorded, using a four-point scale.

Score	Dermal Observations
0	No reaction
1	Scattered mild redness
2	Moderate diffuse redness
3	Intense redness and swelling

Positive Controls: The sensitising potential of hexylcinnamaaldehyde (HCA) was assessed essentially as described above to demonstrate the sensitivity of the strain of animals used and the reliability of the experimental technique. A concentration of 0.3 % w/v HCA in corn oil was used for the intradermal injections and HCA was used undiluted for the topical induction and challenge applications.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortalities were reported.

B. CLINICAL OBSERVATIONS

Clinical observations were made but none were reported.

C. BODY WEIGHT

There were no treatment-related effects on bodyweight during the study.

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

E. SKIN REACTIONS

Induction reactions and duration: Not reported.

Challenge reactions and duration: Following challenge of previously-induced guinea pigs with a 75 % w/v preparation of the glyphosate acid in deionised water, scattered mild redness was seen in three of the twenty test animals and one of the ten control animals. This response is considered to be due to skin irritation following topical challenge. The basis for this conclusion is that an equivalent reaction was seen in one of the ten control animals and the reaction was restricted to the 24-hour clinical observation only, which is characteristic of a mild skin irritation reaction rather than skin sensitisation.

Following challenge of previously-induced guinea pigs with a 30 % w/v preparation of the glyphosate acid in deionised water, no reaction was seen in any of the test or control animals. The net percentage response was calculated to be 0 %.

Positive control: Following challenge of previously induced guinea pigs, scattered mild redness or moderate diffuse redness was observed in 14/20 test animals. Scattered mild redness was seen in two of the ten control animals. The net % response was 50 % and, therefore, HCA was classified as a moderate skin sensitiser which demonstrated the sensitivity of the strain of animals used and the reliability of the experimental technique.

Table 5.2.6-25 Glyphosate Acid: Skin Sensitisation to the Guinea Pig ([redacted] 1996): Summary of skin reactions after challenge

Treatment group	Incidence*			
	75 %**		30 %**	
	24-hours	48 hours	24-hours	48 hours
Main test – test group	3/20	0/20	0/20	0/20
Main test – negative vehicle control	1/10	0/10	0/10	0/10
	Incidence* (100 %**)			
	24-hours		48 hours	
Positive control – test group	14/20		13/20	
Positive control – vehicle control	2/10		0/10	

*: Number of animals with findings / number of animals tested
**: Test substance concentration during challenge phase

III. CONCLUSIONS

Glyphosate acid is not a skin sensitiser under the conditions of the test.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The GLP study is in concordance with the current OECD 406 (1992). Therefore, the study is considered acceptable / reliable and the outcome can be reported as valid.
After challenge treatment with glyphosate (30 %), no skin reactions in guinea pigs were observed after 24 and 48 hours. Therefore, based on the results, glyphosate has no sensitising effect on the skin of the guinea pig in the Maximisation test.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.6/013
Report author	[redacted]
Report year	1995
Report title	HR-001: Dermal sensitisation study in Guinea pigs
Report No	[redacted] 95-0036
Document No	Not reported
Guidelines followed in study	OECD 406 (1992), US EPA FIFRA Guideline Subdivision F (1984)
Deviations from current test guideline (OECD 406, 1992)	Not reported whether clinical signs of toxicity were recorded.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes

Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

For the determination of potential sensitising properties of HR-001 (Glyphosate Technical, Batch: T-941209; Purity: 97.56 %) was applied to the skin of female Hartley strain guinea pig Maximisation test. DNCB (2,4-dinitrochlorobenzene) was used as the positive control substance. Animals were assigned to the following four groups:

- 20 animals to the test substance treatment group, treated with the test substance both at the induction and challenge,
- 20 animals to the negative control group for the test substance, treated with the test substance at the challenge but not at the induction,
- Ten animals to the DNCB treatment group treated with DNCB both at induction and challenge, and
- Ten animals to the negative control group for DNCB, treated with DNCB at the challenge but not at the induction.

Concentrations of 5 %, 25 %, and 25 % of the test substance, and concentrations of 0.1 %, 1 %, and 0.5 % of DNCB were selected as the dose for intradermal induction, epidermal induction, and epidermal challenge, respectively. Skin reactions to the challenge were observed 24 and 48 hours after removal of the patch and dermal sensitisation rates were calculated.

All 20 animals in the test substance treatment group exhibited the reaction of 0 (no reaction).

All 20 animals in the negative control group for the test substance also exhibited score 0. Thus the sensitising rate, i.e. the percentage of animals positively sensitised, was 0 % in the test substance treatment group.

The positive control group administered DNCB had nine animals because one animal died at Day 12 after intradermal induction. These animals exhibited a reaction of score 3 (intense redness and swelling). All ten animals in the negative control group for DNCB exhibited a score of 0. Thus the sensitising rate of DNCB was greater than 100 %. This was sufficient to assure the reliability of this study.

Therefore, based on the results, HR-001 (Glyphosate Technical) has no sensitising effect on the skin of the guinea pig in the Maximisation test.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	HR-001 (Glyphosate technical)
Description:	White crystal
Lot/Batch #:	T-941209
Purity:	97.56 %
Water solubility	12 g/L (25 °C)
Stability of test compound:	Not reported

2. Vehicle and/or positive control:

Paraffin oil (intradermal); white petrolatum (epidermal)

3. Test animals:

Species:	Guinea pig
Strain:	Crj:Hartley
Source:	[redacted]
Age:	6 weeks
Sex:	Female
Weight at dosing:	332 – 423 g
Acclimation period:	8 days
Diet/Food:	Pellet diet GC4 (Oriental Yeast Co., Ltd., Azusawa, Itabashi-ku, Tokyo, Japan), *ad libitum*
Water:	Filtered and sterilized water, *ad libitum*
Housing:	Aluminium cage with wire-mesh floor, 5 animals/cage
Environmental conditions:	Temperature: 23.9 – 24.0 °C Humidity: 51.8 - 56.3 % Air changes: 15/hour Photocycle: 12 hours light/dark cycle

B: Study design and methods

In life dates: 1995-04-19 to 1995-05-13

Animal assignment and treatment:
The test was carried out according to the Maximisation method of Magnusson and Kligman.

A preliminary test was performed to determine the dose levels for intradermal injections and topical exposure. Intradermal injections were administered to four animals at dose levels of 0.5 %, 1 %, 2.5 %, and 5 % (w/v) of the test substance in Freund's Complete Adjuvant (FCA) in sterilized physiological salt solution (SPS). The test substance caused moderate and diffuse redness or weaker irritation at all concentrations.

To determine the epidermal induction and challenge doses, four animals received doses of 10 % and 25 %, and another four animals received doses of 15 % and 20 %; the test substance was applied in a mixture with white petrolatum by a closed patch application. None of the concentrations caused any irritation to the skin.

Based on these results, twenty female Hartley guinea pigs (Crj:Hartley) were exposed to concentrations of 5 %, 25 %, and 25 % glyphosate technical for intradermal induction, epidermal induction, and epidermal challenge, respectively.

The positive control, 2,4-dichlorobenzene (DCNB), was administered to ten females at concentrations of 0.1 %, 1 %, and 0.5 % for intradermal induction, epidermal induction, and epidermal challenge, respectively.

Groups of ten and 20 animals were used for the negative control group for DCNB (treated with DCNB at the challenge but not at the induction) and the negative control group for technical glyphosate (treated with test substance at the challenge but not at the induction), respectively.

Table 5.2.6-26 HR-001: Dermal sensitisation study in Guinea pigs ([redacted], 1995): Animal assignment to the treatment groups

Treatment group	Number of animals
Main Study	
Test group – treated with test substance at both the induction and challenge	20
Negative control group – treated with test substance only at the challenge	20
Positive control group- treated with DNCB at both the induction and challenge	10
Negative control group for DNCB – treated with DNCB only at the challenge	10

For the main test, animals received in the test and positive control groups received three pairs of intradermal injections: 0.1 mL water in oil emulsion of FCA blended with SPS, 0.1 mL test substance or DNCB in paraffin oil, and 0.1 mL test substance or DNCB in FCA blended with SPS. Animals in the negative control groups received similar injections without test substance or DNCB.

On Day 6 after the intradermal induction, 10 % sodium lauryl sulfate in white petrolatum was applied to the shaved skin by open application. On Day 7 after the intradermal induction, the animals in the test and positive control groups were exposed to the test substance and DNCB, respectively; 0.4 g of the preparation was applied to filter paper (2 cm x 4 cm) which was applied to the skin and covered with an occlusive dressing for 48 hours. The negative control groups were treated in a similar way, but without the test substance or DNCB.

On Day 13 after the topical induction, the test and negative control groups were topically exposed to 0.4 g of the test substance as described above for 24-hours. Animals in the positive control and negative control group for DNCB were exposed to 0.4 g DNCB as described above.

Skin reactions to the challenge were recorded 24 and 48 hours after removal of the patch, and dermal sensitisation rates were calculated as [(No. of animals positively sensitised)/(No. of animals examined) x 100]. The skin reaction to the challenge was scored according to the following criteria:

Score	**Reaction**
0	No reaction
1	Scattered mild redness
2	Moderate and diffuse redness
3	Intense redness and swelling

Body weights were measured at the first induction and 48 hours after the removal of the patch. Necropsy was conducted for one animal of the DNCB treatment group that died on Day 12.

II. RESULTS AND DISCUSSION

A. MORTALITY

One animal died in the DNCB treatment group on Day 12 after the intradermal induction. The remaining animals in this group did not show any abnormality in the health condition and the skin reactions were clearly observed.

B. CLINICAL OBSERVATIONS

No signs of systemic toxicity were reported.

C. BODY WEIGHT

No abnormal body weight changes were noted in any animal of the four groups.

D. NECROPSY

One animal died in the DNCB treatment group. At necropsy of the dead animal, consolidation of lung and hydrothorax were noted. These findings were associated with the hindrance of circulation and the respiratory abnormality, which led to the death.

E. SKIN REACTIONS

No data were provided on skin irritations after induction.

Following challenge with 25 % technical glyphosate, no oedema or erythema were observed in test animals. The rate of sensitisation in the test substance treatment group was therefore 0 %.

The rate of sensitisation in the DNCB treatment group was 100 %, which was considered to sufficiently assure the reliability of this study.

Table 5.2.6-27: HR-001: Dermal sensitisation study in Guinea pigs ([redacted], 1995): Summary of skin reactions after challenge

Treatment group	Incidence*			
	No reaction	Scattered mild redness	Moderate and diffuse redness	Intense redness and swelling
	Score 0	1	2	3
Test group – treated with test substance at both the induction and challenge	20/20	0/20	0/20	0/20
Negative control group – treated with test substance only at the challenge	20/20	0/20	0/20	0/20
Positive control group- treated with DNCB at both the induction and challenge	0/10	0/10	0/10	9/10
Negative control group for DNCB – treated with DNCB only at the challenge	10/10	0/10	0/10	0/10

*: number of animals with skin reaction / number of animals tested

III. CONCLUSIONS

Based on the results, it was concluded that glyphosate technical (HR-001) had no dermal sensitising potential in the guinea pig Maximisation test.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The GLP study is in concordance with the current OECD 406 (1992). Therefore, the study is considered acceptable and the outcome can be reported as valid.
After epidermal induction and a challenge treatment with glyphosate technical no skin reactions in any treated or control guinea pigs were observed 48 and 72-hours after the start of the challenge. Therefore, based on the results, glyphosate has no sensitising effect on the skin of the guinea pig in the Maximisation test.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.6/014
Report author	[redacted]
Report year	1994
Report title	Glyphosate: Magnusson & Kligman maximisation study in the guinea pig.
Report No	710/19
Document No	Not reported
Guidelines followed in study	N/A
GLP	N/A
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	In a guinea pig maximisation study, 15 female Dunkin-Hartley guinea pigs were administered 1 % w/v of the test material in archis oil during the induction period. Animals were then challenged with either 50 % w/w of the test material or 25 % w/w of the test material in arachis oil.
Short description of results:	The test material was considered not sensitizing
Reasons for why the study is not considered relevant/reliable or not considered as key study:	A full study report to evaluate was not available.
Reasons why the study report is not available for submission	The notifier has not access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL
Category study in AIR 5 dossier (L docs)	Category 4a

1. Information on the study

Data point:	CA 5.2.6/015
Report author	[redacted]
Report year	1994
Report title	Glyphosate Premix: Magnusson & Kligman Maximisation Study in the Guinea Pig
Report No	545/42
Document No	Not reported
Guidelines followed in study	US EPA Guidelines Section 81-6
Deviations from current test guideline (OECD 406, 1992)	A positive control was not included in the study; nevertheless historical control data not older than 6 months were provided; it was not reported whether clinical signs of toxicity were measured.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Glyphosate premix (Batch: 290-Jak-146-4; Purity: 62.2 % as glyphosate isopropylamine salt; 46.1 % as glyphosate) was tested for its sensitising effect on the skin of the guinea pig in the Maximisation Test. The test-substance concentrations for the main test were selected based on the results of the pre-test. The intradermal induction was performed with a 25 % dilution of the test item in distilled water and an emulsion of Freund's Complete Adjuvant (FCA)/distilled water. The epidermal induction was conducted under occlusion with undiluted test material one week after the intradermal induction. Two weeks after induction the animals were challenged by epidermal application of undiluted test material under occlusive dressing.

The study was performed using one control group consisting of ten animals, and one test group consisting of 20 animals. None of the animals exhibited a positive skin reaction after the challenge treatment. There was no effect on body weight gain.

Historical control data confirm the sensitivity and reliability of the experimental technique within six months compared to the current study. Therefore, based on the results, glyphosate premix has no sensitising effect on the skin of the guinea pig in the Maximisation test.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate premix
Description:	Pale yellow liquid
Lot/Batch #:	290-Jak-146-4
Purity:	62.2 % as glyphosate isopropylamine salt; 46.1 % as glyphosate
Stability of test compound:	Not reported; expiry date: 1995-09-30
2. Vehicle and/ or positive control:	Distilled water

3. Test animals:

Species:	Guinea pig
Strain:	Albino Dunkin-Hartley
Source:	[redacted]
Age:	8 to 12 weeks old
Sex:	Female
Weight at dosing:	340 - 450 g
Acclimation period:	At least 5 days
Diet/Food:	Guinea Pig FDI Diet, supplied by Special Diet Services Limited, Witham, Essex, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Housed 5 test animals/cage; all 6 control animals were housed together
Environmental conditions:	Temperature: 21 - 24 °C Humidity: 47 - 67 % Air changes: 15/hour Photocycle: 12 hours light/dark cycle

B: Study design and methods

In life dates: 1994-03-22 to 1994-05-02

Animal assignment and treatment:
Glyphosate premix was tested for its sensitising effect on the skin of the guinea pig using the Maximisation test according to Magnusson and Kligman. Female Dunkin Hartley guinea pigs with body weights ranging from 340 to 450 g were used. The test substance concentrations for the main study were selected based on the results of the pre-testing. The main study was performed in 20 test animals and 10 control animals.

Table 5.2.6-28 Glyphosate Premix: Magnusson & Kligman Maximisation Study in the Guinea Pig ([redacted], 1994): Animal assignment to the treatment groups

Treatment group	Number of animals
Pretest	
Intradermal Pretest	4
Epidermal Pretest	2
Main Study	
Negative Control Group	10
Test Group	20

The induction phase consisted of a row of 3 injections that were made on each side of the mid-line. The injections (0.1 mL/site) consisted of Freund´s Complete Adjuvant plus distilled water in the ratio of 1:1; a 25 % w/v dilution of test material in distilled water; and a 25 % w/v dilution of test material in 1:1 preparation of Freund's Complete Adjuvant plus distilled water.

One week after (Day 7) the injection phase, an epidermal application was made. Animals were clipped again, and undiluted test material was topically applied to the same shoulder area and covered with an occlusive dressing, which was left in place for 48 hours. The reaction sites were assessed 24 and 48 hours after removal of the bandage.

Two weeks after the topical indication, test and control animals were challenged with an occlusive patch containing 0.1 to 0.2 mL of undiluted test material. To ensure that the maximum non-irritant concentration was used at the challenge, the test material at a concentration of 75 % v/v in distilled water was applied similarly applied to a separate skin site on the right shorn flank. Vehicle was applied alone to the left shorn flank. Approximately 24 and 48 hours after the challenge, the degree of erythema and oedema was quantified.

Control animals were administered intradermal injections using the same procedure noted above for the test animals except the injections were as follows: Freund´s Complete Adjuvant plus distilled water in the ratio of 1:1; distilled water; and Freund´s Complete Adjuvant plus distilled water in the ratio of 1:1. Topical applications used the same procedures as those noted for test animals except that the vehicle alone was applied.

No positive control animals were evaluated during the study. Historical control data were provided.

Body weights were recorded at the start of the main test and on test completion.

Any animal showing erythema at the site of challenge was considered to have shown a positive response.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortality was observed during the study.

B. CLINICAL OBSERVATIONS

Clinical signs of toxicity were not reported.

C. BODY WEIGHT

Body weights and body weight gains of guinea pigs in the test group, between Day 0 and Day 24, were comparable to those of the control animals.

D. NECROPSY

A necropsy was not performed.

E. SKIN REACTIONS

No skin reactions were observed 24 or 48 hours after the challenge treatment with glyphosate premix in the control or test group.

Table 5.2.6-29 Glyphosate Premix: Magnusson & Kligman Maximisation Study in the Guinea Pig ([redacted], 1994): Summary of skin reactions after challenge

Treatment group	Incidence*	
	24-hours	48 hours
Test group – undiluted	0/20	0/20
Test group – 75 %	0/20	0/20
Negative control group	0/10	0/10

*: number of animals with findings / number of animals tested

III. CONCLUSIONS

Based on the results of the study, glyphosate premix is not considered a skin sensitiser.

Assessment and conclusion

Assessment and conclusion by applicant:
Except to the deviation of providing no control group, the GLP study is in concordance with the current OECD TG 406 (1992). Therefore, the study is considered acceptable and the outcome can be reported as valid.
After epidermal induction with 25 % glyphosate technical and a challenge treatment with 75 % no skin reactions in any treated or control guinea pigs were observed 48 and 72-hours after the start of the challenge. Therefore, based on the results, glyphosate has no sensitising effect on the skin of the guinea pig in the Maximisation test.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.6/016
Report author	[redacted]
Report year	1994
Report title	Glyphosate (Alkaloida, Tiszavasvári). Sensitisation in Guinea Pigs
Report No	GHA-94-406/G
Document No	Not reported
Guidelines followed in study	OECD 406 (1981)
Deviations from current test guideline (OECD 406, 1992)	Yes, 8 instead of a minimum of 20 animals used in the treatment group, evaluation of skin reactions after 24, 48 and 72-hours instead of 30 and 54 hours after challenge; age of animals not reported; except of body weight, no individual animal data provided and therefore, no scores on skin reaction of single animals were reported; experimental procedures are only briefly described; no pilot study was conducted and therefore no conclusion on induction and challenge concentrations to be used in the test were provided; the highest concentration to cause mild irritation for induction and the maximum non-irritant concentration for challenge were not determined; it is not known whether a negative control group has been included; limited data on the historical positive control DNCB (2,4-dinitro-chlorobenzene) which is not one of the prefered substances as recommended in the guideline.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Glyphosate (Batch: 36300892; Purity: 97.2 %) was tested for its sensitising effect on the skin of the guinea pig using the modified Buehler test method. During the induction phase, eight male animals were treated with 0.5 mL of an aqueous solution of 1.2 % glyphosate; it was not reported whether a negative control group was evaluated. For the positive control group, results from historical positive control animals treated with dinitrochlorobenzene were used. Applications were made for six hours per day, three days per week, ten times altogether. Two weeks after the final induction animals were challenged with the glyphosate solution. Dermal irritation was scored at 24, 48, and 72-hours after the challenge application.

All animals survived until study termination. There were no body weight changes that were considered treatment-related. The general health of the treated animals was unaffected by treatment.

The combined indices for erythema and oedema were 0.00, 0.00, and 0.00 for the 24, 48, and 72-hour observation periods, respectively, after challenge. No individual animal data on skin reactions were provided.

For the historical positive control group, the combined indices obtained at hours 24, 48, and 72 were 3.83, 3.00, and 1.50, respectively, corresponding to moderate, moderate, and slight sensitisation.

Therefore, based on the results, glyphosate is a non-sensitising substance in guinea pigs.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate
Description:	White or almost white crystalline powder
Lot/Batch #:	36300892
Purity:	97.2 %
Stability of test compound:	Not reported

2. Vehicle and/ or positive control:

Water / 1-chloro-2,4-dinitrobenzene (DNCB)

3. Test animals:

Species:	Guinea pig
Strain:	Albino
Source:	[redacted]
Age:	Not reported
Sex:	Male
Weight at dosing:	Not reported
Acclimation period:	It was reported that animals were quarantined but the duration was not reported.
Diet/Food:	Standard guinea pig chow supplemented with carrots, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually housed in suspended stainless steel cages
Environmental conditions:	Temperature: 20 ± 2 °C Humidity: 45 – 70 % Air changes: 10/hour Photocycle: Not reported

B: Study design and methods

In life dates: 1994-01-17 to 1994-02-23

Animal assignment and treatment:
Glyphosate was tested for its sensitising effect on the skin of the guinea pig using a modified Buehler test method. It was not reported how the test substance concentrations for the main study were selected. The main study was performed in eight test animals. It was not reported whether negative control animals were evaluated. For the positive control group, results from historical positive control animals treated with dinitrochlorobenzene were used.

Table 5.2.6-30 Glyphosate (Alkaloida, Tiszavasvári). Sensitisation in Guinea Pigs ([redacted] 1994): Animal assignment to the treatment groups

Treatment group	Number of animals
Main Study	
Test Group	8

The induction phase consisted of applying the test material directly to the shaved flanks of each guinea pig. The next day, 0.5 mL of the test material in a 1.2 % solution in water was applied by intense rubbing for 30 seconds. The induction was performed a total of ten occasions at two day intervals on the right and left flank, respectively. Animals were inspected over the next days for signs of primary irritancy. After each administration, an occlusive dressing was used to hold the patch in place for six hours.

Two weeks after the last exposure, animals were challenged with the test material. Subsequent skin reactions were scored for both local oedema and erythema at hours 24, 48, 72, and 96. The skin reaction after the challenge was scored according to the to the Draize's method.

Clinical signs of toxicity were observed and recorded. Body weights were determined prior to treatment and weekly thereafter for five weeks.

II. RESULTS AND DISCUSSION

A. MORTALITY

No deaths occurred in during the study period.

B. CLINICAL OBSERVATIONS

No signs of toxicity were observed.

C. BODY WEIGHT

Body weights were unaffected by treatment.

D. NECROPSY

A necropsy was not performed.

E. SKIN REACTIONS

The combined indices for erythema and oedema were 0.00, 0.00, and 0.00 for the 24, 48, and 72-hour observation periods, respectively, after challenge with glyphosate. For the historical positive control group, the combined indices obtained at hours 24, 48, and 72 were 3.83, 3.00, and 1.50, respectively, corresponding to moderate, moderate, and slight sensitisation. No individual scores were reported.

III. CONCLUSIONS

Based on the results of the study, 1.2 % glyphosate solution in water applied epicutaneously on the guinea pig skin proved to be non-sensitising agent.

Assessment and conclusion

Assessment and conclusion by applicant

The GLP study is in concordance with OECD TG 406 (1981). Therefore, the study is considered acceptable and valid. Nevertheless, due to the deviations from the current OECD TG 406 (1992), such as reduced test animal number, no justification of concentration, no individual skin scores provided and limited reporting, the study can be used as supplementary information, only.

After challenge treatment with 1.2 % glyphosate, no skin reactions in guinea pigs were observed after 24, 48 and 72-hours. Therefore, based on the results, glyphosate has no sensitising effect on the skin of the guinea pig in the modified Buehler Test.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

2. Information on the study

Data point:	CA 5.2.6/017
Report author	[redacted]
Report year	1993
Report title	Skin sensitization test in guinea pigs with glyphosate technical 95 % of Excel Industries Ltd.
Report No	[redacted] 1230
Document No	Not reported
Guidelines followed in study	N/A
GLP	N/A
Previous evaluation	PREVIOUSLY submitted (accepted in Monograph, B5, 2000, not re-evaluated in RAR Vol 3, B6, 2015)
Short description of study design and observations:	In a guinea pig maximisation study, 28 male and female English guinea pigs were induced intradermally with 5 % of the test material in propylene glycol and topically with 50 % of the test material in propylene glycol.
Short description of results:	The test material was considered not sensitizing
Reasons for why the study is not considered relevant/reliable or not considered as key study:	A full study report to evaluate was not available.
Reasons why the study report is not available for submission	The notifier has not access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL
Category study in AIR 5 dossier (IUCLID docs)	Category 4a

1. Information on the study

Data point:	CA 5.2.6/054
Report author	[redacted]
Report year	1992
Report title	Dermal Sensitization Study in Guinea Pigs with MON 8722 – Modified Buehler Study Design – (EPA-OECD-EEC-MAFF)
Report No	3044.229
Document No	Not reported
Guidelines followed in study	US EPA Guidelines Section 81-6 (1984); OECD 406 (1987)
Deviations from current test guideline (OECD 406, 1992)	Yes, 10 instead of a minimum of 20 animals used in the treatment group, evaluation of skin reactions 24 and 48 hours instead of 30 and 54 hours after challenge; environmental conditions for animals not fully reported (missing temperature and humidity data); age of animals at study initiation not reported; batch and purity of the test substance not provided; clinical signs of toxicity not reported; assessment of

	sensitivity and reliability of the experimental technique not within six months as recommended in the guideline; DNCB (2,4-dinitro¬chlorobenzene) used as positive control is not one of the preferred substances recommended in the guideline.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

For the determination of potential sensitising properties of MON 8722 (glyphosate, sodium salt, Batch: RUD-9108-3241-F; Purity: not reported) a modified Buehler test was conducted. The test-substance concentrations for the main test were selected based on the results of the pre-test. The epidermal induction was performed with undiluted, moistened test item under occlusive conditions and was repeated once per week for a total of three applications. Fourteen (14) days after induction the animals were challenged by epidermal application of undiluted test material, moistened with saline, under occlusive dressing.

The study was performed using five male and five female Dunkin Hartley albino guinea pigs for the control and the test group, respectively. None of the animals exhibited a positive skin reaction after the challenge treatment.

DNCB (2,4-dinitrochlorobenzene) was used as the positive control substance in a separately conducted study of the same laboratory. These data demonstrate the susceptibility of guinea pigs to sensitisation.

Therefore, based on the results of this study, MON 8722 (glyphosate, sodium salt) has no sensitising effect on the skin of the guinea pig in the modified Buehler Test.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	MON 8722 (Glyphosate, sodium salt)
Description:	White granular powder
Lot/Batch #:	RUD-9108-3241-F
Purity:	Not reported
Stability of test compound:	Not reported

2. Vehicle and/or positive control: Distilled water / dinitrochlorobenzene historical control data

3. Test animals:

Species:	Guinea pig
Strain:	Albino Dunkin-Hartley
Source:	[redacted]
Age:	Not reported
Sex:	Male and female

Weight at dosing:	Male: 356 - 393 g; females: 334 – 391 g	
Acclimation period:	At least 5 days	
Diet/Food:	Agway Prolab Guinea Pig formula provided *ad libitum*	
Water:	Tap water, *ad libitum*	
Housing:	Individually housed in suspended stainless steel cages	
Environmental conditions:	Temperature:	Not reported
	Humidity:	Not reported
	Air changes:	Not reported
	Photocycle:	12 hours light/dark cycle

B: Study design and methods

In life dates: 1991-08-13 to 1991-10-30

Animal assignment and treatment:

MON 8722 was tested for its sensitising effect on the skin of the guinea pig using a modified Buehler test method. The test substance concentrations for the main study were selected based on the results of the pre-testing. In the preliminary test, four animals were treated topically with undiluted test material (100 %) and with concentrations of 50 %, 25 %, and 10 % w/v of the MON 8722 in distilled water. Based on results of the pre-test, the undiluted material was found to be non-irritating and was, therefore, administered at 100 % concentration for both induction and challenge. The main study was performed using five animals per sex for the test and the control group, respectively.

Table 5.2.6-31 Dermal Sensitisation Study in Guinea Pigs with MON 8722 – Modified Buehler Study Design ([redacted], 1992): Animal assignment to the treatment groups

Treatment group	Number of animals	
Pretest	**Males**	**Females**
Epidermal Pretest	2	2
Main Study	**Males**	**Females**
Test Group	5	5
Challenge Irritation Control	5	5
Rechallenge Irritation Control	5	5

One day prior to the induction phase, the hair was clipped from the left side of five male and five female test animals. The next day, the test site was moistened with distilled water and 0.4 g of undiluted test substance was applied to the test site. Approximately six hours after dosing, test sites were wiped with gauze moistened with distilled water. This induction procedure was repeated once per week for a total of three applications. Following each induction, test sites were scored for dermal irritation. Following the induction phase, the test animals were left untreated for a period of 14 days. Challenge and rechallenge controls animals remained untreated throughout the induction phase.

On the day prior to challenge hair was clipped from the posterior left side of the test animals and from the ten previously untreated guinea pigs. Test sites were moistened with distilled water and 0.4 g of undiluted MON 8722 was applied to the test area. An occlusive dressing was then placed over the test site. After six hours of exposure, the test site was wiped with gauze moistened with distilled water. Twenty hours after patch removal, residual hair was removed with a commercial depilatory. Fifteen minutes after application, the commercial depilatory was removed, and test sites were graded for dermal irritation at 24 and 48 hours.

Historical control data from animals exposed to dinitrochlorobenzene was used as positive control.

All animals were observed daily for viability and weekly for clinical signs of toxicity. Body weights were recorded at the start of the main test and on test completion.

Any animal showing erythema (score 1 or higher) at the site of challenge was considered to have shown a positive response. Scores of ± (barely perceptible erythema) were considered equivocal.

The skin reaction to the challenge was scored according to the following criteria:

Score	**Dermal Observations**
0	No reaction
±	Slight patchy erythema
1	Slight, but confluent or moderate patchy erythema
2	Moderate confluent erythema
3	Severe erythema with or without oedema

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortality was observed during the study.

B. CLINICAL OBSERVATIONS

Clinical signs of toxicity were not reported.

C. BODY WEIGHT

Body weights were considered acceptable.

D. NECROPSY

A necropsy was not performed.

E. SKIN REACTIONS

No dermal irritation was observed during the induction phase. No skin reactions were observed 24 or 48 hours after the challenge treatment with MON 8722 in the control or test group.

Table 5.2.6-32 Dermal Sensitisation Study in Guinea Pigs with MON 8722 – Modified Buehler Study Design ([redacted], 1992): Summary of skin reactions after challenge

Treatment group / animal number	Skin reaction (scores)	
	24-hours	48 hours
Test group – undiluted		
Male 1	0	0
Male 2	0	0
Male 3	0	0
Male 4	0	0
Male 5	0	0
Female 1	0	0
Female 2	±	0

Table 5.2.6-32 Dermal Sensitisation Study in Guinea Pigs with MON 8722 – Modified Buehler Study Design ([redacted]1992): Summary of skin reactions after challenge

Female 3	±	±
Female 4	±	0
Female 5	±	0
Challenge irritation controls		
Male 6	0	0
Male 7	±	0
Male 8	0	0
Male 9	0	0
Male 10	0	0
Female 6	0	0
Female 7	0	0
Female 8	0	0
Female 9	0	0
Female 10	0	0

III. CONCLUSIONS

Under the conditions of the study, MON 8722 exhibited no potential to induce dermal sensitisation in guinea pigs.

Assessment and conclusion

Assessment and conclusion by applicant:
The GLP study is in concordance with Guidelines Section 81-6, equivalent to the current OECD 406 (1992). Due to the deviations from the current OECD TG 406 (1992), such as reduced test animal number, no reporting of the test substance purity, the study can be used as supplementary information, only.
After challenge treatment with MON 8722, no skin reactions in guinea pigs were observed after 24 and 48 hours. Therefore, based on the results, glyphosate has no sensitising effect on the skin of the guinea pig in the modified Buehler Test.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.6/019
Report author	[redacted]
Report year	1991
Report title	Luxan Glyphosate Tech.: Magnusson & Kligman Maximisation Study in the Guinea Pig
Report No	349/11
Document No	Not reported
Guidelines followed in study	OECD 406 (1981); Commission Directive 84/449/EEC B.6
Deviations from current test guideline (OECD 406, 1992)	Batch and purity of the test substance not provided; clinical signs of toxicity not recorded; low temperature 15 - 22 °C instead of 20 °C (± 3 °C); humidity of 26 - 64 % instead of 30-70 %.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Glyphosate technical (Batch and Purity: not reported) was tested for its sensitising effect on the skin of the guinea pig in the Maximisation Test. The test-substance concentrations for the main test were selected based on the results of the pre-test. The intradermal induction was performed with a 0.1 % dilution of the test item in distilled water and an emulsion of Freund's Complete Adjuvant (FCA)/distilled water. The epidermal induction was conducted under occlusion with the test item at 50 % one week after the intradermal induction. Two weeks after induction the animals were challenged by epidermal application of the test item at 25 % under occlusive dressing.

The study was performed using one control group consisting of ten animals, and one test group consisting of 20 animals. None of the animals exhibited a positive skin reaction after the challenge treatment. There was no effect on body weight gain.

Formaldehyde was used as the positive control substance in a separately conducted study of the same laboratory. These data demonstrate the susceptibility of guinea pigs to sensitisation. Therefore, based on the results of this study, Glyphosate Technical has no sensitising effect on the skin of the guinea pig in the Maximisation test.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	LUXAN GLYPHOSATE TECHN.
Description:	White powder
Lot/Batch #:	Not reported
Purity:	Not reported
Stability of test compound:	Not reported

2. **Vehicle and/or positive control:**
3. **Test animals:**

Species:	Guinea pig
Strain:	Albino Dunkin-Hartley
Source:	[redacted]
Age:	8 - 12 weeks old
Sex:	Female
Weight at dosing:	328 - 440 g
Acclimation period:	At least 5 days
Diet/Food:	Guinea Pig FDI Diet, Special Diet Services Limited, Witham, Essex, U.K., *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Housed in groups up to 3 animals/cage in solid-floor polypropylene cages furnished with softwood shavings
Environmental conditions:	Temperature: 15 - 22 °C Humidity: 26 - 64 % Air changes: 15/hour Photocycle: 12 hours light/dark cycle

B: Study design and methods

In life dates: 1990-12-12 to 1991-02-03

Animal assignment and treatment:

Glyphosate technical was tested for its sensitising effect on the skin of the guinea pig using the Maximisation test according to Magnusson and Kligman. The test substance concentrations for the main study were selected based on the results of the pre-testing. The main study was performed in 20 test animals and ten control animals.

Table 5.2.6-33 Luxan Glyphosate Tech.: Magnusson & Kligman Maximisation Study in the Guinea Pig ([redacted] 1991): Animal assignment to the treatment groups

Treatment group	Number of animals
Pretest	
Intradermal Pretest	4
Epidermal Pretest	2
Main Study	
Negative Control Group	10
Test Group	20

The induction phase consisted of a row of three injections made of each side of the mid-line on Day 0. One week later an epidermal application was made on Day 7.

On Day 0 the test substance was injected (0.1 mL/site) into the clipped dorsal skin from the shoulder region as: Freund´s Complete Adjuvant and distilled water in a 1:1 ratio; a 0.1 % (w/v) dilution of test material in distilled water; or a 0.1 % (w/v) dilution of test material in a 1:1 preparation of Freund´s Complete Adjuvant and distilled water. On Day 7 the test material was topically applied at a concentration of 50 % to the same

shoulder area and covered with an occlusive dressing, which was left in place for 48 hours. The reaction sites were assessed 24 and 48 hours after removal of the bandage.

The challenge was conducted on Day 21 by an occlusive patch containing 0.1 to 0.2 mL of the test material at a concentration of 25 % w/w in distilled water that was applied to the shorn right flank of each animal for 24-hours. The vehicle alone was similarly applied to the left shorn flank.

Control animals were administered intradermal injections using the same procedure noted above for the test animals except the injections were as follows: Freund´s Complete Adjuvant and distilled water in a 1:1 ratio; distilled water; or Freund´s Complete Adjuvant and distilled water in a 1:1 ratio. Topical applications used the same procedures as those noted for test animals except that the vehicle alone was applied to filter paper. No positive control animals were evaluated.

Body weights were determined at Day 0 through Day 24.

Evaluation criteria for classification as a potential skin sensitiser:

At the 24-hour and/or 48-hour reading, 30 % or more of the test animals exhibit a positive response (scores ≥ 1) in the absence of similar results in the vehicle control group.

The skin reaction to the challenge was scored according to the following criteria:

Score	**Dermal Observations**
0	No reaction
1	Scattered mild redness
2	Moderate and diffuse redness
3	Intense redness and swelling

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortality was reported.

B. CLINICAL OBSERVATIONS

The study report did not provide clinical observations.

C. BODY WEIGHT

All animals showed the expected gain in body weight throughout the study.

D. NECROPSY

A necropsy was not performed.

E. SKIN REACTIONS

No skin reactions were observed 24 or 48 h after the challenge treatment with glyphosate technical (NUP 05068) in the control or test group.
The known contact sensitiser, formaldehyde, produced an 87 % (20/23) sensitisation rate. This was considered to be a satisfactory sensitisation response for this material under the conditions of the test.

Table 5.2.6-34 Luxan Glyphosate Tech.: Magnusson & Kligman Maximisation Study in the Guinea Pig ([redacted] 1991): Skin reactions after challenge**

Treatment group	Incidence*	
	24-hours	48 hours
Test group	0/20	0/20
Negative control group	0/10	0/10

*: Number of animals with findings / number of animals tested
**: Challenge concentration: 25 %

III. CONCLUSIONS

Based on the results of the study, glyphosate is not a skin sensitiser.

Assessment and conclusion

Assessment and conclusion by applicant:
Except to deviations, such as no purity reported, the GLP study is in concordance with OECD 406 (1992). Therefore, the study is considered acceptable and reliable, the outcome can be reported as valid. After challenge treatment with glyphosate, no skin reactions in guinea pigs were observed after 24 and 48 hours. Therefore, based on the results, glyphosate has no sensitising effect on the skin of the guinea pig in the Maximisation test.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.6/020
Report author	[redacted]
Report year	1989
Report title	Glyphosate Tech.: Magnusson & Kligman Maximisation Study in the Guinea Pig
Report No	5887
Document No	Not reported
Guidelines followed in study	OECD 406 (1981); Commission Directive 84/449/EEC B.6; OPPTS 870.2600
Deviations from current test guideline (OECD 406, 1992)	Purity not given; no detailed data on positive control group animals provided; mean humidity reported, only; air change per hour not reported.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Glyphosate technical (Batch: 206-Jak-25-1, Purity: not reported) was tested for its sensitising effect on the skin of the guinea pig in the Maximisation Test. The test-substance concentrations for the main test were selected based on the results of the pre-test. The intradermal induction was performed with a 10 % dilution of the test item in distilled water and an emulsion of Freund's Complete Adjuvant (FCA)/distilled water. The epidermal induction was conducted under occlusion with the test item at 25 % one week after the intradermal induction. Two weeks after induction the animals were challenged by epidermal application of the test item at 25 % under occlusive dressing.

The study was performed using one control group consisting of 20 animals, and one test group consisting of 20 animals. None of the animals exhibited a positive skin reaction after the challenge treatment. There was no effect on body weight gain.

The positive control, 2,4-dinitro-chlorobenzene (DNCB) revealed 100 % positive skin reactions in the positive control group animals.

Therefore, based on the results, glyphosate technical has no sensitising effect on the skin of the guinea pig in the Maximisation test.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate technical
Description:	White powder
Lot/Batch #:	206-Jak-25-1
Purity:	Batch 206-JaK-25-1 reported with 98.6 %, see [redacted], 1991 (see CA 5.2.1/21)
Stability of test compound:	Not reported

2. Vehicle and/or positive control: Distilled water

3. Test animals:

Species:	Guinea pig
Strain:	Albino Dunkin-Hartley
Source:	[redacted]
Age:	Young adults (less than 1 year old)
Sex:	Female (nulliparous and non-pregnant)
Weight at dosing:	302 - 466 g
Acclimation period:	7 days
Diet/Food:	FDI Guinea Pig Diet, supplied by Special Diet Services Limited, Witham, Essex, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Housed five test animals/cage; all six control animals were housed together
Environmental conditions:	Temperature: 19 - 21 °C Humidity: 53 % Air changes: Not reported Photocycle: 12 hours light/dark cycle

B: Study design and methods

In life dates: 1989-06-14 to 1989-07-17

Animal assignment and treatment:

Glyphosate technical was tested for its sensitising effect on the skin of the guinea pig using the Maximisation test according to Magnusson and Kligman. Female Dunkin Hartley guinea pigs with body weights ranging from 302 to 466 g were used. The test substance concentrations for the main study were selected based on the results of the pre-testing. The main study was performed in 20 test animals and 20 control animals.

Table 5.2.6-35 Glyphosate Tech.: Magnusson & Kligman Maximisation Study in the Guinea Pig ([redacted] 1989): Animal assignment to the treatment groups

Treatment group	Number of animals
Pretest	
Intradermal Pretest	2
Epidermal Pretest	2
Main Study	
Negative Control Group	20
Test Group	20

The induction phase consisted of six injections to the scapular region, three in a row made on each side of the mid-line. The anterior and middle injections (0.1 mL/site) consisted of Freund´s Complete Adjuvant (anterior) and a test material (middle). The posterior injection (0.5 mL/site) consisted for the test material emulsified in Freund´s Complete Adjuvant. The test material was injected at a concentration of 10 % (w/v) in distilled water.

Six days after the injection phase, an epidermal application was made. Animals were re-shaved and wetted with a 10 % aqueous solution of sodium lauryl sulphate to provoke mild inflammation. After 24-hours, the test material was topically applied at a concentration of 25 % to the same shoulder area and covered with an occlusive dressing, which was left in place for 48 hours. The reaction sites were assessed 24 and 48 hours after removal of the bandage.

Two weeks after the topical induction, test and control animals were challenged with an occlusive patch containing the test material at a concentration of 25 %.

Control animals were administered intradermal injections using the same procedure noted above for the test animals except the injections were with vehicle (i.e., distilled water). Topical applications used the same procedures as those noted for test animals except that the vehicle alone was applied. No positive control animals were evaluated.

The sensitivity of the strain of guinea pig to a known sensitiser, 2,4-dinitro-chlorobenzene (DNCB), was checked at six month intervals. The most recent positive control test with DNCB was completed on 1988-12-16.

Body weights were recorded at the start of the main test and on test completion.

Any animal showing erythema at the site of challenge was considered to have shown a positive response.

II. RESULTS AND DISCUSSION

A. MORTALITY

One control animal died during the study.

B. CLINICAL OBSERVATIONS

No clinical signs, other than skin reactions induced by treatment, were noted.

C. BODY WEIGHT

Body weights were considered acceptable.

D. NECROPSY

A necropsy was not performed.

E. SKIN REACTIONS

After induction treatment, slight or discrete erythema (score 1) was observed 1 and 24-hours after treatment.

No skin reactions were observed 24 or 48 h after the challenge treatment with glyphosate technical in the control or test group.

After treatment of guinea pigs with the positive control, DNCB 100 % of the test group animals reacted positively. No individual animal data were reported.

Table 5.2.6-36 Glyphosate Tech.: Magnusson & Kligman Maximisation Study in the Guinea Pig ([redacted] 1989): Summary of skin reactions after challenge**

Treatment group	Incidence*	
	24-hours	48 hours
Test group	0/20	0/20
Negative control group	0/19	0/19

*: Number of animals with findings / number of animals tested
**: Challenge concentration: 25 %

III. CONCLUSIONS

There is no evidence from the test results, that Glyphosate Technical is a sensitiser in guinea pigs.

Assessment and conclusion

Assessment and conclusion by applicant:
The GLP study is in concordance with the current OECD 406 (1992). Therefore, the study is considered acceptable and the outcome can be reported as valid.
After epidermal induction with 10 % / 25 % glyphosate technical and a challenge treatment with 25 % no skin reactions in any treated or control guinea pigs were observed 48 and 72-hours after the start of the challenge. Therefore, based on the results, glyphosate has no sensitising effect on the skin of the guinea pig in the Maximisation test.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant.

1. Information on the study

Data point:	CA 5.2.6/021
Report author	[redacted]
Report year	1988
Report title	A Closed-patch Repeated Insult Dermal Sensitization Study in Guinea Pigs (Buehler Method)
Report No	[redacted]-87-218/4470-87
Document No	Not reported
Guidelines followed in study	US EPA Guidelines Section 81-6
Deviations from current test guideline (OECD 406, 1992)	Yes, 10 instead of a minimum of 20 animals used in the treatment group, evaluation of skin reactions 24 and 48 hours instead of 30 and 54 hours after challenge; assessment of sensitivity and reliability of the experimental technique not within six months as recommended in the guideline; DNCB (2,4-dinitro¬chlorobenzene) used as positive control is not one of the preferred substances as recommended in the guideline.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

MON 8750 (Batch: XLH-274; Purity: 86.2 % glyphosate acid) was tested for its sensitising effect on the skin of the guinea pig using a modified Buehler study method. The test-substance concentrations for the main test were selected based on the results of the pre-test. The epidermal induction was performed with undiluted test item moistened with saline under occlusive conditions and was repeated once per week for a total of three applications, each for a duration of six hours. Two weeks after the last induction exposure the animals were challenged by epidermal application of undiluted test material moistened with saline under occlusive dressing for 6 hours.

The study was performed using one control group consisting of five males and five females, and one test group consisting of five animals per sex. None of the animals exhibited a positive skin reaction after the challenge treatment.

DNCB (2,4-dinitrochlorobenzene) was used as the positive control substance in a separately conducted study of the same laboratory. These data demonstrate the susceptibility of guinea pigs to sensitisation.

Based on the results, MON 8750 exhibited no potential to produce dermal sensitisation in guinea pigs.

I. MATERIALS AND METHODS

A. Materials

1. Test material:

Identification: MON 8750

Description: White granular powder

Lot/Batch #:	XLH-274
Purity:	86.2 %
Stability of test compound:	Not reported
2. Vehicle and/or positive control:	Distilled water / dinitrochlorobenzene historical control data
3. Test animals:	
Species:	Guinea pig
Strain:	Albino Dunkin-Hartley
Source:	[redacted]
Age:	3 – 4 weeks (at receipt); 5 – 6 weeks (at study initiation)
Sex:	Male and female
Weight at dosing:	Male: 366 - 427 g; females: 310 - 370 g
Acclimation period:	16 days (Main study)
Diet/Food:	Agway Prolab Guinea Pig formula provided *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually housed in suspended stainless steel cages
Environmental conditions:	Temperature: Approximately 18 – 24 °C Humidity: 30 – 70 % Air changes: Not reported Photocycle: 12 hours light/dark cycle

B: Study design and methods

In life dates: 1987-09-21 to 1987-11-6

Animal assignment and treatment:

MON 8750 was tested for its sensitising effect on the skin of the guinea pig using a modified Buehler study method. Male and female Dunkin Hartley guinea pigs with body weights ranging from 366 to 427 g for males and 310 to 370 g for females were used.
In the preliminary test, six animals were treated topically with undiluted test material (100 %; moistened with saline) and with concentrations of 50 %, 25 %, and 10 % w/v of the test material in distilled water (4 chambers per animal). Based on results of the pretest, the undiluted material was found to be non-irritating and was, therefore, administered at 100 % concentration for both induction and challenge.

The main study was performed using five animals per sex for the test and the control group, respectively.

Table 5.2.6-37 A Closed-patch Repeated Insult Dermal Sensitisation Study in Guinea Pigs (Buehler Method) ([redacted] 1988): Animal assignment to the treatment groups

Treatment group	Number of animals	
Pretest	**Males**	**Females**
Epidermal Pretest	3	3
Main Study	**Males**	**Females**
Test Group	5	5
Irritation Control	5	5

One day prior to the induction phase, the hair was clipped from the back and sides of five male and five female test animals. The next day, the test material was moistened with 0.3 mL saline and 0.3 cc of the test substance was applied to the test site. An occlusive dressing was placed over the test site. Approximately six hours after dosing, excessive test material was wiped off. This induction procedure was repeated once per week for a total of three applications. Following each induction, test sites were scored for dermal irritation. Following the induction phase, the test animals were left untreated for a period of 14 days.

On the day prior to challenge hair was clipped from the posterior left side of the test animals and from the untreated guinea pigs. The test substance was administered in the same manner as in the induction phase, but at a second site, on the left side of the midline. After six hours of exposure, the chambers were removed, and the skin wiped free of any excess material.

An additional group of ten animals (five per sex) served as an irritation control group. These animals were not treated during the induction phase and then received the identical challenge dose.

Historical control data from animals exposed to dinitrochlorobenzene was used as positive control.

Body weights were recorded at the start of the main test and on test completion. All animals were observed twice daily for viability and weekly for clinical signs of toxicity.

Skin reaction after treatment was evaluated 24 and 48 hours after each induction and after termination of challenge. Any animal showing erythema at the site of challenge was considered to have shown a positive response. Scores of ± (barely perceptible erythema) were considered equivocal.

The skin reaction to the challenge was scored according to the following criteria:

Score	**Dermal Observations**
0	No reaction
±	Very slight (barely perceptible) erythema, usually non-confluent
1	Slight (well-defined) erythema, usually confluent
2	Moderate erythema
3	Severe erythema, with or without oedema, necrosis or eschar

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortality was observed during the study.

B. CLINICAL OBSERVATIONS

No adverse clinical signs of toxicity were observed during the study.

C. BODY WEIGHT

Body weights were considered acceptable.

D. NECROPSY

A necropsy was not performed.

E. SKIN REACTIONS

After induction no dermal irritation was observed.

No skin reactions were observed 24 or 48 h after the challenge treatment with MON 8750 in the control or test group.

Historical control data on the rate of sensitisation after treatment with the positive control DNCB revealed 80 to 100 % sensitised animals, which was considered to sufficiently assure the reliability of this study.

Table 5.2.6-38 A Closed-patch Repeated Insult Dermal Sensitisation Study in Guinea Pigs (Buehler Method) ([redacted] 1988): Skin reactions after challenge**

Treatment group	Incidence*	
	24-hours	48 hours
Test group	0/10	0/10
Irritation Control	0/10	0/10

*: Number of animals with findings / number of animals tested
**: Challenge concentration: 25 %

III. CONCLUSIONS

Under conditions of the study, MON 8750 exhibited no potential to produce dermal sensitisation in guinea pigs.

Assessment and conclusion

Assessment and conclusion by applicant:
The GLP study is in concordance with Guidelines Section 81-6, equivalent to the current OECD 406 (1992). Therefore, the study is considered acceptable and valid. Nevertheless, due to the deviations from the current OECD TG 406 (1992), such as low animal number, the study can be used as supplementary information, only.
After challenge treatment with glyphosate, no skin reactions in guinea pigs were observed after 24 and 48 hours. Therefore, based on the results, glyphosate has no sensitising effect on the skin of the guinea pig in the Buehler Test.

Assessment and conclusion by RMS:
RMS should indicate if they agree to the results and conclusions of the applicant

1. Information on the study

Data point:	CA 5.2.6/022
Report author	[redacted]
Report year	1983
Report title	A Dermal Sensitization Study in Guinea Pigs with Glyphosate
Report No	4235-83
Document No	Not reported
Guidelines followed in study	Similar to OECD 406
Deviations from current test guideline (OECD 406, 1992)	10 instead of a minimum of 20 animals used in the test group; age of animals at study initiation not reported; induction treatment on 3 days per week for three weeks instead of once per week for 3 weeks; clinical signs of toxicity not reported; temperature: 21 °C (±3 °C) instead of 20 °C (+ 3 °C).
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category2a

2. Full summary

Glyphosate (Batch: NBP 1782610, Purity: 99.7 %) was tested for its sensitising effect on the skin of the guinea pig using a closed patch technique. The test-substance concentrations for the main test were selected based on the results of the pre-test.

During the induction phase, five male and five female animals received 0.2 mL of undiluted glyphosate; an additional three male and three female animals received saline (negative control) and three males and three females received 1-chloro-2,4-dinitrobenzene (positive control). Applications were made for six hours per day, three days per week for three weeks. Two weeks after the final induction the three male and three female animals exposed to the test material during the induction phase were challenged with 0.2 mL of undiluted glyphosate. The remaining animals served as an irritation control group. Animals in the negative and positive control group also were challenged in a similar manner. Dermal irritation was scored at 24 and 48 hours after each induction application and the challenge application for all animals, including controls. Throughout the study, all animals were observed twice daily for mortality and weekly for clinical signs of toxicity. Body weights were recorded pretest and at termination of the study.

All animals survived until study termination. There were no body weight changes that were considered treatment-related.

No significant irritation was observed after the first five induction exposures to the test material. Beginning on the sixth exposure, very mid irritation was apparent in several animals. Most animals continued to show mild irritation throughout the induction period. These findings were indicative of irritation since no sensitisation occurred during the challenge phase.

All animals challenged with the test material exhibited no dermal response. No dermal responses were observed in negative control animals during the induction and challenge phase.

Positive control animals exhibited slight dermal irritation after the first induction and severe dermal responses beginning after two to three induction exposures. All ten animals showed a positive response

after the challenge confirming the sensitivity and reliability of the experimental technique.

Therefore, glyphosate is not considered a skin sensitiser based on the results of a closed patch technique.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate
Description:	White powder
Lot/Batch #:	NBP 1782610
Purity:	99.7 %
Stability of test compound:	Not reported
2. Vehicle and/or positive control:	Saline / 1-chloro-2,4-dinitrobenzene (DNCB)
3. Test animals:	
Species:	Guinea pig
Strain:	Albino Hartley
Source:	[redacted]
Age:	Not reported
Sex:	Male and female
Weight at dosing:	Male: 420 - 495 g; female: 365 – 451 g
Acclimation period:	29 days
Diet/Food:	Charles River Vitamin C-Fortified Guinea Pig Diet, *ad libitum*
Water:	Automatic watering system, *ad libitum*
Housing:	Individually housed in suspended stainless steel cages
Environmental conditions:	Temperature: 18 - 24 °C Humidity: 30 - 70 % Air changes: Not reported Photocycle: 12 hours light/dark cycle

B: Study design and methods

In life dates: 1982-12-27 to 1983-03-02

Animal assignment and treatment:

Glyphosate was tested for its sensitising effect on the skin of the guinea pig using a closed patch technique, which is similar to the Buehler test method. The test substance concentrations for the main study were selected based on the results of the pre-testing. For the pre-testing, six animals were treated topically with concentrations of 100 %, 50 %, 25 %, and 10 % v/v of the test material in paraffin oil (4 patches per animal). The patches were left in place for six hours, after which they were removed, and the skin was wiped free of any excess material. Based on the results of this study, a 100 % concentration was found to be non-irritating and was therefore, selected for both induction and challenge administration.

The main study was performed in ten test animals and ten negative and positive control animals.

Table 5.2.6-39 A Dermal Sensitisation Study in Guinea Pigs with Glyphosate ([redacted] 1983): Animal assignment to the treatment groups

Treatment group	Number of Animals	Concentration (%)	
		Induction	Challenge
Group IA – Saline (Negative Control)	10 (5 males, 5 females)	100 %	100 %
Group IB – Saline (Irritation Control)	6 (3 males, 3 females)	---	100 %
Group IIA – DNCB (Positive Control)	10 (5 males, 5 females)	0.5 % [a]	0.3 % [b]
Group IIB - DNCB (Irritation Control)	6 (3 males, 3 females)	---	0.3 % [b]
Group IIIA – Glyphosate	10 (5 males, 5 females)	100 %	100 %
Group IIIB – Glyphosate (Irritation Control)	6 (3 males, 3 females)		100 %

a: Vehicle: 80 % ethanol; b: Vehicle: acetone

On the day prior to the first application, the hair on the dorsal and lateral surfaces was clipped with an electric clipper. During the induction phase test material (0.2 cc) was moistened with 0.2 mL of saline, applied to the skin (right side of the midline) and covered with a gauze square. After administration, an occlusive dressing was used to hold the patch in place. After six hours of exposure, the patch was removed, and the skin was wiped free of excess material. This procedure was repeated three times a week for three weeks, for a total of nine exposures.

Fourteen days after the last exposure, animals were challenged with either the test material or control substance using the same administration procedure used in the induction phase, but on the left side of the midline. After six hours of exposure, patches were removed, and the skin was wiped free of excess material.

Irritation control animals were used to differentiate whether dermal reactions were produced by irritation or sensitisation. Irritation control animals used two groups of three males and three females that were subjected to the same challenge procedures noted above.

Throughout the study, animals were observed twice daily for mortality and weekly for clinical signs of toxicity. Body weights were recorded prior to the start of the study and at study termination.

Dermal scores of 1 or greater was considered clearly indicative of sensitisation. Scores of ± (barely perceptible erythema) were considered equivocal, although a high percentage of scores of ± in treated animals with no dermal response in irritation control animals was considered suggestive of sensitisation.

Dermal responses were scored according to the scale presented in the following:

Score	**Dermal observation**
0	No reaction
±	Very slight (barely perceptible) erythema, usually nonconfluent
1	Slight (well-defined) erythema, usually confluent
2	Moderate erythema
3	Severe erythema, with or without oedema, necrosis or eschar formation

II. RESULTS AND DISCUSSION

A. MORTALITY

All animals survived through the study.

B. CLINICAL OBSERVATIONS

The study report did not provide clinical observations.

C. BODY WEIGHT

All animals, control and treated, gained weight throughout the study. Gains in control and treated animals were considered comparable.

D. NECROPSY

A necropsy was not performed.

E. SKIN REACTIONS

No significant irritation was observed after the first 5 induction exposures to the test material. Beginning on the sixth exposure, very mid irritation was apparent in several animals. Most animals continued to show mild irritation throughout the induction period. These findings were indicative of irritation since no sensitisation occurred during the challenge phase. All animals challenged with the test material exhibited no dermal response.

No dermal responses were observed in negative control animals during the induction and challenge phase.

Positive control animals exhibited slight dermal irritation after the first induction and severe dermal responses beginning after two to three induction exposures. All 10 animals showed a positive response after the challenge confirming sensitisation.

Table 5.2.6-40 A Dermal Sensitisation Study in Guinea Pigs with Glyphosate ([redacted] 1983): Summary of skin reactions after challenge

Treatment group	Interval (hr)	Dermal scores					Total number of animals
		0	±	1	2	3	
IA – Saline (Negative Control)	24	10	0	0	0	0	10
	48	10	0	0	0	0	10
IB – Saline (Irritation Control)	24	6	0	0	0	0	6
	48	6	0	0	0	0	6
IIA – DNCB (Positive Control)	24	0	0	2	8	0	10
	48	0	0	7	3	0	10
IIB - DNCB (Irritation Control)	24	4	2	0	0	0	6
	48	6	0	0	0	0	6
IIIA – Glyphosate	24	10	0	0	0	0	10
	48	10	0	0	0	0	10
IIIB – Glyphosate (Irritation Control)	24	6	0	0	0	0	6
	48	6	0	0	0	0	6

III. CONCLUSIONS

Under conditions of this study, glyphosate revealed no dermal sensitisation in guinea pigs.

Assessment and conclusion

Assessment and conclusion by applicant

The study is similar to the current OECD 406 (1992). The study is considered acceptable and valid. Nevertheless, due to the deviations, such as reduced test animal number, different test method, non-GLP, the study can be used as supplementary information, only.

After challenge treatment with glyphosate, no skin reactions in guinea pigs were observed after 24 and 48 hours. Therefore, glyphosate is not considered a skin sensitiser based on the results of a closed patch technique.

Assessment and conclusion by RMS:

RMS should indicate if they agree to the results and conclusions of the applicant.

CA 5.2.7 Phototoxicity

According to Regulation (EU) No 283/2013 a phototoxicity test is required if the substance absorbs in the range of 290-700 nm and is liable to reach the eyes or light-exposed areas of skin. The ultraviolet/visible molar extinction/absorption coefficient of glyphosate is smaller than 10 L/(mol*cm) (CA 2.4/002). No UV/VIS maximum was observed at wavelengths >250 nm. Therefore, no phototoxicity study was performed with glyphosate.

CA 5.3 Short-Term Toxicity

CA 5.3.1 Oral 28–day study

No new subacute oral toxicity studies have been performed. Table 5.3.1-1 summarises the studies performed in rats, mice and dogs previously assessed in the 2001 and 2015 EU glyphosate evaluations.

Table 5.3.1-1: Studies on oral 21-/28–day repeated-dose toxicity with glyphosate

Annex Point	Study	Species Study type	Substance(s)	Reference list-related category[1]	Result
CA 5.3.1/001 CA 5.3.1/002 CA 5.3.1/003	[redacted] 1991 (Study Report) [redacted], 1994 (1st & 2nd Amendment) [redacted] 1991 (Appendix)	Wistar rat 28–day, oral diet (0, 200, 2000, 20000 ppm)	Glyphosate technical (Purity: 96.8 %)	Valid, Category 2a	NOAEL = 2000 ppm (equivalent to 100 mg/kg bw/day)
CA 5.3.1/004	[redacted] 1991	Sprague-Dawley rat 28–day, oral diet (0, 50, 250, 1000, 2500 mg/kg bw/day)	Glyphosate technical (Purity: 99.5 %)	Valid, Category 2a	NOAEL = 2500 mg/kg bw/day
CA 5.3.1/005	[redacted] 1989	Sprague-Dawley rat 28–day, oral diet (0, 30000, 40000, 50000 ppm)	Glyphosate (Purity: 97.67 %)	Supportive, Category 3b	No NOAEL derived
CA 5.3.1/006	[redacted], 1978	CD-1 mouse 28–day, oral diet (80, 235 and 800 mg/kg bw/day)	Glyphosate (Purity: 83 %)	Supportive, Category 3b	NOAEL = 800 mg/kg bw/day
CA 5.3.1/007	[redacted], 1989	Beagle dog 14-day, oral capsule (100, 300 and 1000 mg/kg bw/day)	Glyphosate (Purity: 99.5 %)	Valid, Category 2a	NOAEL = 1000 mg/kg bw/day
CA 5.3.1/008	[redacted], 1982	Beagle dog 5-day, oral gavage and	Isopropylamine salt of glyphosate	Supportive, Category 2a	No NOAEL derived

		capsule (312.5, 625, 1250, 2500 mg/kg bw/day)	(Purity: 62.49 %)		

[1] The category describes the acceptability/reliability of the study within the AIR 5 submission. This category does not describe hazard classification. (For details please refer to the Doc ID: 110054-B-GRG_Jun_2020).

Publications on oral 28–day toxicity

A literature search for the active substance glyphosate was performed in accordance to the provisions of the EFSA Guidance "Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) 1107/2009" and updated Appendix to this Guidance document. The following publications were found relevant and reliable for this section and the summaries are thus presented below and are part of the general discussion at the beginning of the section.

Table 5.3.1-2 summaries the relevant publications.

Table 5.3.1-2: Publications on oral 21–/28–day repeated-dose toxicity with glyphosate

Annex Point	Study	Species Study type	Substance(s)	Result	Comments
CA 5.3.1/009	Gao H. *et al.*, 2019	ICR mouse 28–day, oral gavage (400 mg/kg bw/day)	Glyphosate monoisopropylamine salt (Purity: 96 %)	No NOAEL derived Histopathological effects in kidney (exfoliation of renal tubular cells)	Biochemical methods are not sufficiently described. Only one dose tested *in vivo*. Pathology results from the *in vivo* study are not corroborated by regulatory 90–day toxicity studies dosed 10–17.5 times higher.
CA 5.3.1/010	Milic M. *et al.*, 2018	Wistar rat 28–day, oral gavage (0.1, 0.5, 1.75, 10 mg/kg bw/day)	Glyphosate (Purity: ≤100 %)	No NOAEL derived	Highly variable results within the comet assay.
CA 5.3.1/011	Tang J. *et al.*, 2017	Sprague-Dawley rat 35–day, oral gavage (0, 5, 50, 500 mg/kg bw/day)	Glyphosate (Purity: not reported)	No NOAEL derived	Test material was not sufficiently characterised. Insufficient number of animals. Results not always accurately reported. Results not corroborated by regulatory toxicology studies of similar test durations and dose range.

There are three subacute oral studies in rats available.

The study by [redacted] (1989) was considered not acceptable due to serious reporting deficiencies in the last EU evaluation (2015). Apart from several deviations to the current OECD guideline, the study was well conducted and can provide supportive information for the assessment of repeated-dose toxicity of glyphosate. The study is therefore considered to be supportive. No No Observed Adverse Effect Level (NOAEL) could be derived in this study.

The 28–day repeated-dose toxicity studies by [redacted] (1991) and by [redacted] (1989) in which glyphosate technical was administered to rats via the diet, were both considered acceptable for assessment. Both studies were conducted according to OECD 407 (1981) and in compliance with GLP.

The study by [redacted] (1991) had the apparent deficiency that sexes were combined for reporting of haematological, clinical chemistry parameters and organ weights in the summary tables. However, the corresponding raw data in the report easily allowed for a separate assessment of sexes. In this 4–week toxicity study, groups of male and female Wistar rats were administered technical glyphosate for 28 days via the diet at doses of 0, 10, 100 or 1000 mg/kg bw/day. A high-dose recovery group was included. Dosing Wistar rats via the diet with glyphosate produced slight increases in blood urea nitrogen and glutamic pyruvic transaminase at the high-dose; these changes did not show a clear dose-response relationship and were shown to be reversible during the recovery period. There were no other treatment-related effects at any dose level. Based on this information, the no observed adverse effect level was determined to be 100 mg/kg bw/day in the 2nd Amendment to the study report ([redacted], 1994).

In the study by [redacted] (1989) groups of male and female Sprague-Dawley rats were administered glyphosate technical in the diet at dose levels of 0, 50, 250, 1000 or 2500 mg/kg bw/day for 28 days. Administration of glyphosate via the diet produced slight increases in plasma phosphate levels in males (mid and high dose) and increases in incidence of nephrocalcinosis in females (mid and high dose), both being expected findings in view of the dose levels employed and the phosphate content of glyphosate. In the earlier EU evaluation (2001) a No Observed Effect Level (NOEL) of 50 mg/kg bw/day had been derived based on this finding. These effects were not identified in the subsequent 90–day definitive study and thus were considered spurious. The 2004 JMPR review also concluded the following: "In contrast to a 4–week study in rats conducted at the same testing facility ([redacted], 1989), the incidence of nephrocalcinosis in the 13-week study ([redacted], 1991) was evenly distributed among dose groups and sexes and did not follow a dose–response relationship, and is therefore clearly not treatment-related. Thus, the previous finding was not confirmed". In addition, there were equivocal increases in alanine aminotransferase, alkaline phosphatase and total bilirubin, all of which were regarded as normal responses to increased liver activity at the elevated dose levels of glyphosate. Since the effects observed were considered to be adaptive, the NOAEL was set at 2500 mg/kg bw/day for male and female Sprague-Dawley rats under the conditions of this study.

Thus, despite some slight changes in haematological and clinical chemistry parameters and soft faeces, there were no clear adverse effects in rats after exposure to glyphosate.

The 4–week pilot study by [redacted] (1978) in CD-1 mice was considered not acceptable due to serious reporting deficiencies in the last EU evaluation (2015). The study is therefore included as supportive. All in-life data and gross necropsy observations indicated no adverse effects of glyphosate in mice.

In addition to the rodent studies, there are two subacute studies available with Beagle dogs; one performed with glyphosate and one with the isopropylamine salt of glyphosate.

In the study by [redacted], (1989), glyphosate was administered by gelatin capsule to one male and one female dog for 7 day periods at escalating dose levels of 100, 300 and 1000 mg/kg bw day (Part A) and to one dog of each sex for 14 days at 1000 mg/kg bw/day (Part B). This study was essentially a dose range-finding study conducted in compliance with GLP. Whereas Part B was considered acceptable in the last EU evaluation (2015), Part A was difficult to interpret due to the very unusual test design with increasing doses. Fourteen days of treatment at 1000 mg/kg bw/day in one male and one female dog was well tolerated. There were no clinicopathological, gross pathological or organ weight findings considered attributable to treatment. Based on these results it is concluded that the oral maximum tolerated dose of glyphosate in Beagle dogs is greater than 1000 mg/kg bw/day.

At last, the study by [redacted] (1982) in Beagle dogs does not completely comply to modern standards and

may not be used for the risk assessment of glyphosate. But the study provided useful information on the single and repeated dose toxicity of the isopropylamine salt of glyphosate compared to the toxicity of isopropylamine administered alone. The study is therefore considered valid, but supportive only. Strong gastrointestinal irritation became apparent when the isopropylamine salt of glyphosate was administered to Beagle dogs by gavage and capsule. Administration of isopropylamine alone led to even stronger irritation of gastric and oesophageal mucosa at a much lower dose. A NOAEL was not established for the isopropylamine salt of glyphosate in this study; however, a dosage level of 312.5 mg/kg bw/day was determined as non-emetic.

The small number of valid subacute studies by the oral route is not considered a data gap since there is a sufficient number of fully valid subchronic, i.e. 90–day studies in all relevant species.

Additional to the available subacute studies, a literature search for glyphosate was performed and provided three reliable new publications which are summarised in Table 5.3.1-2.

In the study published by Gao *et al.* (2019) the effect of glyphosate on human proximal tubular epithelial cells was studied *in vitro* and on the mouse kidney *in vivo*. ICR mice were administered glyphosate by gavage at 400 mg/kg bw/day for 28 days. In the *in vitro* assays, glyphosate was found to reduce cell viability, increase the incidence of apoptotic cells with an increase in the expression of apoptosis-related proteins, increase of oxidative stress in a concentration-related manner, increase of the expression of the N-methyl-D-aspartate receptor (NMDAR) and increase Ca^{2+} influx. Kidney histopathology revealed the exfoliation of renal tubular cells. It was postulated by the authors that glyphosate could affect renal tubule epithelial cells via the NMDAR1/$[Ca^{2+}]_i$/ROS pathway. These findings were not corroborated by the available regulatory 28– or 90–day repeated-dose studies up to dose levels of more than 4000 mg/kg bw/day in rats and up to more than 7000 mg/kg bw/day in mice.

In the second 28–day study (Milic *et al.*, 2018), the effects of glyphosate administered by gavage to Wistar rats at daily doses equivalent to 0.1 of the acceptable operator exposure level (AOEL), 0.5 of the consumer acceptable daily intake (ADI), 1.75 (corresponding to the chronic population-adjusted dose, cPAD), and 10 mg kg bw/day (corresponding to 100 times the AOEL) were evaluated. Endpoints investigated in this study were: DNA damage as measured in the alkaline comet assay, oxidative stress and cholinesterase activity. The results of the alkaline comet assays revealed a statistically significant increase in tail length and tail intensity in leucocytes and small and medium sized liver nuclei. With the exception of tail length of small sized liver nuclei no dose-effect relationship was evident. The oxidative stress markers in plasma and liver and cholinesterase activity in plasma did not show a dose related effect either.

Tang *et al.* (2017) investigated the effects of glyphosate on liver function and induction of pathological changes in ion levels and oxidative stress in hepatic tissue. Sprague-Dawley rats were treated by gavage with 0, 5, 50, and 500 mg/kg bw/day for 35 days. Statistically significant effects were found on body weight, body weight gain, organ weight, serum indicators of liver toxicity and histopathology of the liver and the kidney. Significant changes were also reported on markers of oxidative stress and transcription of genes related to inflammation and lipid metabolism. Many of the effects reported were mild in nature and/or didn't show a clear dose-effect relationship.

The publications by Milic *et al.* (2018) and Tang *et al.* (2017) are in line with the results of the summarised repeated-dose studies that did not reveal any clear adverse effects of glyphosate on rodents or non-rodents.

Study summaries on oral 28–day toxicity

1. Information on the study

Data point	CA 5.3.1/001
Report author	[redacted]
Report year	1991 (study report)
Report title	28–day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate (FSG 03090 h/05 March 1990)
Report No	ES.881.28 DDR
Document No	Not reported
Guidelines followed in study	OECD 407 (1981)
Deviations from current test guideline (OECD 407, 2008)	The following organs were not examined in the gross pathology evaluation: epididymides, peripheral nerve, prostate, skeletal muscle and bone, spinal cord, thymus, thyroid, vagina. The following organs were not weighed: testes, epididymides, prostate and seminal vesicles with coagulating glands, thymus, heart, brain, and spleen
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

Data point	CA 5.3.1/002
Report author	[redacted]
Report year	1st amendment (June 1994); 2nd amendment (November 1994)
Report title	Amendment to Final Report. 28–day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate
Report No	ES.881.28 DDR
Document No	Not reported
Guidelines followed in study	OECD 407 (1981)
Deviations from current test guideline (OECD 407, 2008)	The following organs were not examined in the gross pathology evaluation: epididymides, peripheral nerve, prostate, skeletal muscle and bone, spinal cord, thymus, thyroid, vagina. The following organs were not weighed: testes, epididymides, prostate and seminal vesicles with coagulating glands, thymus, heart, brain, and spleen
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

Data point	CA 5.3.1/003
Report author	[redacted]
Report year	1991 (Appendices to study report)
Report title	28–day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate (FSG 03090 h/05 March 1990)
Report No	ES.881.28 DDR
Document No	Not reported
Guidelines followed in study	OECD 407 (1981)
Deviations from current test guideline (OECD 407, 2008)	The following organs were not examined in the gross pathology evaluation: epididymides, peripheral nerve, prostate, skeletal muscle and bone, spinal cord, thymus, thyroid, vagina. The following organs were not weighed: testes, epididymides, prostate and seminal vesicles with coagulating glands, thymus, heart, brain, and spleen. Deviations from the current version of OECD 407 (2008) are basically due to the fact that the study was aligned to an older version of the OECD test guideline 407.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

In a 4–week toxicity study, groups of five male and five female Wistar rats were administered technical glyphosate for 28 consecutive days via the diet at concentrations of 0, 200, 2000, or 20000 ppm (equivalent to 0, 17.6, 178.5, 1894.9 or 1987.5 mg/kg bw/day in males and 21.6, 223.3, 2250.8 or 2129.7 mg/kg/day in females). A high-dose recovery group was administered 20000 ppm for 28 days and then were followed for 14 days without treatment before being sacrificed.

General clinical observations were done daily. Body weights and food consumption were assessed in weekly intervals. Haematological and blood biochemistry parameters were evaluated during week 4 of dosing. At the end of the scheduled period, the animals were killed and subjected to a post-mortem examination and selected organs were weighed and tissues were taken for subsequent histopathology examination.

There was no mortality in any of the study groups during treatment or recovery period. In general, there were no clinical signs of toxicity observed in any of the treatment groups. However, there were a few incidences of urine incontinence in the mid- and high-dose groups but not in the high-dose recovery group. There were no notable intergroup differences in body weights, food consumption, or haematological parameters. With regards to clinical chemistry parameters, there was a statistically significant increase in blood urea nitrogen (BUN) level and in the activity of glutamic pyruvic transaminase (SGPT) at the high-dose level. These changes were not evident in the 14 day recovery group. A significant increase in BUN at all dose levels in females and a significant decrease in calcium in females at the low-dose level were observed. Lastly there was a notable increase in calcium in the high-dose recovery group. There were no notable intergroup differences in organ weights. No gross pathology or histopathology findings attributed to administration of glyphosate were recorded.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Technical Glyphosate
Description:	Solid, odourless white coloured crystals
Lot/Batch #:	FSG 03090 h/05 March 1990 / Batch No. 60
Purity:	96.8 %
Stability of test compound:	Fairly stable for 30 days under ambient temperature and stored in polyethylene lined stainless steel drums

2. Vehicle and/or positive control: Plain diet / none

3. Test animals:

Species:	Rat
Strain:	Wistar
Source:	[redacted]
Age:	8 weeks
Sex:	Male and female
Weight at dosing:	♂ group means 130 – 174 g; ♀ group means 108 – 154 g
Acclimation period:	At least one week
Diet/Food:	Standard "Gold Mohur" brand powdered rat feed, *ad libitum,*
Water:	Deep borewell water passed through activation charcoal filter and exposed to UV rays, *ad libitum*
Housing:	Groups of 5 rats/ sex in steam sterilized standard polypropylene rat cages with stainless steel top grill
Environmental conditions:	Temperature: 23 ± 2°C Humidity: 66 ± 2 % Air changes: 10 – 15/hour 12 hours light/dark cycle

B: Study design and methods

In life dates: November 1990 to January 1991

Animal assignment and treatment:
Groups of five male and five female Wistar rats were administered technical glyphosate for 28 consecutive days via the diet at concentrations of 0 (control), 20, 2000, or 20000 ppm.

Table 5.3.1-3: 28–day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate (FSG 03090 h/05 March 1990) [redacted] 1991): Animal assignment

Test group	Dose Level [ppm]	Males	Females
Control	0	5	5
Low	200	5	5
Intermediate	2000	5	5
High	20000	5	5
High Recovery	20000	5	5

The required amount of finely ground test compound was weighed and mixed manually with 0.5 kg powdered rat feed to prepare the premix. The premix was added in portions to the remaining quantity of feed and mixed. To the control group feed, the powdered rat feed was mixed for 20 minutes. Prepared feed was sampled at different levels for assaying the test compound concentration.

All test groups received diet specifically prepared for the group ad libitum. Animals were treated for seven days a week for four weeks. The high dose recovery group received powdered normal rat feed for two weeks following four weeks of treatment.

Mortality
Each animal was checked for mortality or signs of morbidity twice daily during the treatment period.

Clinical observations
General clinical observations were done twice daily. Cage side observations included changes in skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous system, somatomotor activity and behaviour pattern.

Body weight
Individual body weights were recorded at the end of each week.

Food consumption and utilisation
Daily feed consumption per cage measured during the last two days of the week.

Ophthalmoscopic examination
Ophthalmoscopic eye examinations were not performed.

Haematology and clinical chemistry
One day prior to sacrifice, blood smears from surviving rats were made by tail clipping and the blood smears were stained by Wright's stain. At the end of the study all the surviving animals were fasted overnight (water allowed) and blood was collected from abdominal aorta under ether anaesthesia.

For haematology, differential leucocyte counts were done manually. Fractions of blood were taken for coagulation time. For haematology and plasma separation blood was heparinized. The following haematological parameters were measured: white blood cell (WBC) count, red blood cell (RBC) count, haemoglobin (Hb), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC).

For clinical chemistry analysis the following parameters were measured: glucose, total bilirubin, creatinine, glutamic pyruvic transaminase (SGPT), glutamic oxaloacetic transaminase, enzymatic blood urea nitrogen (BUN), albumin, alkaline phosphatase, total protein, sodium, potassium, calcium, and inorganic phosphorus.

Urinalysis
Not performed.

Sacrifice and pathology
At the end of the study, rats were fasted overnight and sacrificed by total blood collection under either anaesthesia. After sacrificed, a detailed gross necropsy was conducted. The following organs were collected and preserved from every animal: adrenals (both), gonads (both), kidneys (both), and liver. The relative organ weights were determined as a percentage of body weight. Tissue samples from brain, lungs, heart, eye (Bouin's fluid), spleen, lymph nodes, (mesenteric) oesophagus, stomach, small and large intestines, salivary gland, liver, pancreas, kidneys (both), urinary bladder, and adrenals (both) were preserved in neutral buffered formalin for processing and 5 µm sections were stained. The tissues sections were studied

for histopathological changes. All the tissues from all animals in control and high-dose group were subjected to detailed histopathological studies.

Statistics
Body weight and feed consumption were compared using the Bartlett's test for homogeneity of variance and one-way classification analysis of variance (ANOVA) and Dunnet's multiple pairwise comparison. The clinical laboratory analysis data and organ weight data from the control group were compared with the data of treated groups by the Bartlett's test for homogeneity of variance followed by ANOVA, where the variances proved to be heterogeneous, the data was transformed using the appropriate transformation. If ANOVA of homogeneous data was significant, Dunnet's pairwise comparison procedure was used to compare the treated group with the control group individually. All analyses were evaluated at 5 % probability level.

II. RESULTS AND DISCUSSION

A. MORTALITY
There was no mortality in any of the study groups during treatment or recovery period.

B. CLINICAL OBSERVATIONS
In general there were no clinical signs of toxicity observed in any of the treatment groups. However, there were a few incidences of urine incontinence in the mid- and high-dose groups but not in the high-dose recovery group.

C. BODY WEIGHT
Body weight gains were comparable between treatment groups and control animals and there were no statistically significant differences. Results are presented in the following table:

Table 5.3.1-4: 28–day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate (FSG 03090 h/05 March 1990) ([redacted] 1991): Intergroup comparison of mean body weights (both sexes combined)

Time point	Weekly body weight [g]				
	0	1	2	3	4
Dose level [ppm]	Combined Sex (5 males + 5 females)				
0	146±8.77	149±10.38	173±16.84	196±27.18	209±32.01
200	**131±12.89*** ↓	138±17.63	159±20.05	175±21.48	186±28.17
2000	144±13.69	153±14.27	172±20.54	195±29.62	207±40.77
20000	149±15.46	150±16.18	174±26.61	189±30.01	207±42.74
20000 (recovery group)	149±15.95	155±14.61	173±26.79	195±32.11	206±40.81

* Significant at P = 0.05 over control group value.
↓ Decreased

Table 5.3.1-5: 28–day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate (FSG 03090 h/05 March 1990) ([redacted] 1991): Intergroup comparison of mean body weights for males

Time point	Weekly body weight [g]				
	0	1	2	3	4
Dose level [ppm]	Males				
0	150±7.87	156±8.00	186±9.94	220±11.44	238±14.24
200	**139±10.06*** ↓	150±11.87	172±15.56	194±9.49	210±12.92
2000	154±8.99	164±11.08	189±10.26	220±15.65	242±23.87
20000	161±9.65	163±10.06	198±9.49	216±11.17	245±17.81

Table 5.3.1-5: 28–day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate (FSG 03090 h/05 March 1990) ([redacted]., 1991): Intergroup comparison of mean body weights for males

Time point	Weekly body weight [g]				
	0	1	2	3	4
Dose level [ppm]	**Males**				
20000 (recovery group)	162±9.84	165±10.06	195±13.16	221±16.41	240±18.51

* Significant at P = 0.05 over control group value.
Re-assessment performed using a statistical program. Based on the data set, the most appropriate t-test procedure was selected by the program.
↓ Decreased

Table 5.3.1-6: 28–day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate (FSG 03090 h/05 March 1990) ([redacted] 1991): Intergroup comparison of mean body weights for females

Time point	Weekly body weight [g]				
	0	1	2	3	4
Dose level [ppm]	**Females**				
0	142 ± 8.17	142 ± 7.92	159 ± 7.95	172 ± 9.53	181 ± 10.73
200	**122 ± 9.38* ↓**	127 ± 15.66	145 ± 14.60	157 ± 9.12	162 ± 13.37
2000	134 ± 9.53	143 ± 8.67	154 ± 10.24	169 ± 8.07	172 ± 8.53
20000	138 ± 10.95	137 ± 8.07	150 ± 9.32	162 ± 7.21	168 ± 10.33
20000 (recovery group)	136 ± 7.27	145 ± 11.37	151 ± 14.46	168 ± 18.30	172 ± 22.69

* Significant at P = 0.05 over control group value.
Re-assessment performed using a statistical program. Based on the data set, the most appropriate t-test procedure was selected by the program.
↓ Decreased

D. FOOD CONSUMPTION AND TEST SUBSTANCE INTAKE

All groups receiving Glyphosate performed similarly to their respective controls. Test compound intakes are presented in the following table:

Table 5.3.1-7: 28–day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate (FSG 03090 h/05 March 1990) ([redacted] 1991): Feed intake for combined sexes

Time point	Feed intake mean g/rat/day [g]				
	0	1	2	3	4
Dose level [ppm]	**Combined Sex (5 males + 5 females)**				
0	17±2.83	18±0.71	19±1.41	16±1.41	16±1.41
200	16±1.41	16±0.71	15±2.12	16±1.41	15±0.07
2000	16±2.83	16±1.41	19±2.12	16±1.41	19±2.12
20000	13±3.54	17±1.41	18±1.41	15±2.83	22±2.12
20000 (recovery group)	15±2.83	17±4.95	18±2.83	17±0.71	20±4.24

Table 5.3.1-8: 28–day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate (FSG 03090 h/05 March 1990) ([redacted]., 1991): Feed intake for males

Time point	Feed intake mean g/rat/day [g]				
	0	1	2	3	4
Dose level [ppm]	Males				
0	19	18	20	17	17
200	17	15	16	17	15
2000	18	17	20	17	17
20000	15	18	19	17	23
20000 (recovery group)	17	20	21	17	22

Table 5.3.1-9: 28–day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate (FSG 03090 h/05 March 1990) ([redacted] 1991): Feed intake for females

Time point	Feed intake mean g/rat/day [g]				
	0	1	2	3	4
Dose level [ppm]	Females				
0	15	17	18	15	15
200	15	16	13	15	15
2000	14	15	17	15	20
20000	10	16	17	13	20
20000	13	13	17	16	18

Table 5.3.1-10: 28–day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate (FSG 03090 h/05 March 1990) ([redacted] 1991): Test compound intake

	Mean dietary concentration of glyphosate [ppm]				
	Combined sexes (5 males + 5 females)				
	0	200	2000	20000	20000 (recovery group)
Dose [mg/kg bw/day]	0	19.6	200.9	2072.9	2058.6

Table 5.3.1-11: 28–day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate (FSG 03090 h/05 March 1990) ([redacted] 1991): Test compound intake

	Mean dietary concentration of glyphosate [ppm]									
	Males					Females				
	0	200	2000	20000	20000 (re-covery)	0	200	2000	20000	20000 (re-covery)
Dose [mg/kg bw/day]	0	17.6	178.5	1894.9	1987.5	0	21.6	223.3	2250.8	2129.7

E. OPHTHALMOSCOPIC EXAMINATION

Specific eye examinations were not performed.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

There were no treatment-related differences noted in any dose group for most of the parameters examined during the haematology evaluation. Haemoglobin values, however, showed a decrease in the high-dose recovery group when compared with the high-dose groups for males and females.

Table 5.3.1-12: 28–day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate (FSG 03090 h/05 March 1990) ([redacted] 1991): Intergroup comparison of selected haematology parameters (mean ± SD)

Parameter	Combined Sex (5 males + 5 females)				
	Dose level [ppm]				
	0	200	2000	20000	20000 (recovery group)
Hb [g/dL]	16.61±1.80	16.64±1.12	18.46±2.43	16.36±1.98	14.95±0.40 ↓

↓ Decreased, compared to the high-dose group

Table 5.3.1-13: 28–day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate (FSG 03090 h/05 March 1990) ([redacted] 1991): Selected haematology parameters (mean ± SD) for males

Parameter	Males				
	Dose level [ppm]				
	0	200	2000	20000	20000 (recovery group)
Hb [g/dL]	17.12±2.06	16.70±1.40	17.76±1.34	20.44±1.73	14.80±0.34 ↓

↓ Decreased, compared to the high-dose group

Table 5.3.1-14: 28–day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate (FSG 03090 h/05 March 1990) ([redacted] 1991): Selected haematology parameters (mean ± SD) for females

Parameter	Females				
	Dose level [ppm]				
	0	200	2000	20000	20000 (recovery group)
Hb [g/dL]	16.10±1.54	16.58±0.92	14.96±1.44	16.48±0.74	15.10±0.43 ↓

↓ Decreased, compared to the high-dose group

Blood clinical chemistry

There were no treatment-related differences noted in any dose group for most of the parameters examined during the clinical chemistry evaluation. There was a significant increase in SGPT in both sexes at the high-dose level. These changes were not evident in the 14 day recovery group. A significant increase in BUN at all dose levels in females and a significant decrease in calcium in females at the low-dose level were observed. Lastly there was an apparent increase in calcium in the high-dose recovery group, but in the absence of a concurrent control sample the significance of this is in doubt.

Table 5.3.1-15: 28–day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate (FSG 03090 h/05 March 1990) ([redacted] 1991): Intergroup comparison of selected clinical chemistry parameters (mean ± SD)

Parameter	Combined Sex (5 males + 5 females)				
	Dose level [ppm]				
	0	200	2000	20000	20000 (recovery group)
SGPT [IU/L]	32.80±6.76	37.00±7.07	30.20±5.35	**46.50±8.24*↑**	23.90±5.41↓
BUN [mg/dL]	23.60±2.68	**28.10±3.48*↑**	24.50±2.84	**28.10±2.38*↑**	24.20±3.12
Calcium [mg/dL]	9.35±0.14	**9.20±0.08*↓**	9.29±0.12	9.25±0.11	10.00±0.58↑

* Significant at P = 0.05 over control group value
↓ Decreased; ↑ Increased

Table 5.3.1-16: 28–day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate (FSG 03090 h/05 March 1990) ([redacted] 1991): Selected clinical chemistry parameters (mean ± SD) for males

Parameter	Males				
	Dose level [ppm]				
	0	200	2000	20000	20000 (recovery group)
SGPT [IU/L]	36.2±7.29	39.4±8.53 ↑	27.2±5.89 ↓	**50.4±6.88*** ↑	27.0±6.40 ↓
BUN [mg/dL]	24.6±3.51	26.0±3.08 ↑	23.8±1.19	26.2±1.10 ↑	25.2±3.96 ↑
Calcium [mg/dL]	9.26±0.15	9.18±0.08	9.26±0.15	9.18±0.08	10.00±0.74 ↑

* Significant at P = 0.05 over control group value
Re-assessment performed using a statistical program. Based on the data set, the most appropriate t-test procedure was selected by the program.
↓ Decreased; ↑ Increased

Table 5.3.1-17: 28–day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate (FSG 03090 h/05 March 1990) ([redacted] 1991): Selected clinical chemistry parameters (mean ± SD) for females

Parameter	Females				
	Dose level [ppm]				
	0	200	2000	20000	20000 (recovery group)
SGPT [IU/L]	29.4±4.56	34.6±5.03 ↑	33.2±2.68 ↑	**42.6±8.20*** ↑	20.8±0.84
BUN [mg/dL]	22.6±1.14	**30.2±2.59*** ↑	**25.2±3.63*** ↑	**30.0±1.58*** ↑	23.2±1.92
Calcium [mg/dL]	9.44±0.05	**9.22±0.08*** ↓	9.32±0.08	9.32±0.08	10.00±0.46 ↑

* Significant at P = 0.05 over control group value
Re-assessment performed using a statistical program. Based on the data set, the most appropriate t-test procedure was selected by the program.
↓ Decreased; ↑ Increased

G. URINALYSIS
Not performed

H. NECROPSY
Organ weights
There were no intergroup differences in either sex.

Gross pathology
At the high dose, there was an increase incidence of petechiae and ecchymosis and two incidences of focal congestion in the lungs. In the recovery group, there was one occurrence of enteritis and thickening of splenic capsule and two incidences of petechiae in the lungs. These changes appear to be incidental.

Table 5.3.1-18: 28–day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate (FSG 03090 h/05 March 1990) ([redacted]., 1991): Summary incidence of gross pathological findings

Findings	Dose level [ppm]									
	Males					Females				
	0	200	2000	20000	20000 (re-covery group)	0	200	2000	20000	20000 (re-covery group)
Pancreatic lymph node - enlarged	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Lungs – Petechiae	0/5	0/5	0/5	1/5	2/5	0/5	0/5	0/5	0/5	0/5
Lungs – Ecchymosis	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5
Lungs – Focal congestion	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	1/5	0/5
Small intestine – Enteritis	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5
Spleen Constriction – Thickening of capsule	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5

Histopathology
There were no treatment-related findings reported during the histopathological evaluation. Lymphoid hyperplasia in the colon and lungs were more frequently observed in the high-dose group; however, this finding was not considered treatment related because the severity remained unaltered as compared to the control animals.

Table 5.3.1-19: 28–day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate (FSG 03090 h/05 March 1990) ([redacted] 1991): Summary incidence of histopathological findings

Findings	Dose level [ppm]			
	Males		Females	
	0	20000	0	20000
Lungs				
Congestion	4/5	4/5	5/5	5/5
Lymphoid	1/5	5/5	2/5	1/5

Table 5.3.1-19: 28–day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate (FSG 03090 h/05 March 1990) ([redacted] 1991): Summary incidence of histopathological findings

Findings	Dose level [ppm]			
	Males		Females	
	0	20000	0	20000
hyperplasia				
Atelectasis	4/5	4/5	4/5	4/5
Interstitial pneumonia	3/5	2/5	2/5	3/5
Perivascular leucocytic infiltration	0/5	1/5	1/5	0/5
Perivascular lymphocytic aggregation	4/5	3/5	5/5	4/5
Kidneys				
Intestial nephrosis	1/0	0/0	0/0	0/0
Spleen				
Lymphoid hyperplasia	2/5	0/5	0/5	1/5

III. CONCLUSIONS

There was no mortality in any of the study groups during treatment or recovery period. In general, there were no clinical signs of toxicity observed in any of the treatment groups. However, there were a few incidences of urine incontinence in the mid- and high-dose groups but not in the high-dose recovery group. There were no notable intergroup differences in body weights, food consumption, or haematological parameters. With regards to clinical chemistry parameters, there was a statistically significant increase in blood urea nitrogen (BUN) level and in the activity of glutamic pyruvic transaminase (SGPT) at the high-dose level. These changes were not evident in the 14 day recovery group. There were no notable intergroup differences in organ weights. No gross pathology or histopathology findings attributed to administration of glyphosate were recorded.

According to the 2nd amendment to the study report created in November of 1994, the NOAEL was established at 2000 ppm due to the lack of treatment-related effects.

3. Assessment and conclusion

Assessment and conclusion by applicant:

In this 4–week toxicity study, groups of five male and five female Wistar rats were administered technical glyphosate for 28 consecutive days via the diet at concentrations of 0, 200, 2000, or 20000 ppm (equivalent to 0, 17.6, 178.5, 1894.9 or 1987.5 mg/kg bw/day in males and 21.6, 223.3, 2250.8 or 2129.7 mg/kg/day in females). A high-dose recovery group was administered 20000 ppm for 28 days and then were followed for 14 days without treatment before being sacrificed. The study was conducted according to OECD 407 (1981) and in compliance with GLP (no certificate of the competent authority was provided).

The study was well conducted, in compliance with GLP and OECD 407 (1981) and thus, can provide useful information for the assessment of repeated dose toxicity of glyphosate. The study is therefore considered valid.

Dosing Wistar rats via the diet with glyphosate produced slight increases in blood urea nitrogen (BUN) and glutamic pyruvic transaminase (SGPT) at the high-dose; these changes did not show a clear dose-

response relationship and were shown to be reversible during the recovery period. There were no treatment-related effects at any dose level with regards to mortality, clinical signs of toxicity, body weight, haematology, organ weight and gross and histopathological findings. Based on this information, the no observed adverse effect level is determined to be the mid dose level 2000 ppm (178.5 mg/kg bw/day in males and 223.3 mg/kg bw/day in females).

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.3.1/004
Report author	[redacted]
Report year	1991
Report title	Glyphosate: 4 Week Dietary Toxicity Study in Rats
Report No	5626
Document No	Not reported
Guidelines followed in study	OECD 407 (1981)
Deviations from current test guideline (OECD 407, 2008)	Dose levels exceed the 1000 mg/kg bw/day limit dose. Reticulocytes, platelet count, total cholesterol, urea and bile acids not assessed. T3, T4 and TSH were not determined. Urinalysis not performed. Only liver, kidneys, adrenals, testes, epididymides were weighed. Only liver, heart, kidneys, spleen and adrenals from the control and high dose group were examined histopathologically. Deviations from the current version of OECD 407 (2008) are basically due to the fact that the study was aligned to an older version of the OECD test guideline 407.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

In a 4–week toxicity study, groups of five male and five female Sprague-Dawley rats were administered technical glyphosate for 28 consecutive days via the diet at concentrations calculated to achieve dose levels of 0 (control), 50, 250, 1000 or 2500 mg/kg bw/day.

General clinical observations were done daily and detailed clinical examinations weekly. Body weights and food consumption were assessed in weekly intervals. Water consumption was monitored by visual inspection throughout the study. Haematological, blood biochemistry parameters were evaluated during week 4 of dosing. At the end of the scheduled period, the animals were killed and subjected to a full post-mortem examination and selected organs were weighed and tissues were taken for subsequent histopathology examination.

There was one premature death, an intermediate dose group male that died during the week 4 blood sampling. Incidences of soft faeces were noted in high dose males during weeks 3 and 4. There were no notable intergroup differences in body weight, food consumption, water consumption, or haematological parameters. There were equivocal increases in alanine aminotransferase (ALT) (males at 250, 1000 and 2500 mg/kg bw/day; females at 2500 mg/kg bw/day), alkaline phosphatase (males at 250, 1000 and 2500 mg/kg bw/day; females at 1000 and 2500 mg/kg bw/day) and total bilirubin (females at 2500 mg/kg bw/day), all of which were regarded as normal responses to increased liver activity at the elevated dose levels of glyphosate. Plasma phosphate was slightly increased in 1000 and 2500 mg/kg bw/day males. There were no notable intergroup differences in organ weights. No gross pathology findings attributed to administration of glyphosate were recorded. Histopathological changes were limited to nephrocalcinosis in 250, 1000 and 2500 mg/kg bw/day females. The increases in plasma phosphate and nephrocalcinosis were considered expected findings in view of the dose levels employed and the phosphate content of glyphosate.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Technical Glyphosate
Description:	White powder
Lot/Batch #:	161-JRJ-131-2
Purity:	99.5 %
Stability of test compound:	Not indicated
2. Vehicle and/or positive control:	Plain diet / none
3. Test animals:	
Species:	Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	Approx. 4 weeks on arrival
Sex:	Male and female
Weight at dosing:	♂ group means 154 – 161 g; ♀ group means 108 – 115 g
Acclimation period:	8 days
Diet/Food:	SDS Rat and Mouse No.1 Expanded Fine Ground Diet, *ad libitum,*
Water:	Tap water, *ad libitum*
Housing:	2 or 3 rats per suspended polypropylene cage with stainless steel wire grid top and bottom.
Environmental conditions:	Temperature: 18 – 22 °C Humidity: 55 ± 10 % Air changes: 15 – 20/hour 12 hours light/dark cycle

B: Study design and methods

In life dates: 1988-12-21 to 1989-01-18

Animal assignment and treatment:
Groups of five male and five female Sprague-Dawley rats were administered technical glyphosate diet for 28 consecutive days via the diet at concentrations calculated to achieve dose levels of 0 (control), 50, 250, 1000 or 2500 mg/kg bw/day.

Table 5.3.1-20: Glyphosate: 4 Week Dietary Toxicity Study in Rats ([redacted] 1991): Study design

Test group	Dose Level [mg/kg bw/day]	Males	Females
Control	0	5	5
Low	50	5	5
Intermediate I	250	5	5
Intermediate II	1000	5	5
High	2500	5	5

The test diets were prepared weekly by direct admixture of test material to untreated diet and blending for 20 minutes in a diet mixer. Samples of diets prepared for week 1 of the study were analysed with regard to stability, concentration and homogeneity. Data proving 21-day stability of glyphosate were generated prior to commencement of the study. The concentration of glyphosate in the diet was adjusted weekly in order to achieve a constant dose level in mg of test material per kg of the animal's body weight per day.

Mortality
Each animal was checked for mortality or signs of morbidity twice daily during the treatment period.

Clinical observations
General clinical observations were done daily and detailed clinical examinations weekly.

Body weight
Body weights were assessed during the week prior to starting treatment and in weekly intervals thereafter.

Food consumption and utilisation
Food consumption was assessed in weekly intervals. Water consumption was monitored by visual inspection throughout the study.

Ophthalmoscopic examination
Ophthalmoscopic eye examinations were not performed.

Haematology and clinical chemistry
Blood samples from all rats were taken during week 4 dosing from the retro-orbital sinus under light anaesthesia. These samples were submitted for haematological and clinical chemistry examination. The following haematological parameters were measured: Haemoglobin, haematocrit, red blood cell (RCB) count, leucocyte count and differential and coagulation via tail snip.

For clinical chemistry analysis the following parameters measured: Glucose, blood urea nitrogen, total protein, albumin, globulin, albumin/globulin ratio, creatinine, calcium, phosphate, sodium, potassium, chloride, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, total bilirubin and plasma and RBC cholinesterase.

Sacrifice and pathology
After 28 of consecutive treatment, all surviving animals were sacrificed via carbon dioxide followed by exsanguination and subjected to a gross pathological examination. The following organ weights were determined: Adrenals, kidneys, liver, and testes and epididymides.

Tissue samples were taken from the following organs and preserved: all gross lesions, adrenals, heart, kidneys, liver, ovaries (with fallopian tubes), spleen, testes (plus epididymides). Liver, heart, kidneys, spleen and adrenals from the control and high dose group were examined histopathologically. Kidneys from the intermediate I, Intermediate II and low dose female groups were also subjected to histopathological evaluation.

Statistics
Haematology, clinical chemistry, organ weight and body weight data were statistically analysed for homogeneity of variance using the 'F-max' test. If the group variances appeared homogeneous, a parametric ANOVA was used and pairwise comparisons made via Student's t-test using Fisher's F-protected LSD. If the variances were heterogeneous, then transformations such as log and rank were used to achieve homogeneity, or a non-parametric test such as Kruskal-Wallis ANOVA was used. Organ weights were also analysed conditional on body weight (i.e. analysis of covariance). Histopathology data were analysed by Fisher's Exact Probability test.

II. RESULTS AND DISCUSSION

A. MORTALITY
There was one premature death, a male from the 250 mg/kg bw/day dose group that died during the blood sampling in week 4. This was regarded as unrelated to test substance administration.

B. CLINICAL OBSERVATIONS
Three out of 5 high dose males had soft faeces during weeks 3-4 or in week 4 only. There were no other effects observed in males or females from the other dose groups considered related to treatment.

C. BODY WEIGHT
The 2500 mg/kg bw/day dose group showed a slight but consistent reduction in body weight gain throughout the dosing period. This reduction did not reach statistical significance. The 2500 mg/kg bw/day dose female group showed a slight but consistent reduction in body weight gain throughout the four weeks of dosing. The difference did not attain statistical significance. These reductions in body weight gain were considered unrelated to treatment based on the absence of notable differences in body weight gain in the other dose groups and in final group body weights. Results are presented in the following table:

Table 5.3.1-21: Glyphosate: 4 Week Dietary Toxicity Study in Rats ([redacted] 1991): Intergroup comparison of mean body weights and body weight gain

Dose level [mg/kg bw/day]	Initial body weight [g] Week 0	Final body weight [g] Week 4	Weight gain [g]	Weight gain [% of controls]
Males				
0	154	351	197	-
50	↑158	↑368	↑210	↑107
250	↑157	↑376	↑219	↑111
1000	↑161	↓349	↓188	↓95
2500	↑157	↓336	↓179	↓91
Females				
0	115	228	113	-
50	↓109	↓214	↓105	↓93
250	↓110	↑234	↑124	↑110
1000	↓108	↓212	↓104	↓92
2500	↓113	↓214	↓101	↓89

D. FOOD CONSUMPTION AND TEST SUBSTANCE INTAKE
There were no notable intergroup differences in either sex. All groups receiving glyphosate performed similarly to if not better than their respective controls.

The concentration of glyphosate in the diet was adjusted weekly in order to achieve a constant dose level in mg of test material per kg of animal's body weight per day. Test compound intakes are presented in the following table:

Table 5.3.1-22: Glyphosate: 4 Week Dietary Toxicity Study in Rats ([redacted], 1991): Test compound intake

Test group	Dietary concentration [mg/kg bw/day]	Achieved dietary concentration [mg/kg bw/day]	
		Males	Females
Control	0	0	0
Low	50	49 ± 5.8	55 ± 7.9
Intermediate I	250	255 ± 9.6	277 ± 17.1
Intermediate II	1000	1034 ± 58.2	1047 ± 86.4
High	2500	2592 ± 226.9	2614 ± 318.4

E. OPHTHALMOSCOPIC EXAMINATION

Specific eye examinations were not performed.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

There were no treatment-related differences noted in any dose group.

Blood clinical chemistry

In males , there was a mild equivocal increase in alanine aminotransferase (ALT) of +21 %, +34 %, +25 % and alkaline phosphatase (AP) of +35 %, +23 %, +60 % in the 250, 10000 and 2500 mg/kg bw/day groups, respectively. Phosphate was also slightly increased in the 1000 mg/kg bw/day (+13 %) and 2500 mg/kg bw/day (+16 %) dose groups.

In females, ALT was significantly increased in the 2500 mg/kg bw/day group (+31 %), as was total bilirubin (+63 %). Alkaline phosphatase was increased in the 1000 and 2500 mg/kg bw/day dose groups but did not reach statistical significance and there was no relationship to dose level of Glyphosate. There were slight increases in sodium in the 50, 1000 and 2500 mg/kg bw/day dose groups (all +1 %) and in chloride in the 50 mg/kg bw/day group (+2 %) but these were considered due to chance (see table below):

Table 5.3.1-23: Glyphosate: 4 Week Dietary Toxicity Study in Rats ([redacted] 1991): Intergroup comparison of selected clinical chemistry parameters (mean ± SD)

Parameter	Dose level [mg/kg bw/day]				
	0	50	250	1000	2500
Males					
ALT [U/L]	68 ± 7	↑71 ± 13	**↑82* ± 8**	**↑91** ± 11**	**↑85* ± 9**
AP [U/L]	575 ± 84	↑624 ± 66	**↑779* ± 217**	↑707 ± 59	**↑921*** ± 162**
Total bilirubin [µmol/L]	0.9 ± 0.3	↑1.0 ± 0.4	0.9 ± 0.5	↓0.8 ± 0.2	↑1.0 ± 0.4
Phosphate [mmol/L]	2.44 ± 0.20	↓2.24 ± 0.16	↑2.64 ± 0.17	**↑2.76** ± 0.19**	**↑2.82** ± 0.15**
Sodium [mmol/L]	145 ± 1	145 ± 1	↑146 ± 1	↑146 ± 2	145 ± 1
Chloride [mmol/L]	97 ± 1	↑98 ± 1	↑98 ± 2	↑98 ± 1	↑99 ± 1
Females					
ALT [U/L]	59 ± 13	↓52 ± 8	↑74 ± 13	↑67 ± 14	**↑77* ± 12**
AP [U/L]	485 ± 87	↑492 ± 184	↓469 ± 103	↑701 ± 79	↑687 ± 289
Total bilirubin [µmol/L]	0.8 ± 0.2	0.8 ± 0.2	↑1.1 ± 0.2	↑1.0 ± 0.2	**↑1.3** ± 0.3**
Phosphate [mmol/L]	2.28 ± 0.30	↓2.19 ± 0.10	↑2.38 ± 0.18	↑2.46 ± 0.22	↑2.32 ± 0.20
Sodium [mmol/L]	144 ± 1	**↑145** ± 1**	↑145 ± 1	**↑145** ± 1**	**↑145* ± 1**
Chloride [mmol/L]	98 ± 1	**↑100* ± 1**	98 ± 1	↑99 ± 2	**↑100** ± 1**

* Statistically significant from controls ($p < 0.05$);
** Statistically significant from controls ($p < 0.01$);
*** Statistically significant from controls ($p < 0.001$)

G. NECROPSY

Organ weights

There were no intergroup differences in either sex.

Gross pathology

There were no findings attributed to administration of glyphosate.

Histopathology

Findings related to glyphosate exposure were limited to the kidneys of females. Very mild to mild nephrocalcinosis (mineral deposition) was noted at the corticomedullary junctions in 40 % of the 250 mg/kg bw/day females, 40 % of the 1000 mg/kg bw/days females and 80 % of the 2500 mg/kg/day females. This finding was not present in the 50 mg/kg bw/day or control females (See Table below):

Table 5.3.1-24: Glyphosate: 4 Week Dietary Toxicity Study in Rats ([redacted] 1991): Summary incidence of histopathological findings

Finding	Dose level [mg/kg bw/day]									
	Males					Females				
	0	50	250	1000	2500	0	50	250	1000	2500
Nephro-calcinosis	0/5	-	-	-	0/5	0/5	0/5	2/5	2/5	4/5

III. CONCLUSIONS

There was one premature death, an intermediate dose group male that died during the week 4 blood sampling. Incidences of soft faeces were noted in high dose males during weeks 3 and 4. There were no notable intergroup differences in body weight, food consumption, water consumption, or haematological parameters. There were equivocal increases in alanine aminotransferase (ALT) (males at 250, 1000 and 2500 mg/kg bw/day; females at 2500 mg/kg bw/day), alkaline phosphatase (males at 250, 1000 and 2500 mg/kg bw/day; females at 1000 and 2500 mg/kg bw/day) and total bilirubin (females at 2500 mg/kg bw/day), all of which were regarded as normal responses to increased liver activity at the elevated dose levels of glyphosate. Plasma phosphate was slightly increased in 1000 and 2500 mg/kg bw/day males. There were no notable intergroup differences in organ weights. No gross pathology findings attributed to administration of glyphosate were recorded. Histopathological changes were limited to nephrocalcinosis in 250, 1000 and 2500 mg/kg bw/day females. The increases in plasma phosphate and nephrocalcinosis were considered expected findings in view of the dose levels employed and the phosphate content of glyphosate.

The No Effect Level (NOEL) was set at 50 mg/kg bw/day in the study report.

3. Assessment and conclusion

Assessment and conclusion by applicant:

In this study groups of male and female Sprague-Dawley rats were administered glyphosate via the diet at dose levels of 0, 50, 250, 1000 or 2500 mg/kg bw/day for 28 days according to OECD 407 (1981).

Dosing Sprague-Dawley rats via the diet with glyphosate produced slight increases in plasma phosphate levels in males (1000 and 2500 mg/kg bw/day) and increases in incidence of nephrocalcinosis in females (≥250, 1000 and 2500 mg/kg bw/day), both being expected findings in view of the dose levels employed and the phosphate content of glyphosate. In addition, there were equivocal increases in alanine aminotransferase (males at ≥250 mg/kg bw/day; females at 2500 mg/kg bw/day), alkaline phosphatase (males at ≥250 mg/kg bw/day; females at 1000 and 2500 mg/ kg bw/day) and total bilirubin (females at

2500 mg/kg bw/day), all of which were regarded as normal responses to increased liver activity at the elevated dose levels of glyphosate.

Since the effects observed were considered to be adaptive, the NOAEL was set at 2500 mg/kg bw/day for male and female Sprague-Dawley rats under the conditions of this study.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.3.1/005
Report author	[redacted]
Report year	1989
Report title	Range-finding Study of Glyphosate Administered in Feed to Sprague-Dawley Rats
Report No	[redacted]-8921
Document No	Not reported
Guidelines followed in study	Not reported; in general compliance to OECD 407 (1981)
Deviations from current test guideline (OECD 407, 2008)	Temperature-range of the housing conditions was below 22 ± 3 °C; no sensory reactivity tests were performed; no haematology/clinical chemistry were performed; no organ weights were determined; histopathology was performed on liver and kidneys only.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 3b

2. Full summary

Executive Summary
Glyphosate (Lot XLI-203; 97.67 % pure) was administered in the diet to four groups of five male and five female Charles River Sprague-Dawley rats at target concentrations of 0, 30000, 40000 or 50000 ppm (equivalent to 0, 1921.1, 2634.1 or 3278.1 mg/kg bw/day for males and 0, 2310.6, 3256.4 or 4150.2 mg/kg bw/day for females) for four weeks. Food and water were available *ad libitum*. Diets were prepared once at the beginning of the study.
All animals were observed twice daily for mortality and moribundity. Detailed observations for clinical signs of toxicity were performed weekly. Body weights and food consumption were determined each week. All animals were sacrificed and given a gross necropsy at the end of the study. The liver and kidneys from each animal were preserved; these organs were examined microscopically for control and high dose animals.

No animals died during the course of the study. Slightly reduced body weight gains were noted in both sexes at all three dose levels, although significant reductions consistently occurred only in high dose males and females (9.6 and 9.0 %, respectively, after 4 weeks). Food consumption (g/day) was reduced for mid and high dose males during the first week of the study. Food intake for treated females was comparable to

controls throughout the study. The only clinical signs of toxicity were soft stool and/or diarrhoea, which occurred in both sexes at all dose levels; nine-of-ten low dose animals had loose stools, all mid dose rats had loose stools and/or diarrhoea, while diarrhoea was the predominant sign in high dose animals during the last three weeks of the study. Gross and microscopic pathology examinations revealed no treatment-related lesions.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate (T880068)
Description:	White powder
Lot/Batch #:	XLI-203
Purity:	97.67 %
Stability of test compound:	Not reported

2. Vehicle and/or positive control: Diet / none

3. Test animals:

Species:	Albino rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	Ca. 9 weeks
Sex:	Male and female
Weight at dosing:	♂ 259.9 – 340.3 g; ♀ 187.7 – 237.2 g
Acclimation period:	27 days
Diet/Food:	Purina Mills Certified RODENT CHOW # 5002, *ad libitum*
Water:	Water (St. Louis public water supply), *ad libitum*
Housing:	Individual suspended stainless steel cages
Environmental conditions:	Temperature: 18 – 26 °C Humidity: 40 – 70 % Air changes: Not reported 12 hours light/dark cycle

B: Study design and methods

In life dates: 1988-07-25 to 1988-08-22

Animal assignment and treatment:
The test material was offered on a continuous basis in the basal diet to groups of 5 Sprague-Dawley rats per sex for a minimum of 28 days. Dietary concentrations were 0, 30000, 40000 and 50000 ppm (equivalent to 0, 1921.1, 2634.1 and 3278.1 mg/kg bw/day for males and 0, 2310.6, 3256.4 and 4150.2 mg/kg bw/day for females).

Table 5.3.1-25: Range-finding Study of Glyphosate Administered in Feed to Sprague-Dawley Rats ([redacted], 1989): Study design

Test group	Dietary concentration [ppm]	Average dietary concentration [mg/kg bw/day]	Males	Females
Control	0	♂: 0; ♀: 0	5	5
Low	30000	♂: 1921.1; ♀: 2310.6	5	5
Mid	40000	♂: 2634.1; ♀: 3256.4	5	5
High	50000	♂: 3278.1; ♀: 4150.2	5	5

Preparation of the test diet
Purina Mills Certified RODENT CHOW #5002 was mixed for 10 minutes using a Hobart HCM-450 mixer. A batch size of 12 kg per dose was produced once during this study.

Mortality
Animals were checked twice daily for mortality and moribundity.

Clinical observations
Detailed observations for clinical signs of toxicity were performed weekly.

Body weight
The weight of each animal was recorded once weekly.

Food consumption
The quantity of food consumed by each animal was recorded once each week.

Sacrifice and pathology
All animals were killed and necropsied. External and internal investigations were performed on opened internal cavities and organs *in situ* and then removed. Hollow organs were opened and examined.

Histopathology
The following tissues were processed and examined histopathologically from all Control and High dose animals.
The following tissues were investigated: Kidneys and liver.

Statistics
The following statistical procedures were used to detect statistically significant differences between treated animals and their respective controls:
Dunnett's Multiple Comparison Test (two-tailed): Body weights, food consumption
Fisher's Exact Test with Bonferroni Inequality Procedure: Incidence of microscopic lesions
Other statistical routines used for some data were: Bartlett's Test to evaluate homogeneity of variances, Analysis of Variance to determine if the sample (group) means could be considered as an estimate of a common population, and Grubbs' Test to detect outliers.

II. RESULTS AND DISCUSSION

A. ACHIEVED DOSAGES
Please refer to the table above Table 5.3.1-25.

B. MORTALITY
There were no animals found dead or killed *in extremis* in any group during the treatment period.

C. CLINICAL OBSERVATIONS

Clinical signs that were considered related to treatment included soft stool and/or diarrhoea, and were seen in animals of both sexes at all three dose levels.
Frequently, the lowest dose level animals had soft stools and the middle dose level animals had soft stools and diarrhoea. The highest dose level animals experienced incidences of marked diarrhoea. No other treatment-related clinical signs were noted.

D. BODY WEIGHT

Slightly reduced body weight gains occurred for both sexes at all three dose levels. However, differences in body weights between treated and control groups were less than 10 %. This should be set in context to the fact that the dietary inclusion levels represented 3 %, 4 % and 5 % by mass of the feed offered, and consequently the calorific value of the feed was concomitantly reduced.

Table 5.3.1-26: Range-finding Study of Glyphosate Administered in Feed to Sprague-Dawley Rats ([redacted], 1989): Body weight means [g]

	Dose group [ppm]							
	Males				Females			
	0	30000	40000	50000	0	30000	40000	50000
Study mean	360.9	↓355.0	↓342.9	↓335.0	234.2	↓225.2	↓225.9	↓219.8
% Difference from control (study mean)	-	-1.7	-5.0	-7.2	-	-3.8	-3.6	-6.2
% Difference from control (final body weight)	-	-2.3	-5.9	-9.6	-	-5.9	-4.5	-9.0
% Gain from initial	27.2	↓22.9	↓20.8	↓15.4	23.1	↓15.4	↓17.8	↓12.6

Table 5.3.1-27: Range-finding Study of Glyphosate Administered in Feed to Sprague-Dawley Rats ([redacted], 1989): Summary of body weight data [g]

	Dose group [ppm]							
	Males				Females			
	0	30000	40000	50000	0	30000	40000	50000
Pretest	313.3 ± 24.25	↑316.6 ± 20.59	↓310.6 ± 30.37	↓312.0 ± 19.24	206.6 ± 15.32	↑207.2 ± 18.09	↓206.1 ± 14.72	205.6 ± 16.02
Day 6	337.7 ± 28.26	↓332.6 ± 21.14	↓322.0 ± 39.20	↓311.0 ± 26.37	224.8 ± 14.34	↓217.6 ± 17.22	↓216.1 ± 13.47	↓212.2 ± 14.98
Day 16	372.0 ± 30.40	↓360.4 ± 19.92	↓345.8 ± 46.67	↓341.2 ± 27.13	240.0 ± 15.27	↓231.0 ± 18.44	↓230.0 ± 16.64	↓223.4 ± 18.70
Day 23	383.0 ± 32.10	↓375.5 ± 21.05	↓361.0 ± 47.99	↓350.7 ± 31.36	245.4 ± 16.88	↓231.1 ± 17.27	↓234.5 ± 12.54	↓226.3 ± 18.16
Day 28	398.6 ± 35.77	↓389.5 ± 21.79	↓375.1 ± 48.90	↓360.2 ± 35.90	254.3 ± 16.93	↓239.2 ± 19.29	↓242.8 ± 19.85	↓231.5 ± 20.93

Table 5.3.1-28: Range-finding Study of Glyphosate Administered in Feed to Sprague-Dawley Rats ([redacted] 1989): Summary of cumulative body weight changes [g]

	Dose group [ppm]							
	Males				Females			
	0	30000	40000	50000	0	30000	40000	50000
Day 1 – 8	24.3 ± 6.21	↓15.8 ± 5.62	**↓11.4* ± 11.23**	**↓-1.0** ± 7.27**	18.2 ± 5.70	↓10.3 ± 7.83	↓10.0 ± 7.18	**↓6.6* ± 4.52**
Day 1 – 16	58.6 ± 8.38	↓43.6 ± 11.90	**↓36.2* ± 16.35**	**↓29.2** ± 9.00**	33.4 ± 10.54	↓23.8 ± 6.12	↓23.9 ± 5.87	**↓17.8** ± 4.76**
Day 1 – 23	69.6 ± 6.89	↓58.7 ± 8.48	↓50.4 ± 17.91	**↓38.7** ± 12.49**	38.8 ± 10.73	**↓23.9* ± 7.14**	↓28.3 ± 8.18	**↓20.7** ± 6.46**

Table 5.3.1-28: Range-finding Study of Glyphosate Administered in Feed to Sprague-Dawley Rats ([redacted], 1989): Summary of cumulative body weight changes [g]

	Dose group [ppm]							
	Males				Females			
	0	30000	40000	50000	0	30000	40000	50000
Day 1 – 28	85.3 ± 12.31	↓72.7 ± 6.98	↓64.5 ± 19.13	**↓48.2** ± 16.94**	47.7 ± 7.51	**↓32.0* ± 9.10**	↓36.7 ± 12.08	**↓25.8** ± 7.07**

* Statistically significant from control (Dunnett's test; $p \leq 0.05$);
** Statistically significant from control (Dunnett's test; $p \leq 0.01$)

E. FOOD CONSUMPTION

Food consumption was reduced on a g/day basis for males at the two highest dietary levels as compared to their respective controls during the first week of testing.

After that, food consumption was comparable to that of the control animals during the remainder of the study. Food intake for females was comparable to control throughout the study.

Table 5.3.1-29: Range-finding Study of Glyphosate Administered in Feed to Sprague-Dawley Rats ([redacted], 1989): Food consumption [g/day]

	Dose group [ppm]							
	Males				Females			
	0	30000	40000	50000	0	30000	40000	50000
Day 1 – 8	23.6 ± 1.98	↓20.9 ± 2.18	**↓19.5* ± 2.88**	**↓17.0** ± 1.92**	18.5 ± 1.63	↓15.7 ± 0.69	↓16.0 ± 1.93	↓16.8 ± 4.46
Day 8 – 16	24.3 ± 1.98	↓24.0 ± 1.50	↓23.9 ± 3.29	↓23.0 ± 0.96	18.8 ± 2.34	↓17.4 ± 0.86	↓18.5 ± 2.32	↓17.9 ± 0.90
Day 16 – 23	24.6 ± 2.21	↑25.0 ± 0.99	↑24.7 ± 3.38	↑25.5 ± 0.65	19.1 ± 1.24	↓18.1 ± 0.43	↑19.5 ± 2.12	↓18.8 ± 1.65
Day 23 – 28	23.8 ± 2.42	↓23.5 ± 0.66	↑24.5 ± 3.06	↑24.3 ± 2.31	20.7 ± 3.92	↓17.1 ± 0.74	↓18.2 ± 2.73	↓18.6 ± 0.37

* Statistically significant from control (Dunnett's test; $p \leq 0.05$);
** Statistically significant from control (Dunnett's test; $p \leq 0.01$)

Table 5.3.1-30: Range-finding Study of Glyphosate Administered in Feed to Sprague-Dawley Rats ([redacted], 1989): Mean food consumption [g/day]

	Dose group [ppm]							
	Males				Females			
	0	30000	40000	50000	0	30000	40000	50000
Study mean	24.1	23.4	23.2	22.5	19.3	17.1	18.1	18.0
% Difference from control (study mean)	n.a.	-2.9	-3.7	-6.6	n.a.	-11.4	-6.2	-6.7

n.a.: not applicable

F. NECROPSY

Gross pathology

There were no treatment-related changes observed at necropsy.

Histopathology

There were no microscopic lesions related to treatment.

III. CONCLUSIONS

No animals died during the course of the study. Slightly reduced body weight gains were noted in both sexes at all three dose levels, although significant reductions consistently occurred only in high dose males and females (9.6 and 9.0 %, respectively, after 4 weeks). Food consumption (g/day) was reduced for mid and high dose males during the first week of the study. Food intake for treated females was comparable to controls throughout the study. The only clinical signs of toxicity were soft stool and/or diarrhoea, which occurred in both sexes at all dose levels; nine-of-ten low dose animals had loose stools, all mid dose rats had loose stools and/or diarrhoea, while diarrhoea was the predominant sign in high dose animals during the last three weeks of the study. Gross and microscopic pathology examinations revealed no treatment-related lesions.

According to the study author, the body weight reduction may have been secondary to the described gastrointestinal effects, rather tha a direct toxic response to treatment. This interpretation was supported by the absence of any corresponding changes in food intake, survival, gross and/or microscopic pathology.

3. Assessment and conclusion

Assessment and conclusion by applicant:
In this study, glyphosate was administered in the diet to four groups of five male and five female Sprague-Dawley rats at target concentrations of 0, 30000, 40000 or 50000 ppm (equivalent to 0, 1921.1, 2634.1 or 3278.1 mg/kg bw/day for males and 0, 2310.6, 3256.4 or 4150.2 mg/kg bw/day for females) for four weeks in general compliance to OECD 407.

Significant incidences of soft stools and/or diarrhoea were noted for both sexes at all three exposure levels with the highest dose group most affected. Slightly reduced body weight gains occurred for both sexes at all three dose levels but were at all times less than 10 % of the corresponding controls, and were probably in part due to the lower calorific value of the diet offered (due to high level of glyphosate inclusion). This body weight reduction may have been secondary to the observed gastrointestinal effects. This interpretation is supported by the absence of any corresponding changes in food intake, survival, gross or microscopic pathology. No other significant effects were noted in this study.

Selection of dose levels for a longer term study should consider secondary effects (nutritional imbalance, dehydration and death) often related to diarrhoea.

Based on the results of this 28-day range-finding study, no robust NOAEL can be derived.

Assessment and conclusion by RMS:
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1. Information on the study

Data point	CA 5.3.1/006
Report author	[redacted]
Report year	1978
Report title	A Four Week Pilot Study with Glyphosate in Mice
Report No	77-2110
Document No	Not reported
Guidelines followed in study	No guideline followed, similar to OECD 407 (1981)
Deviations from current test	No sensory reactivity was investigated; no haematology or clinical

guideline (OECD 407, 2008)	chemistry was performed; organ weights were not determined; histopathology was not performed.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No (pre-GLP).
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 3b

2. Full summary

Executive Summary

This study was conducted to select dose levels for a 90–day sub-chronic study of glyphosate. The test substance was administered orally via the diet to mice at target dose levels of 100, 300 or 1000 mg/kg bw/day (approximately 80, 235 or 800 mg/kg bw/day actual achieved dose, after correction for test substance purity). All survivors were necropsied after four weeks of test substance administration.

Animals were observed for mortality, clinical signs, body weight changes, food consumption and gross pathology.

All in-life data (physical observations, body weight and food consumption) and gross necropsy observations indicated no adverse effects at any of the dose levels administered.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification: Glyphosate
Description: Course white powder
Lot/Batch #: XHI-162
Purity: 83 %
Stability of test compound: Not reported

2. Vehicle and/or positive control: Diet / none

3. Test animals:

Species: Mouse
Strain: CD-1, COBS (ICR derived)
Source: [redacted]
Age: 7 weeks
Sex: Male and female
Weight at dosing: ♂ 28 g (25 – 31 g); ♀ 22 g (20 – 24 g)
Acclimation period: 18 days
Diet/Food: Standard laboratory diet (Purina Laboratory Chow®) *ad libitum*; fresh food presented twice weekly.
Water: Water, *ad libitum*
Housing: Individually in elevated stainless steel wire mesh cages.

Environmental conditions:	Temperature:	Not reported
	Humidity:	Not reported
	Air changes:	Not reported

B: Study design and methods

In life dates: 1978-03-13 to 1978-04-12

Animal assignment and treatment:
The test material was offered on a continuous basis in the basal diet to groups of 5 CD-1 mice per sex for 30 days. Dietary concentrations were 0, 100, 300 and 1000 mg/kg bw/day (approximately 80, 235 and 800 mg/kg bw/day after correction for test substance purity).

Table 5.3.1-31: A Four Week Pilot Study with Glyphosate in Mice ([redacted], 1978): Study design

Test group	Target dietary concentration [mg/kg bw/day][1]	Achieved compound intake [mg/kg bw/day]	Males	Females
Control	0	0	5	5
Low	100	80	5	5
Mid	300	235	5	5
High	1000	800	5	5

[1] Active substance-dosing based on 98.5 % activity of the technical material. Upon analysis of the test material by the sponsor, glyphosate was assayed at 83 % active.

Analysis of the test diet
Appropriate amounts of compound (adjusted by most recent weekly body weight and food consumption data) and standard laboratory diet were mixed weekly. A 4 oz. sample of each dietary level plus control diet was taken from each weekly batch of diet prepared. In addition, when feeders were filled, two extra feeder jars of each dietary level plus control feed were placed in an empty animal cage immediately adjacent to the last animal in each group and sampled after 7 days. All feed samples (the 4 oz. samples taken at each diet preparation from each batch as well as the entire contents of the two extra feeders for Groups I – IV) were stored frozen at [redacted]. Samples were packed on dry ice and sent to Monsanto Agricultural Products Company for analysis on 18 April 1978.

Mortality
Mice were checked twice daily for mortality.

Clinical observations
All animals were examined for gross signs of toxicological or pharmacological effects twice daily. Additionally, all animals received a detailed physical examination for signs of local or systemic toxicity and pharmacological effects once each week.

Body weight
The weight of each animal was recorded twice prior to treatment, weekly during treatment and terminally (after fasting).

Food consumption and compound intake
The quantity of food consumed by each animal was recorded once prior to treatment and weekly during treatment.
Please refer to the table above for information on the compound intake.

Sacrifice and pathology
All animals were killed by exsanguination under ether anaesthesia. Tissues with gross abnormalities were preserved in 10 % neutral buffered formalin and held for possible future examination.

Statistics
Body weight and food consumption data were analysed. Mean values of all dose groups were compared to control at each time interval.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF THE FORMULATED DIETS
The analysis of the formulated diets was in the responsibility of the sponsor.

B. MORTALITY
All animals survived the duration of the study.

C. CLINICAL OBSERVATIONS
There were no physical observations noted which were considered related to the administration of the test material.

D. BODY WEIGHT
Slightly lower mean body weights, as compared to control, were noted for males receiving 300 or 1000 mg/kg bw/day through week two. This only achieved statistical significance during the first week in high dose animals. The high dose males has slightly lower mean body weight pre-test and as a consequence. These differences were not considered related to the administration of Glyphosate.

Mean body weights for males receiving 100 mg/kg bw/day and all test substance-treated females were considered comparable to those of the controls throughout the course of the study.

Table 5.3.1-32: A Four Week Pilot Study with Glyphosate in Mice ([redacted], 1978): Summary of body weight data [g]

Week	Dose group [mg/kg bw/day]							
	Males				Females			
	0	100	300	1000	0	100	300	1000
-1	24.8 ± 1.6	24.8 ± 1.8	↑25.4 ± 1.8	↓24.4 ± 0.9	19.4 ± 0.5	19.4 ± 0.5	↓19.2 ± 0.8	↑19.6 ± 0.9
0	29.8 ± 0.8	↓28.4 ± 2.2	↓27.8 ± 1.9	**↓27.6* ± 1.8**	21.6 ± 0.9	↓21.2 ± 0.8	↑22.4 ± 1.1	↑21.8 ± 1.4
1	32.6 ± 1.5	↓31.6 ± 1.7	↓31.0 ± 1.6	↓31.4 ± 1.7	23.8 ± 0.8	↑24.2 ± 1.1	↑24.4 ± 0.9	↑24.6 ± 1.5
2	33.8 ± 1.3	↓32.6 ± 2.3	↓32.0 ± 2.0	↓31.8 ± 2.0	25.2 ± 0.8	↑25.6 ± 1.5	↑26.0 ± 1.4	↑26.6 ± 2.1
3	33.2 ± 1.6	↑33.6 ± 2.2	↑33.4 ± 1.9	33.2 ± 1.9	26.4 ± 1.5	↓25.2 ± 1.6	↑26.6 ± 0.9	↑26.6 ± 1.8
4	34.4 ± 1.3	↓34.0 ± 2.3	↓33.6 ± 1.7	34.4 ± 1.9	28.2 ± 1.5	↓26.6 ± 1.8	↓27.2 ± 1.1	↓27.6 ± 2.2

E. FOOD CONSUMPTION AND COMPOUND INTAKE
Food consumption values for all animals receiving Glyphosate were generally comparable to those of the controls throughout the study.

Appropriate amounts of test material, based on 98.5 % activity, were mixed fresh weekly in Purina Laboratory Chow® to yield dose levels of 100, 300 and 1000 mg of test material per kg of body weight per

day. Upon analysis by the sponsor, glyphosate assayed at an activity of 83 %. Consequently, the mean compound consumption values are approximately 80 % of the proposed dose levels.

F. NECROPSY

Gross pathology
Macroscopic post mortem observations did not reveal any changes considered related to the administration of test material.

III. CONCLUSIONS

All in-life data (physical observations, body weight and food consumption) and gross necropsy observations indicated no adverse effects at any of the dose levels administered.

3. Assessment and conclusion

Assessment and conclusion by applicant:
In this range-finding, the test substance glyphosate was administered orally via the diet to CD-1 mice at doses of approximately 80, 235 or 800 mg/kg bw/day of active substance similar to OECD 407 (1981).

All in-life data (physical observations, body weight and food consumption) and gross necropsy observations indicated no adverse effects of glyphosate at any of the dose levels administered.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.3.1/007
Report author	[redacted]
Report year	1989
Report title	Glyphosate: Oral Maximum Tolerated Dose Study in Dogs
Report No	5660
Document No	Not reported
Guidelines followed in study	U.S. EPA FIFRA 82-1
Deviations from current test guideline:	This study was essentially a dose range-finding study for which no guideline is available.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary
This study was conducted to determine the oral maximum tolerated dose for glyphosate in Beagle dogs for dosing subsequent repeated dose toxicity studies in this species. Glyphosate was administered by gelatin capsule to one male and one female dog for 7 day periods at escalating dose levels of 100, 300 and 1000

mg/kg bw daily (Part A) and to one dog of each sex for 14 days at 1000 mg/kg bw/day (Part B).

Clinical observations were made daily. Body weights were assessed twice weekly and food consumption recorded daily. For part A, laboratory investigations of haematology and clinical chemistry were performed on all the dogs before dosing started and after 7 days of treatment with each dose level. For part B, blood samples were taken before dosing and after 14 days of dosing. Urine samples were collected pre-trial and the day prior to sacrifice (part B only). Samples were collected over the final 17 hours of a 21 hour period of water deprivation. Faecal analysis for occult blood was performed at the time of the urine collections. At the end of the scheduled dosing periods, the animals were sacrificed and subjected to a full examination *post mortem*. Selected organs were weighed and tissues were taken for possible subsequent histopathology examination that was not performed.

There were no mortalities. There were no clinical signs of toxicity considered treatment related after 21 days of dosing up to 1000 mg/kg bw/day (Part A). Other than loose faeces in the one female during the dosing period, there were no other clinical observations considered related to treatment during 14 days dosing at 1000 mg/kg bw/day (Part B). Body weight profiles or food consumption were not affected by administration of glyphosate. No haematological effects considered related to treatment were observed. A slight increase in alanine aminotransferase occurred in the two male dogs treated with glyphosate and a slight reduction in cholesterol levels in dogs of both sexes that were not considered treatment related. There were no effects of glyphosate administration on urine parameters or occult blood detected in faeces in dogs of either sex. At necropsy, there were no gross lesions observed attributable to treatment with glyphosate with dose escalation or 14 days dosing at 1000 mg/kg bw/day. There was a reduction in absolute and relative spleen weight in the male dog with dose escalation to 1000 mg/kg bw/day but not in the male dog treated with glyphosate for 14 days. Microscopic examination of tissues was not performed.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate
Description:	White powder
Lot/Batch #:	161-JRJ-131-2
Purity:	99.5 %
Stability of test compound:	Not indicated

2. Vehicle and/or positive control: No controls utilised.

3. Test animals:

Species:	Dog
Strain:	Beagle
Source:	[redacted]
Age:	4 months
Sex:	Male and female
Weight at dosing:	Part A: ♂ 8.5 kg ♀ 8.6 kg; Part B: ♂ 9.2 kg ♀ 9.3 kg
Acclimation period:	At least 2 weeks.
Diet/Food:	Special Diet Services (SDS) complete dry Diet A, 400 g/day approximately on hour after dosing.
Water:	Tap water, *ad libitum*
Housing:	Individually in pens

Environmental conditions: Temperature: ~17 °C (14 – 22 °C transient extremes)
Humidity: ~42 % (18 – 56 % transient extremes)
Air changes: 15 per hour
12 hours light/dark cycle

B: Study design and methods

In life dates: Part A 1989-01-31 to 1989- 02-21; Part B 1989-02-28 to 1989-03-14

Animal assignment and treatment:
This study was essentially a dose range-finding study to determine the oral maximum tolerated dose of glyphosate for setting dose levels for subsequent chronic dog studies. Dose levels up to a maximum limit level of 1000 mg/kg bw/day were investigated. Dose levels were selected based on available acute toxicology data. The study was conducted in two parts, A and B. For part A, one male and one female dog were dosed orally by gelatin capsule at escalating dose levels of 100, 300 and 1000 mg/kg bw/day. Each dose level was administered for 7 consecutive days. For part B, one male and one female were dosed orally by gelatin capsule with 1000 mg/kg bw/day for 14 consecutive days. Daily multiple capsule dosing was necessary for the 1000 mg/kg bw/day dose level. One batch of the test material (Batch no. 161-JRJ-131-2, purity 99.5 %) was used during the course of the study. Kruger hard, clear gelatin capsules (35 × 17 mm) were used for the formulation of individual animal daily doses. Encapsulation of the bulk powder into individual animal daily doses was carried out weekly. Individual doses were calculated and prepared on the basis of the animals' most recently recorded body weight.

Table 5.3.1-33: Glyphosate: Oral Maximum Tolerated Dose Study in Dogs ([redacted] 1989): Study design

Test group	Dose Level [mg/kg bw/day]	Males	Females
Part A	100 (7 days)	1	1
	300 (7 days)		
	1000 (7 days)		
Part B	1000 (14 days)	1	1

Mortality
Each animal was checked for mortality or signs of morbidity during daily clinical observations.

Clinical observations
A check for clinical signs of toxicity at regular intervals throughout each working day, generally early morning and as late as possible each day.

Body weight
The body weight of each animal was recorded twice weekly.

Food consumption and utilisation
Individual food consumption was recorded daily.

Ophthalmoscopic examination
Ophthalmoscopy was not performed.

Haematology and clinical chemistry
For part A, laboratory investigations of haematology and clinical chemistry were performed on all the dogs before dosing started and after 7 days of treatment with each dose level. For part B, blood samples were

taken before dosing and after 14 days of dosing. All blood samples were taken from the jugular vein after the dogs had been fasted overnight.

EDTA was used as an anti-coagulant for evaluation of all haematology parameters with the exception of prothrombin time for which citrate was used. The following haematological parameters were measured: Haemoglobin, haematocrit, red blood cell count, mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), reticulocyte count, total white blood cell and differential counts, platelet count and prothrombin time.

For clinical chemistry evaluations, heparin was used as an anti-coagulant and plasma analysed for the following parameters: Blood urea nitrogen, glucose, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, sodium, potassium, calcium, chloride, creatinine, total protein, protein electrophoresis, albumin, albumin/globulin ratio, cholesterol, alkaline phosphatase, phosphate and total bilirubin.

Urinalysis/Faecal analysis
Urine samples were collected (part B only) pretrial and the day prior to sacrifice using metabolism cages. Samples were collected over the final 17 hours of a 21 hour period of water deprivation. The following parameters were measured: Appearance, volume, pH, specific gravity, proteins, glucose, ketones, blood pigments, bilirubin and urobilinogen. Microscopic examination of the spun urine deposit was performed for the presence of epithelial cells, white blood cells, red blood cells, crystals, casts, organisms and abnormal constituents. Faecal analysis for occult blood was performed at the time of the urine collections.

Sacrifice and pathology
After completion of the respective dosing periods, the animals were sacrificed by intravenous pentobarbitone sodium followed by exsanguination and subjected to a gross pathological examination. Any macroscopic findings were recorded. Terminal body weights were recorded.

The following organ weights were determined: heart, liver, kidneys and spleen.

Tissue samples were taken from the following organs and preserved in buffered formalin: All gross lesions, adrenals, heart, kidneys, liver, ovaries, spleen, testes and thymus. These tissues were retained as a contingency for subsequent microscopic examination.

Statistics
Not applicable, only one animal per each sex used for each phase of the study and each animal was its own control.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities.

B. CLINICAL OBSERVATIONS
Part A: There were no treatment related signs observed after 7 days dosing at 100, 300 or 1000 mg/kg bw/day.

Part B: Other than loose faeces in the female during the course of the dosing period, no treatment related signs were observed during the 14 days of dosing at 1000 mg/kg bw/day. The incidence of the loose faeces was not reported.

C. BODY WEIGHT
Part A: Body weight profiles were considered to be satisfactory for both dogs during the treatment period.

Part B: Body weight profiles were considered to be satisfactory for both dogs during the treatment period.

D. FOOD CONSUMPTION AND TEST SUBSTANCE INTAKE

Part A: There were no effects on food consumption from treatment with glyphosate.

Part B: There were no effects on food consumption from treatment with glyphosate.

E. OPHTHALMOSCOPIC EXAMINATION

Not performed.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

Part A: There was a mild reduction in haemoglobin in the male with a mild increase in reticulocytes on Day 22 after the final dosing up to 1000 mg/kg bw/day that was considered related to the repeated blood sampling up to this study day. There were no findings considered to be treatment-related.

Part B: There were no treatment related effects in either dog after 14 days of dosing at 1000 mg/kg bw/day.

Table 5.3.1-34: Glyphosate: Oral Maximum Tolerated Dose Study in Dogs ([redacted], 1989): Selected haematology parameters (individual values)

Parameter	Dose Level [mg/kg bw/day]					
	Male			Female		
	100	300	1000	100	300	1000
Haemoglobin [g/dL]						
Part A						
Pre-test	12.1			13.9		
Day 7	12.0			14.0		
Day 14		10.9			13.6	
Day 22			11.6			13.8
Part B						
Pre-test	12.9			14.2		
Day 22			13.9			15.1
Reticulocytes [%]						
Part A						
Pre-test	0.2			0.3		
Day 7	0.3			0.2		
Day 14		0.9			0.6	
Day 22			1.7			1.3
Part B						
Pre-test	1.2			1.5		
Day 22			1.2			0.6

Blood clinical chemistry

Part A: There were no effects considered related to treatment at the 3 dose levels. A mild increase in alanine aminotransferase (ALT) was observed in the male dog and cholesterol levels were slightly reduced in both male and female animals but these were not considered treatment related effects.

Part B: There were no treatment related effects. A mild increase in alanine aminotransferase was observed in the male dog but was not considered treatment related.

Table 5.3.1-35: Glyphosate: Oral Maximum Tolerated Dose Study in Dogs ([redacted], 1989): Selected Clinical chemistry parameters (individual values)

Parameter	Dose Level [mg/kg bw/day]					
	Male			Female		
	100	300	1000	100	300	1000
ALT [IU/L]						
Part A						
Pre-test	27			22		
Day 7	45			23		
Day 14		49			22	
Day 22			54			21
Part B						
Pre-test	44			23		
Day 22			60			30
Cholesterol [mmol/L]						
Part A						
Pre-test	4.3			4.8		
Day 7	3.7			4.2		
Day 14		3.6			4.1	
Day 22			3.7			4.0
Part B						
Pretest	3.6			4.0		
Day 22			3.6			3.1

G. URINE ANALYSIS/FAECAL ANALYSIS

Part A: Not performed.

Part B: There were no treatment related effects for urine and faeces were negative for occult blood.

H. NECROPSY

Organ weights

Part A: The absolute and relative weights of the spleen were reduced for the male dog. The absolute and relative weight of the spleen for the female and weights of other organs for both sexes were considered to be within normal historical ranges for dogs in this facility.

Part B: Values for the organs weighted were considered to be within normal limits.

Table 5.3.1-36: Glyphosate: Oral Maximum Tolerated Dose Study in Dogs ([redacted], 1989): Spleen Weight - Absolute and relative to body weight (individual values)

Parameter	Dose Level [mg/kg bw/day]					
	Male			Female		
	100	300	1000	100	300	1000
Absolute spleen weight [g]						
Part A						
Day 22			26.91			47.75
Part B						
Day 22			56.21			41.94
Relative spleen weight [% of body weight]						

Table 5.3.1-36: Glyphosate: Oral Maximum Tolerated Dose Study in Dogs ([redacted], 1989): Spleen Weight - Absolute and relative to body weight (individual values)

Parameter	Dose Level [mg/kg bw/day]					
	Male			Female		
	100	300	1000	100	300	1000
Part A						
Day 22			0.29			0.49
Part B						
Day 22			0.58			0.47

Gross pathology
Part A: No lesions attributable to treatment with glyphosate were detected.

Part B: No lesions attributable to treatment with glyphosate were detected.

Histopathology
Not performed for Parts A or B.

III. CONCLUSIONS

There were no mortalities. There were no clinical signs of toxicity considered treatment related after 21 days of dosing up to 1000 mg/kg bw/day (Part A). Other than loose faeces in the one female during the dosing period, there were no other clinical observations considered related to treatment during 14 days dosing at 1000 mg/kg bw/day (Part B). Body weight profiles or food consumption were not affected by administration of glyphosate. No haematological effects considered related to treatment were observed. A slight increase in alanine aminotransferase occurred in the two male dogs treated with glyphosate and a slight reduction in cholesterol levels in dogs of both sexes that were not considered treatment related. There were no effects of glyphosate administration on urine parameters or occult blood detected in faeces in dogs of either sex. At necropsy, there were no gross lesions observed attributable to treatment with glyphosate with dose escalation or 14 days dosing at 1000 mg/kg bw/day. There was a reduction in absolute and relative spleen weight in the male dog with dose escalation to 1000 mg/kg bw/day but not in the male dog treated with glyphosate for 14 days. Microscopic examination of tissues was not performed.

Based on these results it was concluded that the oral maximum tolerated dose of glyphosate in Beagle dogs is in excess of 1000 mg/kg bw/day. The report recommends, however, that the high dose level in any subsequent sub-chronic toxicity study should not exceed 1000 mg/kg bw/day as multiple capsule dose administration would be the limiting factor.

3. Assessment and conclusion

Assessment and conclusion by applicant:
In this study, glyphosate was administered by gelatin capsule to one male and one female dog for 7 day periods at escalating dose levels of 100, 300 and 1000 mg/kg bw daily (Part A) and to one dog of each sex for 14 days at 1000 mg/kg bw/day (Part B). This study was essentially a dose range-finding study conducted in compliance with GLP regulations (no certificate of the competent authority was provided).

Oral administration of glyphosate by gelatin capsule to one male and one female dog for 7 day periods at escalating dose levels of 100, 300 and 1000 mg/kg bw/day produced no adverse effects. Fourteen days of treatment at 1000 mg/kg bw/day in an additional male and female dog was also well tolerated. There were no clinicopathological, gross pathological or organ weight findings considered attributable to treatment. It is unclear, however, if a slight increase in blood alanine aminotransferase in the two male dogs could potentially be related to treatment with glyphosate but this increase did not exceed twice the pretreatment levels in these dogs and thus would not be considered adverse.

Based on these results it is concluded that the oral maximum tolerated dose of glyphosate in Beagle dogs is greater than 1000 mg/kg bw/day.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.3.1/008
Report author	[redacted]
Report year	1982
Report title	Range Finding Study of MON 0139 and Isopropylamine Administered Orally to Dogs
Report No	[redacted]-2155
Document No	Not reported
Guidelines followed in study	None specified
Deviations from current test guideline:	This study was a dose range-finding study for which there are no guidelines available. The examinations were confined to the occurrence of clinical signs, determination of body weight and food consumption and to a gross pathological examination at necropsy.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	GLP was not compulsory when study was conducted/ Study appears in compliance with FDA GLP requirements 21 CFR Part 58./Study conducted at Monsanto Environmental Health Laboratory, St. Louis, Missouri, U.S.A
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The purposes of this study were: 1) to determine the potential toxicity of MON 0139 (the isopropylamine salt of glyphosate) and isopropylamine (IPA) alone administered orally to dogs, and 2) to provide information to assist in determination of dose levels for a subsequent 6-week study of both materials administered to dogs. Five male and four female purebred beagle dogs were administered MON 0139 and/or isopropylamine (IPA) by gavage or gelatin capsules. Dosages of MON 0139 ranged from 312.5 to 2500 mg/kg bw/day in single doses or daily doses for 5 days. No animals died, and all dogs were killed and necropsied after varying observation periods. Mild weight loss and reduced food consumption occurred on and shortly after treatment days with MON 0139, however, both effects were reversible. Diarrhoea was seen at all dose levels of MON 0139 and emesis at all but the lowest dose. Two dose levels of IPA were given: 72 mg/kg bw as a single treatment to a pair of dogs, and 19.43 mg/kg bw/day for five days to a single dog. Emesis, bloody emesis and loose stools were observed. IPA treatment resulted in severe oedema, haemorrhage, and necrosis of the rugae in the stomachs of the higher dose level dogs. Mucosal erosions of the stomach and oesophagus were observed in the lower level dog.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	MON 0139 (Isopropylamine salt of glyphosate); Isopropylamine
Description:	Clear Liquid (MON 0139); Clear, slightly amber liquid (Isopropylamine)
Lot/Batch #:	LUTR-12011 (MON 0139); Luling 2-81 (Isopropylamine)
Purity:	62.49 % (MON 0139); 99.7 % (Isopropylamine)
Stability of test compound:	Not evaluated

2. Vehicle and/or positive control: No controls utilised.

3. Test animals:

Species:	Dog
Strain:	Beagle
Source:	[redacted]
Age:	6–7 months
Sex:	Male and female
Weight at dosing:	♂ 7.3–10.0 kg; ♀ 7.5–9.4 kg when put on test
Acclimation period:	At least one month
Diet/Food:	Ralston-Purina Certified Dog Chow® #5006, 400 g/day
Water:	Tap water, *ad libitum*
Housing:	Individually in stainless steel cages
Environmental conditions:	Temperature: 20 – 24 °C (68 – 75 °F) Humidity: 45 – 65 % Air changes: Not indicated 12 hours light/dark cycle

B: Study design and methods

In life dates: 1981-03-24 to 1981-05-18

Animal assignment and treatment:
Five male and four female purebred beagle dogs were administered MON 0139 or Isopropylamine (IPA) neat by gavage or gelatin capsules. Individual animals received dosages of MON 0139 as a single dose and/or daily doses for 5 days (See table below).

Due to the preliminary nature of this range-finding study, there were several additions and deviations to the study protocol.

The test materials were given neat by gavage or in gelatin capsules at dosages of 312.5, 625, 1250 or 2500 mg/kg bw/day (MON 0139), or 72 or 19.43 mg/kg bw/day (IPA). Analysis of the physical state, chemical content or stability of the test material was not required by the protocol. All but one animal were assigned to groups of two animals (1 male, 1 female). The study protocol indicated that the test material would be administered by gavage and the study was started in that manner. However, when the volume being given was reduced to approximately 7 mL, the form of delivery was changed to the less traumatic method of gelatin capsule administration. With gavage, dosing of the neat test substance was followed with

administration of an equivalent amount of water. Blue food colouring was added to capsules in some cases to help ascertain the presence of test material in vomitus. Three dogs were given isopropylamine alone to determine the level of toxicity of this test substance alone.

Single dose trials:
The first pair of dogs received single doses of 2500 mg/kg bw of MON 0139 by gavage using Tygon tubing. To determine the effect of food in the stomach, the male dog had not been fed that day, while the female dog had food available before and after dosing. On the third day following dosing, in an attempt to administer a dose that would not produce emesis, the same two dogs were re-weighed and given 1250 mg/kg bw of MON 0139. Both dogs had been fed before dosing to determine if the presence of food in the stomach might mitigate emesis. After 4 days, this pair of dogs were again dosed with 1250 mg/kg bw of MON 0139. This time, however, both dogs were fasted before dosing and offered food immediately afterwards. In a final trial with this pair of dogs the dosage was reduced to 625 mg/kg bw with fasting prior to and feed immediately after dosing. These dogs were then observed for 3 weeks, sacrificed and necropsied.

Repeated dosing trials:
As a result of the single dose trials, it was decided to administer doses of 625 mg/kg bw/day for 5 days in gelatin capsules to a new pair of dogs (1 male, 1 female). These dogs were fasted before dosing and fed after dosing. Dose volumes were adjusted for body weight changes each day of treatment. These dogs were sacrificed in good condition after 3 weeks and necropsied.

A third pair of dogs were dosed at 625 mg/kg bw/day of MON 0139 for 5 days and a fourth pair was dosed at 312.5 mg/kg bw/day for 5 days. Several drops of blue food colouring were added to each capsule each day to help determine the presence of test material in the vomitus. These dogs were also fasted prior to and fed after dosing. The dogs treated at a dose level of 312.5 mg/kg bw/day were observed for nearly 2 weeks after treatment and the dogs treated at a dose level of 625 mg/kg bw/day were observed for 1 week.

At the end of the observation period for the two dogs dosed with MON 0139 at 312.5 mg/kg bw/day, it was decided to administer to these dogs the approximately equimolar amount of isopropylamine (IPA) alone contained in the 312.5 mg/kg bw/day dosage of MON 0139. The dosage of IPA given was determined to be 72 mg/kg bw as a single dose given neat in gelatin capsules. Both dogs were fasted before dosing and food was made available after dosing. Within 5 minutes both dogs vomited copious amounts of mucus and frank blood. This continued for 30 minutes, after which time both animals were sacrificed for humane reasons.

In a final trial, one new male dog was given daily doses of IPA diluted to 62.49 % in water via gelatin capsule for five days. The dosage (19.43 mg/kg bw/day) was calculated to be equivalent to the amount of IPA that would be present in a dosage of 75 mg/kg bw/day of MON 0139. The dog was fasted prior to and fed after dosing. The dog was sacrificed and necropsied the day after the last dose.

Table 5.3.1-37: Range Finding Study of MON 0139 and Isopropylamine Administered Orally to Dogs ([redacted], 1982): Study design

Dose	Number of doses	Dogs	Observation period
Mon 0139			
2500 mg/kg bw	1	1♂[a], 1 ♀[a]	3 days
1250 mg/kg bw	1	1♂[a], 1 ♀[a]	4 days
1250 mg/kg bw	1	1♂[a], 1 ♀[a]	2 days
625 mg/kg bw	1	1♂[a], 1 ♀[a]	3 weeks
625 mg/kg bw/day	5	1♂[b], 1 ♀[b]	3 weeks
625 mg/kg bw/day	5	1♂[c], 1 ♀[c]	2 weeks
312.5 mg/kg bw/day	5	1♂[d], 1 ♀[d]	3 weeks

Table 5.3.1-37: Range Finding Study of MON 0139 and Isopropylamine Administered Orally to Dogs ([redacted] 1982): Study design

Dose	Number of doses	Dogs	Observation period
Mon 0139			
Isopropylamine			
72 mg/kg bw	1	1♂[d], 1 ♀[d]	35 minutes (dogs sacrificed in extremis)
19.43 mg/kg bw/day	5	1♂[e]	5 days

Superscripts a, b, c, d indicate the same individual animals. For example, animals with superscript "a" received several different dose levels of the substance during the study.

Mortality
Each animal was observed a minimum of twice daily.

Clinical observations
Each animal was observed a minimum of twice daily.

Body weight
Animals were weighed on each day of dosing and weekly thereafter.

Food consumption and utilisation
Individual food consumption was estimated usually daily.

Ophthalmoscopic examination
Ophthalmoscopy was not performed.

Haematology and clinical chemistry
Haematological and clinical chemistry evaluations were not performed.

Urinalysis/Faecal analysis
Urine and faecal analyses were not performed.

Sacrifice and pathology
All animals were killed at the termination of their portion of the study and were necropsied. Survivors were fasted overnight prior to sacrifice in order to facilitate gross observation of intestinal and gastric organs. No organs were weighed. Only one kidney and two lymph nodes were retained but not examined microscopically.

Statistics
Statistical evaluation of the data was not performed since there were no more than two animals/dose/sex in a group.

II. RESULTS AND DISCUSSION

A. MORTALITY
Animals receiving MON 0139 at all doses survived to the end of the observation period. 2 dogs that received IPA alone, 72 mg/kg, (Equimolar to 312.5 mg/kg MON 0139) were killed in extremis within 30 minutes of dosing.

B. CLINICAL OBSERVATIONS
The first pair of dogs that received single doses of 2500 mg/kg bw of MON 0139 vomited approximately 30 minutes post dosing; the male's vomitus was clear with mucus present; the female's contained moist food

and mucus. The same two dogs given 1250 mg/kg bw of MON 0139 vomited, the female approximately 50 minutes post dosing and the male 2 hours post dosing. There were no other indications of toxicity for the following four days. This pair was again dosed with 1250 mg/kg bw of MON 0139 which led to vomiting about one hour post dosing. The female vomited and had diarrhoea the next day and the male did likewise two days after dosing. This pair of dogs, after receiving 625 mg/kg bw did not vomit but the male had loose stools later in the day and on the following day. The male had one occurrence of diarrhoea during the final week before sacrifice.

For the second pair of dogs dosed with 625 mg/kg bw/day of Mon 0139 for 5 days, both dogs vomited material, presumably food, test material and gelatin. This usually occurred within 30 minutes after dosing. The male vomited each day of dosing, the female on the second and third days only. The female also had diarrhoea the last three days of dosing and the fourth day's stool had a greenish, mucoid appearance. The female had diarrhoea twice more during the observation period.

The third pair of dogs that received 625 mg/kg bw/day of MON-0139 for 5 days, also showed emesis. Both dogs vomited bluish material which usually occurred within 30 minutes after dosing. Diarrhoea occurred in both dogs on one day the week following treatment. The bluish vomitus colour and green stools observed for these animals was considered due to the tracking food colour added to the dose.

The fourth pair of dogs dosed at 312.5 mg/kg bw/day of MON-0139 for 5 days did not vomit at any time. The female at this dose level had diarrhoea on the second and third day of dosing and both dogs had green stools, probably due to the food colouring.

Both dogs dosed with IPA at 72 mg/kg bw as a single dose vomited copious amounts of mucus and frank blood within 5 minutes of dosing. This continued for 30 minutes, after which time it was deemed desirable to humanely sacrifice both animals.

The final male dog given daily doses of IPA at 19.43 mg/kg bw/day (diluted to 62.49 % in water) for 5 days vomited each of the first three days of dosing, with blood in the vomitus on days 2 and 3. Loose stool was the only observation on days 4 and 5 of dosing.

C. BODY WEIGHT

For dogs dosed for 5 days at 625 mg/kg bw/day of MON 0139 and observed for 3 weeks, the male's weight was steady for two weeks then increased by 1 kg during the final week of observation. The female also gained weight, 1.2 kg during the final week. The second pair of dogs dosed for 5 days at 625 mg/kg bw/day of MON 0139, lost a small amount of weight, the male 0.2 kg and the female 0.3 kg by the fifth day. The male regained lost weight within three days following the last day of treatment. However, the female had several episodes of diarrhoea and did not regain the lost weight for 2 weeks.

D. FOOD CONSUMPTION AND TEST SUBSTANCE INTAKE

For the first pair of dogs that received single doses of 2500 mg/kg bw of MON 0139, the male dog consumed all his food the 2 days following dosing; the female ate approximately one-fourth to one-third of her ration the following day and all of it the second day after dosing.

The pair of dogs given 625 mg/kg bw/day of MON 0139 for 5 days and observed for 3 weeks ate an average of one-half of their food during the 5 days of treatment. Both dogs' appetites improved; they ate an average of nearly three-fourths of their rations the second week and all of them the third week. The other pair of dogs given 625 mg/kg bw/day of MON-0139 for 5 days and observed for 2 weeks ate approximately 60 % of the food offered during the 5 days of treatment. The following week, the dogs consumed nearly all of their rations.

Food consumption for the pair of dogs dosed at 312.5 mg/kg bw/day of MON 0139 for 5 days averaged nearly 80 % of the food offered. The following week, the dogs consumed nearly all of their rations.

The final male dog on this study given daily doses of 19.43 mg/kg bw/day of IPA (diluted to 62.49 % in water) for 5 days daily ate all of the food offered to him.

E. OPHTHALMOSCOPIC EXAMINATION
Not performed.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY
Haematology
Not performed.

Blood clinical chemistry
Not performed.

G. URINALYSIS/FAECAL ANALYSIS
Not performed.

H. NECROPSY
Organ weights
Organ weights were not determined.

Gross pathology
For the pair of dogs dosed once with 625 mg/kg bw of MON 0139 and observed for 3 weeks, neither of the animals had any gross lesions at necropsy.

For dogs dosed at 625 mg/kg bw/day for 5 days and observed for 3 weeks, gross necropsy revealed no lesions for either dog.

For dogs dosed at 625 mg/kg bw/day for 5 days and observed for 2 weeks, gross necropsy revealed no lesions in the female animal. The male dog had dark areas, approximately 0.5 to 1 cm in diameter, in both submandibular lymph nodes.

No gross lesions were reported for the pair of dogs dosed at 312.5 mg/kg bw/day of MON 0139 for 5 days.

The pair of dogs dosed once with 75 mg/kg bw of IPA via capsule and humanely sacrificed after 30 minutes showed severe oedema, haemorrhage, and necrosis of the rugae of the stomach at necropsy.

The final male dog on this study given daily doses of 19.43 mg/kg bw/day of IPA (diluted to 62.49 % in water) for 5 days showed diffuse, eroded areas in the mucosal layer of the cardiac portion of the stomach, and two focal erosions were seen in the oesophageal mucosa at gross necropsy the day following the last treatment.

Histopathology
Not performed.

III. CONCLUSIONS

Five male and four female purebred beagle dogs were administered MON 0139 and/or isopropylamine (IPA) by gavage or gelatin capsules. Dosages of MON 0139 ranged from 312.5 to 2500 mg/kg bw/day in single doses or daily doses for 5 days. No animals died, and all dogs were killed and necropsied after varying observation periods. Mild weight loss and reduced food consumption occurred on and shortly after treatment days with MON 0139, however, both effects were reversible. Diarrhoea was seen at all dose levels of MON 0139 and emesis at all but the lowest dose. Two dose levels of IPA were given: 72 mg/kg bw as a single treatment to a pair of dogs, and 19.43 mg/kg bw/day for five days to a single dog. Emesis, bloody emesis and loose stools were observed. IPA treatment resulted in severe oedema, haemorrhage, and

necrosis of the rugae in the stomachs of the higher dose level dogs. Mucosal erosions of the stomach and oesophagus were observed in the lower level dog. A no-effect level was not established for MON 0139 in this study; however, a dosage level of 312.5 mg/kg bw/day was determined that was non-emetic. A preliminary evaluation of the toxicity of IPA was accomplished.

3. Assessment and conclusion

Assessment and conclusion by applicant:

This study was a dose range-finding study conducted in a small number of dogs performed primarily to determine an oral dose of MON 0139 that could be tolerated by dogs to provide data for setting doses for subsequent repeated-dose studies in this species. In addition, preliminary comparative information was obtained on the toxicity of MON 0139 (the isopropylamine salt of glyphosate) and of isopropylamine (IPA) alone administered orally to dogs.

Individual dogs of each sex were administered several dose levels of MON 0139 and/or isopropylamine (IPA) once or daily for 5 days. Dosing was by gavage or gelatin capsules with varying regimens of fasting and feeding before and after dosing to try to control emesis. Dosages of MON 0139 ranged from 312.5 to 2500 mg/kg bw/day in single doses or daily doses for 5 days.

No animals receiving MON 0139 died. Mild body weight loss and reduced food consumption occurred on and shortly after treatment days with MON 0139, however, both effects were reversible. Diarrhoea was seen at all dose levels of MON 0139 and emesis at all but the lowest dose. Two dose levels of IPA were given: 72 mg/kg bw as a single treatment to a pair of dogs, and 19.43 mg/kg bw/day for five days to a single dog. Emesis, bloody emesis and loose stools were observed. IPA treatment resulted in severe oedema, haemorrhage, and necrosis of the rugae in the stomachs of the higher dose level dogs (these dogs were sacrificed *in extremis* on humane grounds 30 minutes after dosing). Mucosal erosions of the stomach and oesophagus were observed in the lower IPA dose level dog.

A NOAEL was not established for MON 0139 in this study; however, a dosage level of 312.5 mg/kg bw/day was determined as non-emetic.

Assessment and conclusion by RMS:

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Publications on oral 28–day toxicity

1. Information on the study

Data point:	CA 5.3.1/009
Report author	Gao, H. *et al.*
Report year	2019
Report title	Activation of the N-methyl-D-aspartate receptor is involved in glyphosate-induced renal proximal tubule cell apoptosis
Document No	doi.org/10.1002/jat.3795 E-ISSN: 1099-1263
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary of the study according to OECD format

Glyphosate-based herbicides have been used worldwide for decades and have been suggested to induce nephrotoxicity, but the underlying mechanism is not yet clear. In this study, a human renal proximal tubule cell line (HK-2) was treated with glyphosate for 24 hours at concentrations of 0, 20, 40 and 60 µM. A cell culture model and an animal exposure model were used to investigate the influences of glyphosate on renal proximal tubule cells and the potential role of NMDAR in response to glyphosate exposure.

Materials and methods

Chemicals – Glyphosate monoisopropylamine salt (purity 96 %), as a 40 % w/w solution in water, purchased from Millipore Sigma, St. Louis, USA.

Cell culture and treatment - The human renal proximal tubular epithelial cell line HK-2 was obtained from the American Type Culture Collection. The cells were cultured in RPMI 1640 medium supplemented with 10 % foetal bovine serum and 1 % penicillin/streptomycin in a humidified incubator with 5 % CO_2 at 37 °C. Glyphosate monoisopropylamine salt solution (40 % w/w in water) was used to prepare a glyphosate stock solution (200 mM), which was then was diluted in complete medium to the final concentrations. HK-2 cells were exposed to glyphosate at 0, 20, 40, 50, 60, 70, 80, 90 or 100 µM for 24 hours. For intervention experiments, HK-2 cells were pretreated with MK-801 at 100 µM for 12 hours, BAPTA-AM at 2 µM for 12 hours or NAC at 2 mM for 12 hours followed by treatment with glyphosate at 40 µM.

Cytotoxicity assays - Cell viability and death were evaluated by Cell Counting Kit-8 (CCK-8) and lactate dehydrogenase (LDH) cytotoxicity assay kit following the manufacturer's instructions. To determine cell viability, HK-2 cells were seeded into a 96-well plate (1×10^4 cells/mL) and then exposed to various concentrations of glyphosate for 24 hours. Cell-free medium and cells treated with water served as the blank and solvent controls, respectively. A mixture of 10 µL of CCK-8 solution and 90 µL of culture medium was added to each well and then incubated at 37 °C for 2 hours. Optical density at 450 nm was measured with a Synergy™ HT Microplate Reader. During the detection of glyphosate cytotoxicity, cell supernatants from each well were collected and then incubated with the LDH assay solutions for 30 minutes at 25 °C. Cell-free medium and cells treated with water served as the blank and solvent controls, respectively. Cells treated with lysis buffer and lysis buffer alone served as the positive and positive blank controls, respectively. Optical density at 490 nm was measured using a Synergy™ HT Microplate Reader.

Annexin V-fluorescein isothiocyanate/propidium iodide apoptosis assay - Cell apoptosis was measured using an Annexin V-fluorescein isothiocyanate apoptosis detection kit. Glyphosate- or vehicle-treated HK-2 cells were harvested and suspended in binding buffer. Annexin V-fluorescein isothiocyanate (5 µL) was added to the cells, followed by the addition of propidium iodide (PI, 5 µL). Water served as the solvent control, and apoptosis inducer A (Apopida) in the Apoptosis Inducer Kit served as the positive control. Cells without Annexin V and PI were used as negative controls. Subsequently, the cells were labeled for 15 minutes at 37 °C. The fluorescence intensity of Annexin V and PI was recorded using a flow cytometer. Data from 10,000 events per sample were analysed using FlowJo™ software.

Cellular reactive oxygen species measurement - Intracellular ROS production was measured by 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) using flow cytometry. HK-2 cells were incubated with 100 nM DCFH-DA for 30 minutes at 37 °C. Cells were then harvested and resuspended in basal medium. Water and hydrogen peroxide were used as the solvent and positive controls, respectively. Cells without the DCFH-DA probe were used as a negative control. The fluorescence intensity of 3×10^4 cells per sample was acquired using a flow cytometer at an excitation wavelength of 488 nm and an emission wavelength of 530 nm. Data were analysed using FlowJo™ software.

Detection of intracellular Ca^{2+} levels -
Intracellular Ca^{2+} concentration was analysed using a Fluo-4/AM fluorescent probe. The cells were incubated with 2 µM Fluo-4/AM in Hanks' balanced salt solution at 37 °C for 30 minutes and then suspended in Hanks' balanced salt solution and incubated at 37 °C for an additional 20 minutes. H_2O and ionomycin (5 µM) were used as the solvent and positive controls, respectively. Cells without Fluo-4/AM

probe were used as a negative control. Cell analysis was performed on a flow cytometer at an excitation wavelength of 488 nm and an emission wavelength of 525 nm. The $[Ca^{2+}]_i$ value is represented by the mean fluorescent intensity.

Western blotting - Untreated and treated cells were washed three times with ice-cold PBS and then lysed by RIPA lysis buffer containing 1 % protease inhibitors. The cell lysate was centrifuged and the supernatant was collected. The total cellular protein concentration was determined using a BCA assay kit. Equal amounts of total proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis through a 12 % gel and then transferred to polyvinylidene difluoride membranes. The membranes were blocked with 5 % nonfat milk in PBS + Tween 20 (pH 7.5) at room temperature for 2 hours. Subsequently, the membranes were washed with PBS + Tween 20 and incubated with primary antibodies against human NMDAR1, Bcl-2, Bax, Bcl-xl, Bad, cleaved caspase-3 or β-tubulin overnight at 4 °C. The membranes were then incubated with antirabbit horseradish peroxidase-conjugated IgG antibodies for 2 hours at room temperature. The protein bands were visualized using an ECL detection kit. β- Tubulin served as an internal loading control.

Animals and treatment - Adult male ICR mice (aged 8 weeks) were obtained from the Shanghai Jiesijie Laboratory Animal Company (Shanghai, China) with license number SCXK (Hu)2013-0006. The mice were provided food and water ad libitum and maintained in a controlled environment at a temperature of 24 ± 1 °C, humidity of 45 ± 5 % and a 12-hour light/dark cycle. The animals were randomly assigned to the control group or the glyphosate exposure group (6 mice per group). The mice in the glyphosate exposure group received 400 mg/kg bw/day glyphosate via oral gavage once per day for a period of 28 days. The mice in the control group received distilled water. Body weight and food intake were measured daily. Urine was collected once a week using metabolic cages. At the end of the exposure period, blood was collected from the orbital venous plexus under anesthesia to prepare serum. Then, the animals were sacrificed and kidneys excised and washed with saline. The kidney samples were fixed by immersion in a 4 % paraformaldehyde solution for 24 hours at 4 °C for histology, immunohistochemistry and TUNEL examinations. The remaining samples were snap-frozen in liquid nitrogen and maintained at −80 °C for subsequent laboratory analysis.

Urine biomarker measurement - Urine and serum were frozen and stored at −80 °C immediately after collection and centrifugation. Urine creatinine, uric acid, urea and serum creatinine levels were determined using the respective assay kits. Urine β2-microglobulin and albumin levels were measured using enzyme-linked immunosorbent assay kits.

Superoxide dismutase, catalase, glutathione peroxidase and malondialdehyde measurements - At the end of glyphosate treatment, the culture supernatants of treated and untreated HK-2 cells for the *in vitro* experiment or renal tissue for the *in vivo* experiment were freshly collected on ice for measurements of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and malondialdehyde (MDA) activities using the corresponding assay kits. SOD activity was determined according to the xanthine oxidase method, CAT activity was determined using the colorimetric method, GSH-Px activity was determined according to the ultraviolet spectrophotometric method, and MDA levels were determined using the thiobarbituric acid method. All experiments were performed following the manufacturer's instructions.

Kidney histopathology – Kidney tissue fixed for histopathological examination was dehydrated and embedded in paraffin. Tissue sections of 5 μm were mounted on poly-L-lysine-coated slides, deparaffinised with xylene, and stained with hematoxylin and eosin. A trained pathologist blinded to the treatments evaluated the tissue slides using an optical microscope.

Immunohistochemistry - Because the chemical structure of glyphosate and that of its metabolite AMPA are similar to glycine and glutamate, which are agonists of the N-methyl-D-aspartate receptor (NMDAR), the potential role of the NMDAR pathway in mediating the proapoptotic effect of glyphosate on proximal tubule cells was investigated. The kidney samples fixed for immunohistochemical examination were

dehydrated, embedded in paraffin and cut in 5-µm thick slices. The slides were then deparaffinised and incubated with rabbit monoclonal anti-NMDAR1 primary antibodies at 4 °C overnight, washed in PBS and incubated with goat antirabbit IgG-horseradish peroxidase secondary antibodies for 50 minutes at room temperature. Next, 50 mL of 3,3'- diaminobenzidine was added to each kidney section, which was stained for 5 minutes. After the slides were washed, they were counterstained with hematoxylin for 3 minutes. The slides were then mounted and examined under a microscope. NR1 expression was quantified using Image-Pro Plus 6.0 software. Two negative controls were used: PBS treatment in place of primary antibody and an isotype-matched nonspecific antibody (normal rabbit IgG). Brain tissue from mice was used as a positive control.

TUNEL assay - Apoptotic cells were detected with a TUNEL assay kit according to the manufacturer's instructions. 4'-6-diamidino-2-phenylindole was used for counterstaining. Kidney tissue samples fixed for TUNEL examination were dehydrated, embedded in paraffin and cut in tissue sections of 5 µm. The sections were then deparaffinised, rehydrated and treated with Protease K, after which they were incubated with the TUNEL reaction mixture in a humidified chamber at 37 °C for 2 hours. The sections were washed with PBS (pH 7.4) to terminate the reaction and then treated with 4'-6-diamidino-2-phenylindole. Sections treated with DNase before TUNEL examination served as a positive control, and sections without terminal deoxynucleotidyl transferase were used as a negative control. From each section, 5 randomly selected fields (200 × magnification) were photographed with a fluorescence microscope. The number of TUNEL-positive cells in each field was counted using Image-Pro Plus 6.0 software and divided by the field area. The average apoptotic cell density of the 5 fields was then obtained for each group.

Results

Cytotoxicity and apoptosis in HK-2 cells – Glyphosate reduced statistically significantly the number of viable cells at ≥ 40 µM and increased LDH release from 40 µM on. In addition, the cytotoxic effect of glyphosate on HK-2 cells was found to be dose- and time-dependent upon prolongation of the exposure times. Cell viability of both the 40 and 60 µM exposure groups was reduced but still greater than 50 % after 24 hours of exposure. Therefore, subsequent experiments were conducted with 0, 20, 40 and 60 µM with 24-hour exposure periods. In comparison with the control group, the glyphosate exposure groups showed increased percentages of apoptotic cells (Annexin V+, PI+/−), with significant differences occurring at concentrations greater than 40 µM. Furthermore, the expression of apoptosis-related proteins was investigated using Western blotting where in comparison to the control group glyphosate exhibited upregulated proapoptotic proteins (Bax and Bad) at 20 and 40 µM while downregulated antiapoptotic proteins (Bcl-2 and Bcl-xl) were evident at 40 and 60 µM. Cleaved caspase-3 levels were significantly upregulated at 40 and 60 µM.

Oxidative stress, NMDAR1 expression and calcium influx in HK-2 cells - In comparison with the control group, glyphosate increased statistically significantly cellular ROS levels at 40 and 60 µM. SOD, CAT and GSH-Px levels were statistically significantly reduced whereas there was a statistically significant increase in the MDA level. Glyphosate exposure increased NMDAR1 expression in a dose-dependent manner. Because NMDAR is involved in calcium influx and calcium homeostasis, $[Ca^{2+}]_i$ levels were determined after glyphosate exposure using flow cytometry-based measurement with the Ca^{2+}- sensitive probe Fluo-4/AM. It was found that glyphosate exposure increased $[Ca^{2+}]_i$ levels.

Effects after pretreatment with MK-801 - To examine the role of NMDAR in glyphosate-induced calcium influx and apoptosis, HK-2 cells were pretreated with the NMDAR inhibitor MK-801 at 100 µM for 12 hours followed by treatment with 40 µM glyphosate for 24 hours. MK-801 attenuated the upregulation of $[Ca^{2+}]_i$ in the HK-2 cells after 24 hours of glyphosate treatment. Inhibition of NMDAR was found to attenuate ROS increase in these cells and significantly decrease glyphosate-induced apoptosis.

Effects after pretreatment with BAPTA-AM and N-acetylcysteine - To examine the relationship between glyphosate-induced calcium influx and apoptosis, HK-2 cells were pretreated with 2 µM of an intracellular calcium chelator (BAPTA-AM) for 12 hours to decrease $[Ca^{2+}]_i$. Cells pretreated with BAPTA-AM and exposed to 40 µM glyphosate had lower $[Ca^{2+}]_i$ levels than cells exposed to glyphosate alone. BAPTA-AM

pretreatment also significantly decreased glyphosate-induced apoptosis and ROS. To determine the role of ROS in glyphosate-induced apoptosis of HK-2 cells, cells were pretreated with 2 mM of a ROS scavenger (N-acetyl cysteine, NAC) for 12 hours before glyphosate treatment. NAC pretreatment was found to reduce glyphosate-induced ROS levels and apoptosis.

NMDAR1 expression and kidney damage - Glyphosate was administered orally to ICR mice for 28 days at a daily dose of 400 mg/kg bw to investigate its effects on the kidney *in vivo*. No statistically significant difference was found in body weight gain and relative liver and kidney weight between the control and the glyphosate exposure groups. Histopathological examination of the kidney identified exfoliation of renal tubular cells. The TUNEL assay confirmed the increase in renal tubular cell apoptosis in mice exposed to glyphosate. No statistically significant changes were observed in urine creatinine, uric acid, urea nitrogen, serum creatinine and blood urea nitrogen levels. A transient increase in urine albumin was observed after 7 and 14 days of treatment and urinary β2- microglobulin levels were statistically significantly increased after 7, 21 and 28 days of treatment. In addition, statistically significant reductions in the levels of SOD, CAT and GSH-Px, and a statistically significant increase in MDA was observed in the kidneys of the glyphosate treated group. Besides, the average optical density for NMDAR1 was found to be increased in kidneys from glyphosate treated mice.

Discussion and conclusion

Glyphosate exposure was found to increase the production of ROS. In *in vitro* and *in vivo* it was shown that MDA levels increased and that the activities of the major endogenous antioxidant enzymes SOD, CAT and GSH-Px decreased as a result of glyphosate exposure which is indicative of the disturbance of the pro-oxidant/antioxidant balance. To understand how this balance is disturbed the role of the N-methyl-D-aspartate receptor (NMDAR) was investigated because the structure of glyphosate is similar to that of glycine and glutamate which are both agonists of this receptor. NMDAR has been reported to mediate some renal diseases, such as hyperhomocysteinemia-induced glomerulosclerosis, gentamicin nephrotoxicity and lipopolysaccharide-induced renal insufficiency. In this study, using the human renal tubular epithelial cell line HK-2, the increased expression of NMDAR1 protein was demonstrated upon glyphosate exposure. In the animal tests, a clear upregulation of NMDAR1 in renal tissue was observed in tubular epithelial cells using immunohistochemical staining. Accompanied by the increase in NMDAR1 upon glyphosate exposure, $[Ca^{2+}]_i$, oxidative stress markers and apoptosis were all increased. Blocking NMDAR not only ameliorated glyphosate-induced increases in $[Ca^{2+}]_i$ and ROS levels but also attenuated apoptosis. The increase in β2-microglobulin in the urine as observed in the *in vivo* study is indicative of an impairment of tubular reabsorption. From the results of this study it can be concluded that glyphosate could affect renal tubule epithelial cells via the NMDAR1/$[Ca^{2+}]_i$/ROS pathway both *in vitro* and *in vivo*. These findings provide a theoretical basis and reference data to assess the risk of glyphosate and to explore the ethiology of chronic kidney disease.

3. Assessment and conclusion

Assessment and conclusion by applicant:

In this study the effect of glyphosate on human proximal tubular epithelial cells was studied *in vitro* and on mouse kidney *in vivo*. Tubular epithelial cells (HK-2) *in vitro* were exposed to glyphosate at concentrations ranging from 20 to 100 µM whereas mice were orally treated with glyphosate at 400 mg/kg bw/day for 28 days. The endpoints investigated for the *in vitro* study were cell viability, apoptosis, oxidative stress, intracellular Ca^{2+}, expression of the N-methyl-D-aspartate (NMDA) receptor and expression of proteins involved in apoptosis. The endpoints explored in the *in vivo* study in mice were kidney pathology biomarkers, oxidative stress in kidney tissue, kidney histopathology, NMDA receptor immunochemistry and apoptosis in kidney tissue. Glyphosate was found to reduce cell viability, increase the incidence of apoptotic cells with an increase in the expression of apoptosis-related proteins, increase of oxidative stress in a concentration-related manner, increase of the expression of the NMDA receptor and increase Ca^{2+} influx. Kidney histopathology in mice treated with glyphosate at 400 mg/kg bw/day for 28 days revealed the exfoliation of renal tubular cells. It is postulated by the authors that glyphosate could affect renal tubule epithelial cells via the NMDAR1/$[Ca^{2+}]_i$/ROS pathway. The effects described in this study are not corroborated by regulatory 90–day repeated dose toxicity studies where no renal effects were seen in rats dosed up to more than 4000 mg/kg bw/day and mice dosed up to more than 7000 mg/kg bw/day.

Reliability criteria for *in vitro* toxicology studies

Publication: Gao *et al.*, 2019	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity of 96 % as glyphosate monoisopropylamine salt. Source: Millipore Sigma, St. Louis, USA.
Only glyphosate acid or one of its salts is the tested substance	Y	
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	Y	
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	Not reported	
Test concentrations in physiologically acceptable range (< 1 mM)	Y	Concentration range *in vitro* from 20 to 100 µM. Only one dose (400 mg/kg bw/day) was used in the oral toxicity study in mice.
Cytotoxicity tests reported	Y	
Biochemical methods described	Y?	Some could be better documented.
Positive and negative controls	Y	
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	

Reliability criteria for *in vitro* toxicology studies

Publication: Gao *et al.*, 2019	Criteria met? Y/N/?	Comments
Dose-effect relationship reported	Y	*In vitro* but not *in vivo* (only one dose used).
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Reliability not assignable		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because some of the biochemical methods are not sufficiently described, only one dose was used in the *in vivo* study and the pathology results from the *in vivo* study are not corroborated by regulatory 90–day repeated dose toxicity studies where no renal effects were seen in rats dosed up to more than 4000 mg/kg bw/day and mice dosed up to more than 7000 mg/kg bw/day.		

1. Information on the study

Data point:	CA 5.3.1/010
Report author	Milic, M. *et al.*
Report year	2018
Report title	Oxidative stress, cholinesterase activity, and DNA damage in the liver, whole blood, and plasma of Wistar rats following a 28–day exposure to glyphosate
Document No	DOI: 10.2478/aiht-2018-69-3114 E-ISSN: 1848-6312
Guidelines followed in study	None
Deviations from current test guideline	No
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary of the study according to OECD format

In this 28 day-study, the effects of the herbicide glyphosate administered by gavage to Wistar rats at daily doses equivalent to 0.1 of the acceptable operator exposure level (AOEL), 0.5 of the consumer acceptable daily intake (ADI), 1.75 (corresponding to the chronic population-adjusted dose, cPAD), and 10 mg kg-1 body weight (bw) (corresponding to 100 times the AOEL) were evaluated. At the end of each treatment, the body and liver weights were measured and compared with their baseline values. DNA damage in leukocytes and liver tissue was estimated with the alkaline comet assay. Oxidative stress was evaluated using a battery of endpoints to establish lipid peroxidation via thiobarbituric reactive substances (TBARS) level, level of reactive oxygen species (ROS), glutathione (GSH) level, and the activity of glutathione peroxidase (GSH-Px). Total cholinesterase activity and the activities of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) were also measured. The exposed animals gained less weight than control. Treatment resulted in significantly higher primary DNA damage in the liver cells and leukocytes. Glyphosate exposure significantly lowered TBARS in the liver of the AOEL, ADI, and cPAD groups, and in plasma in the AOEL and cPAD group. AChE was inhibited with all treatments, but the AOEL and ADI groups significantly differed from control. Total ChE and plasma/liver ROS/GSH levels did not significantly differ from control, except for the 35 % decrease in ChE in the AOEL and ADI groups and a significant drop in liver GSH in the cPAD and 100xAOEL groups. AOEL and ADI blood GSH-Px activity

dropped significantly, but in the liver it significantly increased in the ADI, cPAD, and 100xAOEL groups vs. control.

Materials and methods

Chemicals - Glyphosate (purity of ≤100 %), purchased under the brand name PESTANAL®, a registered trademark of Sigma-Aldrich Laborchemikalien GmbH, Germany.

Animals - Thirty male Wistar rats were obtained from the breeding unit at IMROH and were kept under pathogen-free, steady-state microenvironmental conditions in polycarbonate cages with 40-60 % humidity at 22 °C and normal 12-hour light/dark cycle. The animals had free access to standard Good Laboratory Practice-certified food and tap water. At 3 months of age, the rats were weighted, inspected, and judged to be healthy and fit for the experiment by a licensed veterinarian at IMROH.

Experimental design - Rats were randomly divided into 6 dose groups of 5 animals each and treated orally by gavage for 28 days. Glyphosate was tested at 0.1, 0.5, 1.75 and 10 mg/kg bw/day, doses corresponding respectively with the AOEL from EFSA, the ADI, the chronic population-adjusted dose (cPAD) from EPA and 100 times the AOEL. Ethyl methanesulfonate (EMS) dissolved in PBS served as the positive control and was administered at 300 mg/kg bw over the last 3 days of the experiment. PBS served as the negative control. Body weight was monitored once a week and the glyphosate doses adjusted accordingly. Survival and clinical signs of toxicity were monitored on a daily basis. All animals were humanely sacrificed and dissected on day 29, 24 hours after the last dose. All animals were subjected to necropsy and examined for gross pathological changes.

Sample collection and preparation - Liver was excised, rinsed with cold PBS (pH 7.4) and weighed. Livers were then washed in cold TBS buffer (pH 7.5) to remove as much blood as possible, homogenised in a 50 mM potassium PBS (pH 7.4) with 1 mM EDTA, and centrifuged at 20,000 x g at 4°C for 30 minutes to obtain the supernatant. In a separate procedure, a small portion of the liver tissue was minced in a chilled mincing solution to obtain a cellular suspension. The cell suspension was left a few seconds for large clumps to settle, and the supernatant was used to prepare comet slides. This was performed within 60 minutes from sacrifice to avoid confounding necrotic changes. Blood samples were collected directly from the heart into heparinised vacutainers with an extra addition of Li-heparin and mixed vigorously. Samples were then kept at 4°C pending further processing. Plasma for the biochemical assays was prepared by centrifugation of heparinized blood at 976 x g at 4°C for 10 minutes and kept at -20°C pending analysis.

Alkaline comet assay in leucocytes and liver cells - Two microgels were prepared per tissue per animal. Slides were marked with a randomly generated code. For each slide, an aliquot of 10 µL of the cell suspension was mixed with low melting point agarose (LMPA, 0.5 %) dissolved in TBS buffer. "Sandwich" agarose microgels made of four layers were prepared on microscopic slides. Slides were pre-coated with 1 % normal melting point agarose (NMPA) and air-dried. The second gel layer of 0.6 % NMPA was then applied. The third layer consisted of 0.5 % LMPA mixed with heparinized whole blood (10 µL per slide) or 10 µL of liver cell suspension per slide. Finally, 0.5 % LMPA was applied as the top layer over the gel-embedded cells. After solidification of the gel on ice-cold metal tray, the slides were submerged in freshly prepared cold lysing solution (pH 10) at 4°C overnight. The slides were quickly washed with distilled water and left in a vertical Coplin jar with chilled electrophoresis buffer (pH >13) at 4°C for 10 minutes. The slides were then transferred into a horizontal electrophoresis unit. After electrophoresis, the slides were washed 3 times with neutralisation buffer (pH 7.5). All gels were dehydrated with 70 % and 96 % ethanol, respectively, air dried, and stored at room temperature. Before scoring, the slides were stained with ethidium bromide (20 µg/mL) and analysed with a fluorescent microscope at 200x magnification using the Comet Assay IV image analysis system equipped with appropriate filters. Three hundred cells (2 x 150 nucleoids) were scored in total for each animal and sample. Medium-sized cells (parenchymal cells or hepatocytes, between 30 and 40 µm of head length) and small-sized cells (non-parenchymal cells, <30 µm of head length) were recorded separately. Areas near slide margins were not scored. DNA damage was measured as comet DNA tail intensity (% of DNA in tail) and tail length (TL, expressed in µm, measured from the estimated edge of the comet head). Comets with small or non-existing head and large, diffuse tails

(cells with >80 % DNA in the tail) were excluded from analysis. The frequency of such comets ("hedgehogs" or "clouds") was determined based on visual scoring among 100 nucleoids per sample.

Determination of ROS levels in plasma and liver - ROS levels in plasma and liver homogenates were measured using 2′,7′-dichlorofluorescein diacetate (DCF-DA). The acetate group of DCFH-DA gets DCFH-DA into the cells or organelles, and once inside, it is removed by cellular esterases, producing reduced DCFH which then can be oxidised by peroxides to form fluorescent oxidised DCF that can be measured spectrophotometrically. Plasma samples and 1 % liver tissue homogenate were prepared by dilution with ice-cold PBS (pH 7.4). Black 96-well plates were filled with 0.07 mL of PBS, and 0.03 mL of 1 % liver tissue homogenate or with 0.1 mL of 10 % plasma in quintuplicate for each glyphosate concentration and sample type. 20 µL of 0.12 mM DCFH-DA dye in PBS was then added to each well, and the plates were incubated at 37°C for 30 minutes. Control for dye autofluorescence was prepared without the addition of dye. Control samples were included in each experiment. Samples were analysed using a Victor3™ multilabel plate reader at an excitation wavelength of 485 nm and emission wavelength of 535 nm. The readings were expressed as relative fluorescence units (RFU).

Determination of lipid peroxidation in plasma and liver - The concentration of thiobarbituric reactive substances (TBARS), as a measure of lipid peroxidation, was determined. 5 µL of butylated hydroxytoluene (BHT, 0.2 % w/v) and 750 µL of phosphoric acid (1 % v/v) were added to 50 µL of sample. After mixing, 250 µL of thiobarbituric acid (TBA, 0.6 % w/w) and 445 µL of water were added, and the reaction mixture was incubated at 90°C for 30 minutes. The mixture was cooled, and absorbance measured at 532 nm using a Shimadzu UV probe spectrophotometer. TBARS concentrations were calculated using the standard curves for 1,1,3,3-tetramethoxypropane, obtained by increasing its concentrations, and expressed as µM.

Determination of GSH in plasma and liver - GSH levels were analysed with a fluorogenic bimane probe using monochlorobimane (MBCl), which reacts specifically with GSH to form a fluorescent adduct. Plasma samples and liver tissue homogenates were prepared as previously described for ROS determination. 20 µL of 0.24 mM MBCl dye in PBS was then added to react at 37 °C for 20 minutes. The amount of GSH in the samples was analysed using a Victor3™ multilabel plate reader at an excitation wavelength of 355 nm and emission wavelength of 460 nm. Control samples were included in each experiment. The readings were expressed as relative fluorescence units (RFU). Each sample analysis was performed in quintuplicate.

Determination of GSH-Px activity in whole blood and liver - GSH-Px activity in whole blood and in the supernatant of liver homogenate were determined in accordance with the European standardised method. The amount of GSH oxidised by t-butyl hydroperoxide was determined based on the decrease in β-NADPH absorbance at 340 nm, measured by spectrophotometry. One unit of GSH-Px was the number of micromoles of β-NADPH oxidised per minute. Its activity in whole blood was expressed per gram of haemoglobin (U g_{Hb}^{-1}), and in liver homogenate per gram of total protein (U $g_{protein}^{-1}$).

Protein quantification - Proteins were quantified according to the method of Bradford, using bovine serum albumin as the standard.

Cholinesterase activity in plasma - Plasma samples were analysed for total ChE, AChE, and BChE activities in a 0.1 M sodium phosphate buffer (pH 7.4) at 25°C using ATCh (1.0 mM) and DTNB (0.3 mM) as described by Ellman *et al*. To distinguish between AChE and BChE activities the BChE-selective inhibitor ethopropazine (20 µM) was used. Increase in absorbance was monitored at 412 nm over 4 minutes by means of a Cecil 9000 spectrophotometer. Enzyme activities were expressed as IU g/protein.

Statistical analysis - Statistical analysis was run on Dell Statistica software STATISTICA, version 13.2. The data were first evaluated with descriptive statistics. The results were expressed as means ± standard deviation, and for the comet assay also medians and ranges (min-max) were used. Relative liver weights were logarithmically transformed and analysed with one-way ANOVA. For pairwise organ comparison the *post-hoc* Tukey's HSD test was used. Normality was tested with the Levene's test. Since the results of the alkaline comet assay were not normally distributed even after logarithmic transformation, the non-

parametric Mann-Whitney U test was used. For multiple comparisons of cholinesterase activities, TBARS, and GSH-Px activities between the glyphosate and control groups the Kruskal-Wallis test was used. ROS and GSH levels were compared between the groups using the non-parametric Mann-Whitney U test. P values of ≤ 0.05 were considered statistically significant.

Results and Discussion

Changes in body and liver weight - No premature death or any clinical signs of systemic toxicity in male adult Wistar rats was observed in any of the groups dosed with glyphosate for 28 days. Gross necropsy did not reveal any treatment-related findings. All glyphosate-treated animals showed a similar weight gain throughout the 28–day treatment period, with significant difference between the day before treatment and at the end of treatment. Compared to the negative control animals, the glyphosate-treated animals gained considerably less body weight. There were no statistically significant changes in relative liver weight.

Alkaline comet assay in leucocytes and liver cells - Glyphosate-treated rats had higher primary DNA damage in leukocytes when compared to control in tail length and tail intensity. While tail length was significantly greater at all dose levels, only the lowest dose tested resulted in significantly higher mean tail intensity. One reason for that could be high standard deviations. It is worth noting that glyphosate caused greater DNA damage in liver cells than in the leukocytes. The greatest liver cell DNA damage (as tail intensity) occurred at the lowest and the highest dose both in parenchymal and non-parenchymal liver cells. In fact, the group exposed at 10 mg/kg bw/day suffered even greater damage in medium-sized liver cells than the positive control.

ROS levels in plasma and liver - No statistically significant difference was found in ROS levels in plasma between the glyphosate dose groups and control. A small decrease in ROS of around 7 % was seen in the groups with the highest glyphosate doses. Liver tissue showed a similar pattern with a decrease in ROS at the highest dose levels which was statistically significant at 10 mg/kg bw/day. ROS levels in the liver were 100 times greater than in the plasma.

Lipid peroxidation in plasma and liver - TBARS concentrations in plasma and liver were decreased in all glyphosate-dosed groups when compared to control.

GSH levels in plasma and liver – In plasma, no statistically significant difference was evident between the glyphosate-dosed groups and the control group. In the liver, however, a statistically significant decrease in GSH was seen in the two highest dose groups.

GSH-Px activity in whole blood and liver - GSH-Px activity in whole blood was statistically significantly decreased at 0.5 and 1.75 mg/kg bw/day but not at 10 mg/kg bw/day. In contrast, GSH-Px activity in the liver was statistically significantly increased at 0.5, 1.75 and 10 mg/kg bw/day.

Cholinesterase activity in plasma - Glyphosate did not significantly affect total cholinesterase (ChE) activity, even though there was a decrease about 35 % at 0.1 and 0.5 mg/kg bw/day when compared to control. Acetyl cholinesterase (AChE) activity in turn, was statistically significantly decreased at 0.1 and 0.5 mg/kg bw but not at the higher dose levels. There was no statistically significant change in activity of butyl cholinesterase in the glyphosate dose groups when compared to the control group.

Discussion and conclusion

This study suggests that sub-chronic exposure to glyphosate mostly affects DNA in the liver and white blood cells. General oxidative stress was not confirmed, while total cholinesterase activity showed some, but inconsistent, changes from control. Exposure to environmentally relevant levels of glyphosate, presumably not harmful to humans, seems to have different effects from exposure to much higher doses, especially where oxidative stress is concerned. In this study it has been demonstrated that even without evidence of oxidative stress, small doses allowed for human exposure can produce significant primary DNA damage and inhibit AChE, which may both be related to indirect action through glycine substitution.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Glyphosate was orally administered to male rats at 0.1, 0.5, 1.75 and 10 mg/kg bw for 28 days to investigate its effect on DNA damage, oxidative stress and cholinesterase activity. The endpoints of this study were DNA damage as measured in the alkaline comet assay, ROS in plasma and liver, lipid peroxidation in plasma and liver, GSH in plasma and liver, GSH-Px activity in whole blood and liver and total cholinesterase, acetyl cholinesterase and butyl cholinesterase activity in plasma. The results of the alkaline comet assays revealed a statistically significant increase in tail length and tail intensity in leucocytes and small and medium sized liver nuclei. With the exception of tail length of small sized liver nuclei no dose-effect relationship was evident. Tail intensity of the leucocytes could not be assessed because of the very high variability of the results. From the results of the oxidative stress markers in plasma and liver and cholinesterase activity in plasma it can be concluded that there was no dose-related effect. In summary, the results do not allow a conclusion on effects of glyphosate on DNA damage, oxidative stress and cholinesterase activity.

Reliability criteria for *in vivo* toxicology studies

Publication: Milic *et al*., 2018.	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	Y	*In vivo* Comet assay was compliant with OECD TG 489.
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity of ≤100 %). Source: PESTANAL®, a registered trademark of Sigma-Aldrich Laborchemikalien GmbH, Germany
Only glyphosate acid or one of its salts is the tested substance	Y	
AMPA is the tested substance	N	
Study		
Test species clearly and completely described	Y	
Test conditions clearly and completely described	Y	
Route and mode of administration described	Y	Oral by gavage.
Dose levels reported	Y	0.1, 0.5, 1.75 and 10 mg/kg bw/day, positive and negative control included.
Number of animals used per dose level reported	Y	5 males per dose group.
Method of analysis described for analysis test media	N	
Validation of the analytical method	N	
Analytical verifications of test media	N	
Complete reporting of effects observed	Y	Limited to body weight, organ weight, DNA damage, oxidative stress and ChE activity.
Statistical methods described	Y	
Historical control data of the laboratory reported	N	

Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Reliability not assignable		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because of the high variability of the results of the comet assay, although the conduct of this assay was in general compliant with OECD test guideline 489.		

Assessment and conclusion by RMS:
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1. Information on the study

Data point:	CA 5.3.1/011
Report author	Tang, J. *et al.*
Report year	2017
Report title	Ion imbalance Is Involved in the Mechanisms of Liver Oxidative Damage in Rats Exposed to Glyphosate
Document No	doi.org/10.3389/fphys.2017.01083 ISSN: 1664-042X
Guidelines followed in study	None
Deviations from current test guideline	No
GLP/Officially recognised testing facilities	Non-GLP
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary of the study according to OECD format

This study aimed to investigate the effects of glyphosate on rats' liver function and induction of pathological changes in ion levels and oxidative stress in hepatic tissue. Sprague-Dawley rats were treated orally with 0, 5, 50, or 500 mg/kg body weight of the glyphosate (GLP). After 5 weeks of treatment, blood and liver samples were analysed for biochemical and histomorphological parameters. The various mineral elements content in the organs of the rats were also measured. Significant decreases were shown in the weights of body, liver, kidney and spleen between the control and treatment groups. Changes also happened in the histomorphology of the liver and kidney tissue of GLP-treated rats. The GLP resulted in an elevated level of glutamic-oxalacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) and IL-1b in the serum. Besides, decreased total superoxide dismutase (T-SOD) activity and increased malondialdehyde (MDA) contents in the serum, liver, and kidney indicated the presence of oxidative stress. Moreover, increase of hydrogen peroxide (H_2O_2) level and catalase (CAT) activity in the serum and liver and decrease of glutathione (GSH) and glutathione peroxidase (GSH-Px) activity in the kidney tissue further confirmed the occurrence of oxidative stress. The results of RT-PCR showed that the mRNA expressions of IL-1α, IL-1β, IL-6, MAPK3, NF-κB, SIRT1, TNF-α, Keap1, GPX2, and Caspase-3 were significantly increased in the GLP-treated groups compared to the control group. Furthermore, PPARα, DGAT, SREBP1c, and SCD1 mRNA expressions were also remarkably increased in the GLP-treated groups compared to the control group

Materials and methods

Chemicals - Glyphosate was obtained from Shanghai Ryon Biological Technology Co. Ltd (Shanghai, China). Purity was not reported.

Animals - Male Sprague-Dawley rats of 8 weeks old and weighing 180–220 g were purchased and were allowed to acclimate for at least one week prior to testing. All rats were housed in separate cages and had unrestricted access to food and water throughout the study.

Animal treatment and sample collection - Rats were randomly assigned to 4 groups (n = 8/group) and were orally administered glyphosate by gavage at 5, 50, and 500 mg/kg bw/day for 35 days. Distilled water was used as the negative control. Twenty-four hours after the last gavage, rats were weighed and sacrificed. Blood samples were collected from the jugular vein and placed at 37 °C for one hour before being centrifuged for biochemical assays. Liver, kidney, spleen, heart, lungs, brain, adrenal glands, muscle and fat tissue were collected, rinsed with PBS, dried and weighed. A piece of liver and right kidney was used for morphometric analysis and another piece was used to prepare homogenates for the analysis of parameters of oxidative stress or frozen in liquid nitrogen for subsequent qualitative reverse transcription polymerase chain reaction (RT-PCR).

Histological preparation - Samples of liver and kidney were fixed in 4 % formaldehyde solution for 24 hours, dehydrated in alcohol, clarified with xylene, and embedded in paraffin. Paraffin blocks were sectioned into 5µm slices and stained with hematoxylin-eosin (HE) for microscopic examination.

Biochemical evaluation - Liver and kidney homogenate and serum were used for the assessment of liver function (serum GOT and GPT) and oxidative stress (total SOD, MDA, H_2O_2, CAT, GSH, and GSH-peroxidase or GSH-px). The activity of serum GOT and GPT was assayed according to the method that is normally applied in clinical biology. The analysis of total SOD activity was based on SOD-mediated inhibition of nitrite formation from hydroxyammonium in the presence of O^{2-} generators (xanthine/xanthine oxidase). Total SOD activity was expressed in U/mg protein. MDA was evaluated by the thiobarbituric acid reactive substances method (TBARS) and the results were expressed in nmol/mg protein. GSH-px activity was estimated by the determination of reduced GSH in the enzymatic reaction. GSH-px activity was expressed in U/mg protein. CAT activity was assayed by the method developed by Aebi, and calculated as nM H_2O_2 consumed/min/mg of protein. Protein concentrations in the supernatant were measured according to the Coomassie Brilliant Blue method.

Serum Cytokine Measures - Serum levels of IL-1β and IL-6 were determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit.

Quantitative RT-PCR (qRT-PCR) analysis - Total RNA was extracted from tissue using the reagent box of Total RNA Kit, according to the manufacturer's instructions. The concentration of RNA was measured by spectrophotometry and the purity was ascertained by the A 260/A 280 ratio. Total RNA from each sample was reverse transcribed to cDNA with an Omniscript® Reverse Transcription kit with Oligo-dT primers according to the manufacturer's instructions and used for RT-PCR. The target fragments were quantified by real-time PCR with 100 ng of the cDNA template. Each sample was tested in duplicate. The gene expression data were normalized to β-actin expression. For each real-time PCR assay, the threshold cycle Ct was determined for each reaction. Ct values for each gene of interest were normalized to the housekeeping gene, β-actin and PCR amplification efficiencies were taken into account by amplifying various amounts of target cDNA for each reaction. The fold differences in mRNA expression of samples were relative to the internal control sample, which was included in all runs.

Ion Concentration - The concentrations of Al, Fe, Cu, Zn, and Mg in liver, kidney, spleen, lung, heart, muscle, brain, and fat tissue were determined by inductively coupled plasma optical emission spectrometry using nitric acid–perchloric acid–based wet digestion. Approximately 200 µL or 0.5 g of each sample was digested with nitric acid (75 %) and perchloric acid (25 %) in a microwave digester. The same part of the

organ was used from the control and treated animals.

Statistical Analysis - The data were expressed as mean ± standard error of the mean (SEM) and were analysed by one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test, which was performed with GraphPad Prismsoftware. Differences were considered to be statistically significant when $p < 0.05$.

Results

Body weight and body weight gain were statistically significantly decreased at 500 mg/kg bw/day and at 50 and 500 mg/kg bw/day, respectively. Absolute and relative organ weight was statistically significantly reduced for liver, spleen and kidney at 500 mg/kg bw/day.
Liver sections of rats exposed to glyphosate showed apoptosis of some hepatocytes, focal necrosis and mononuclear cell infiltration. At 5 mg/kg bw/day rats showed mild periportal expansion and apoptosis of some hepatocytes. At 50 and 500 mg/kg bw/day apoptosis of some hepatocytes and monocyte infiltration was observed in liver tissue. In the kidney, marked histological changes were observed, including proximal and distal tubular necrosis and glomerular toxicity. The histologic score of hepatic and renal damage was statistically significantly increased at all dose levels. Serum activity of GOT and GPT was statistically significantly increased at 500 mg/kg bw/day.

Assessment of oxidative stress - In serum, total SOD activity was statistically significantly decreased at 500 mg/kg bw/day. MDA content was significantly increased at 50 mg/kg bw/day but not at 500 mg/kg bw/day and CAT activity was significantly increased at 500 mg/kg bw/day. In liver, total SOD activity was statistically significantly decreased and H_2O_2 levels increased at 500 mg/kg bw/day. In kidney, total SOD and GSH-px activities were significantly decreased at 50 and 500 mg/kg bw/day. A statistically significant decrease in GSH levels was observed at 50 mg/kg bw/day but not at 500 mg/kg bw/day.

Serum IL-1β and IL-6 levels - In serum, the level of IL-1β was statistically significantly increased at 500 mg/kg bw/day. There was no statistically significant change for IL-6.

Expression of genes related to inflammation in the Liver - Hepatic IL-1α and IL-1β mRNA expression were statistically significantly increased at all dose levels. IL-6, MAPK3, SIRT1, TNF-α, GPX2, and *Caspase*-3 mRNA expression was significantly increased at 50 and 500 mg/kg bw/day. NF-κB mRNA expression showed only a significant increase 50 mg/kg bw/day and *Keap*1 mRNA expression was only increased at 5 mg/kg bw/day.

Expression of genes related to lipid metabolism in the liver - PPARα, *SREBP1c*, and *SCD1* mRNA expression were significantly increased at 50 and 500 mg/kg bw/day and *DGAT* mRNA expression was significantly increased at 500 mg/kg bw/day.

Concentration of Al, Fe, Cu, Zn and Mg in tissues – The Al concentration was statistically significantly increased in liver at 50 and 500 mg/kg bw/day but significantly decreased in lung at 50 mg/kg bw/day and in muscle at 500 mg/kg bw/day. Fe was significantly increased at 5 mg/kg bw/day only in liver, at 50 mg/kg bw/day only in kidney and spleen and at 500 mg/kg bw/day in lung. Cu was significantly increased in brain and fat tissue at 500 mg/kg bw/day and Zn was only significantly increased in liver at 50 and 500 mg/kg bw/day. Mg levels were only increased in brain tissue at 500 mg/kg bw/day.

Discussion and Conclusions

The results of this study showed that exposure to glyphosate for 35 days at doses up to 500 mg/kg bw/day led to a statistically significant reduction in body weight and body weight gain and in absolute and relative weight of liver, kidney and spleen. Histopathological examination of the tissues also revealed effects in liver and kidneys. Liver effects were corroborated by a significant increase in GOT and GPT. The results of this study showed that SOD activity was significantly decreased in serum, liver and kidney of rats treated

with glyphosate when compared with the control group. MDA content was significantly increased in serum and kidney. CAT activity was also significantly elevated in serum and H_2O_2 levels were increased in liver tissue, suggesting oxidative stress. Taken together, the data demonstrated that glyphosate exposure could result in liver and kidney damage due to oxidative stress. The level of IL-1β was significantly increased at 500 mg/kg bw/day. Therefore, the relationship was investigated between oxidative stress and the transcription of genes related to inflammation. In this study, mRNA expression of *IL-1α, IL-1β, IL-6, MAPK3, NF-κB, SIRT1, TNF-α, Keap1, GPX2* and *Caspase-3* were all increased upon exposure to glyphosate. Genes related to lipid metabolism such as *PPARα, SREBP1c, DGAT*, and *SCD1* were significantly upregulated in rats exposed to glyphosate. The results from this study show that the liver toxicity induced by glyphosate is mediated by inflammation, oxidative stress and lipid related pathways. Tissue concentrations of Al, Fe and Zn were significantly increased in liver tissue of rats exposed to glyphosate. Concentrations of Fe were also increased in kidney, spleen, and lung tissue although not always in a dose-related manner. Al concentration was decreased in lung and muscle tissue whereas Cu concentrations were increased in brain and fat tissue and Mg in brain tissue of rats exposed to glyphosate. Combined, these results suggest that glyphosate exposure impaired the ion-balance of Al, Fe, Mg, Cu, and Zn.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The objective of this study was to investigate the toxicity, oxidative stress and metal ion concentrations in tissues of rats after oral exposure to glyphosate for 35 days at doses up to 500 mg/kg bw/day. Oxidative stress was studied by the determination of markers of oxidative stress such as SOD, CAT, H_2O_2, MDA, GSH and GSH-px and the transcription of genes related to inflammation and lipid metabolism. Statistically significant effects were found on body weight, body weight gain, organ weight, serum indicators of liver toxicity and histopathology of the liver and the kidney. Significant changes were also reported on markers of oxidative stress and transcription of genes related to inflammation and lipid metabolism. Many of the effects reported were mild in nature and/or didn't show a clear dose-effect relationship. Also the effects on metal ion concentrations in organ tissues were not always consistent and often didn't show a dose-effect relationship. Moreover, the findings are not corroborated by the regulatory studies of similar test durations and dose ranges.

Reliability criteria for *in vivo* toxicology studies		
Publication: Tang *et al*., 2017	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	Number of animals per dose level lower than minimum required for 4–week testing
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	?	Not described in sufficient detail
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	N	Only source reported: Shanghai Ryon Biological Technology Co. Ltd., China.
Only glyphosate acid or one of its salts is the tested substance	Y	
AMPA is the tested substance	N	
Study		
Test species clearly and completely described	Y	
Test conditions clearly and completely described	Y	

Reliability criteria for *in vivo* toxicology studies		
Route and mode of administration described	Y	
Dose levels reported	Y	
Number of animals used per dose level reported	Y	
Method of analysis described for analysis test media	N	
Validation of the analytical method	N	
Analytical verifications of test media	N	
Complete reporting of effects observed	Y	Sometimes inaccurate reporting of data in tables. Results not concordant with short term regulatory toxicology studies
Statistical methods described	Y	
Historical control data of the laboratory reported	N	
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Not reliable		
This publication is considered relevant but reliable with restrictions because the test material was not sufficiently characterised, the number of animals used for this study duration is not sufficient, the results were not always accurately reported and are not corroborated by regulatory toxicology studies of similar test durations and dose ranges.		

Assessment and conclusion by RMS:
.

CA 5.3.2 Oral 90–day study

No new subchronic toxicity studies by the oral route have been performed in rats, mice or dogs. Table 5.3.2-1 summarises the studies performed in rats and mice previously assessed in the 2001 and 2015 EU glyphosate evaluations.

Table 5.3.2-1: Studies on oral 90–day repeated-dose toxicity with glyphosate in rodents

Annex Point	Study	Species Study type	Substance(s)	Reference list-related category§	Result
CA 5.3.2/001 CA 5.3.2/002	[redacted] 1996 (Study Report) [redacted] 1996 (Appendix)	Alpk: APfSD rat 90–day, oral diet (0, 1000, 5000, 20000 ppm)	Glyphosate acid (Purity: 97.4 %)	Valid, Category 2a	NOAEL = 5000 ppm (equivalent to 413.5 mg/kg bw/day for males and 446.9 mg/kg bw/day for females)
CA 5.3.2/003	[redacted] 1996	Sprague-Dawley rat 90–day, oral diet (0, 1000, 10000, 50000 ppm)	Glyphosate technical (Purity: 95.3 %)	Valid, Category 2a	NO(A)EL = 1000 ppm (equivalent to 79 mg/kg bw/day for males and 90 mg/kg bw/day for females)
CA 5.3.2/004	[redacted] 1995	Sprague-Dawley (Crj:CD) rat 13-week, oral diet (0, 3000, 10000, 30000 ppm)	Glyphosate technical (Purity: 95.0 – 97.56 %)	Valid, Category 2a	NOAEL = 3000 ppm (equivalent to 168.4 mg/kg bw/day for males and 195.2 mg/kg bw/day for

Table 5.3.2-1: Studies on oral 90–day repeated-dose toxicity with glyphosate in rodents

Annex Point	Study	Species Study type	Substance(s)	Reference list-related category§	Result
					females)
CA 5.3.2/005 CA 5.3.2/006 CA 5.3.2/007	[redacted] 1993 (Vol. 1, Study Report) [redacted] 1993 (Vol. 2, Pathology Report) [redacted], 1993 (Vol. 3, Histopathology Report)	Sprague-Dawley rat 90–day, oral diet (0, 2000, 6000, 20000 ppm)	Glyphosate acid (Purity: 97.5 %)	Valid, Category 2a	NOAEL = 6000 ppm (equivalent to 371.9 mg/kg bw/day for males and 481.2 mg/kg bw/day for females)
CA 5.3.2/008 CA 5.3.2/009 CA 5.3.2/010	[redacted] 1992 (Study Report) [redacted], 1992 (Appendix) [redacted], 1994 (1st & 2nd Amendment)	Wistar rat 90–day, oral diet (0, 200, 2000, 20000 ppm)	Glyphosate technical (Purity: 96.8 %)	Valid, Category 2a	NOAEL = 2000 ppm (equivalent to 147.3 mg/kg bw/day for males and 195.7 mg/kg bw/day for females)
CA 5.3.2/011	[redacted] 1991	Sprague-Dawley rat 90–day, oral diet (0, 30, 300 and 1000 mg/kg bw/day)	Glyphosate (Purity: 98.6 %)	Valid, Category 2a	NOAEL = 300 mg/kg bw/day
CA 5.3.2/012	[redacted] 1990	CD rat 90–day, oral diet (0, 2000, 5000, 7500 ppm)	Glyphosate technical (Purity: 98.1 %)	Valid, Category 2a	NOAEL = 7500 ppm (equivalent to 482.1 mg/kg bw/day for males and 647.3 mg/kg bw/day for females)
CA 5.3.2/013	[redacted] 1989	CD rat 13-week, oral diet (0, 2000, 3000, 5000, 7500 ppm)	Glyphosate technical (Purity: 97.1 %)	Valid, Category 2a	NOAEL = 7500 ppm (equivalent to 375 mg/kg bw/day in males and females)
CA 5.3.2/014	[redacted], 1987	Sprague-Dawley rat 90–day, oral diet (0, 1000, 5000 and 20000 ppm)	Glyphosate (Purity: 95.21 %)	Valid, Category 2a	NOAEL = 19000 ppm (equivalent to 1267 mg/kg bw/day for males and 1623 mg/kg bw/day for females)
CA 5.3.2/015	[redacted], 1985	Wistar rat 90–day, oral gavage (0, 300, 1200, 2400 mg/kg bw/day)	Glyphosate (Purity: not reported)	Invalid, Category 4b	NOAEL = 1200 mg/kg bw/day
CA 5.3.2/016	[redacted], 1981	Wistar rat 90–day, oral diet (0, 1000, 3000, 10000 ppm)	Glyphosate (Purity: 96.8 %)	Invalid, Category 4b	NOAEL = 1000 ppm (equivalent to 102 mg/kg bw/day for males and 105.4 mg/kg bw/day for females)
CA 5.3.2/017	[redacted], 1995	SPF ICR (Crj:CD-1) mouse 13-week, oral	Glyphosate technical (Purity: 97.56 %)	Valid, Category 2a	NOAEL = 10000 ppm (equivalent to 1221 mg/kg bw/day for males and 1486

Table 5.3.2-1: Studies on oral 90–day repeated-dose toxicity with glyphosate in rodents

Annex Point	Study	Species Study type	Substance(s)	Reference list-related category§	Result
		diet (0, 5000, 10000, 50000 ppm)			mg/kg bw/day for females)
CA 5.3.2/018	[redacted], 1991	CD-1 mouse 13-week, oral diet (0, 200, 1000, 4500 mg/kg bw/day)	Glyphosate (Purity: 98.0 – 99.5 %)	Valid, Category 2a	NOAEL = 4500 mg/kg bw/day
CA 5.3.2/019	[redacted] 1979	CD-1 mouse 13-week, oral diet (0, 5000, 10000, 50000 ppm)	Glyphosate (Roundup® technical) (Purity: 98.7 %)	Supportive, Category 3b	NOAEL = 50000 ppm (equivalent to approx. 9707.0 for males and 14858.2 for females)

§: The category describes the acceptability/reliability of the study within the AIR 5 submission. This category does not describe hazard classification. (for details please refer to the Doc ID: 110054-B-GRG_Jun_2020)

There are nine valid 90–day oral studies in rats of different strains available for the assessment of subchronic toxicity of glyphosate via dietary administration ([redacted], 1996; [redacted], 1996; [redacted], 1995; [redacted], 1993; [redacted], 1992; [redacted], 1991; [redacted], 1990; [redacted], 1989; [redacted], 1987). In the last EU evaluation (2015), shortcomings in some of these studies were pointed out. In the study by [redacted] (1989) which was still considered acceptable, the actual mean dietary intake had not been calculated. This could be remedied in part by applying the appropriate diet conversion factor of 20 as published by Derelanko (The Toxicologist's Pocket Handbook, 2nd Ed., 2008). The same holds true for the study by [redacted] (1992) where the mean dietary intake had been calculated in the 2nd Amendment (1994) but not for the recovery group. Here the missing data were calculated based on the corresponding raw data from the report. In addition, there are two studies in rats ([redacted], 1985; [redacted], 1981) which are considered not acceptable due to serious reporting deficiencies.

Depending on experimental conditions and dose ranges, the NOAELs derived from the nine studies ranged from 79 and 90 mg/kg bw/day for males and females, respectively ([redacted], 1996) to 1267 and 1623 mg/kg bw/day for males and females, respectively [redacted], 1987). In three studies the NOAEL was determined to be between 100 and 200 mg/kg bw/day. In the remaining five studies higher NOAELs were found. Lowest observed adverse effect levels (LOAELs) ranged from about 370 to 2000 mg/kg bw/day. NOEL/NOAEL levels established in the 90-day dietary studies in rats varied between approximately 80 and 1600 mg/kg bw/day and because the salivary gland alterations are considered adaptive the lowest low observed effects of treatment were in the range of 550 mg/kg bw day ([redacted], 1995). Over the range of available studies, the appropriate overall NOAEL was 5000 ppm (equivalent to 414/447 mg/kg bw/day in male and female rats respectively). Mice appeared to be less sensitive, with substantially higher NOAEL values, the lowest of which was 600 mg/kg bw/day.
Summarising all these results, glyphosate was shown to be of low toxicity after dietary administration in rats.

In addition, there are two valid 90–day feeding studies available that were conducted in the mouse ([redacted], 1995; [redacted], 1991). A third 90–day feeding study in mice was considered to be supportive due to major reporting deficiencies ([redacted], 1979). In all of these mouse studies, the NOAEL was above 1000 mg/kg bw/day indicating that mice might be less sensitive towards glyphosate administration as compared to rats.

In a 90–day feeding study in Alpk:APfSD rats, administration of glyphosate acid at the high dose level of approx. 1600 and 1800 mg/kg bw/day in males and females, respectively, resulted in reduced growth (males

only) and associated changes in clinical chemistry, e.g. elevated plasma ALT and ALP levels (1996). The latter also provided limited evidence for an altered liver metabolism which was not associated with any histopathological change. This observation is supported by increased liver weights at the high dose level in another study (1996).

Soft stools and diarrhoea, together with occasionally reduced body weight gain and food consumption, suggest irritation of the gastrointestinal tract at high dose levels. Decreased urinary pH was noted in some studies at high doses, with blood in the urine reported in the study by (1996). In some oral rat studies and in one mouse study, cellular alterations in salivary glands were observed upon histopathological examination. The applicant believes these salivary gland findings are a non-adverse adaptive response to treatment with a low pH diet (See CA 5.8.2/002). Overall the mouse is less sensitive than the rat with effects observed on body weight only at very high dose levels.

Additionally, caecum distension and an increase in caecum weight were observed in both a 13 week rat study (1995) and a 13 week mouse study 1995). This effect appeared to be dose related at very high dose levels in both species but was not associated with any corollary histopathological changes and is therefore of uncertain toxicological relevance. In contrast, in another 13 week dietary rat study 1996) mucosal atrophy of the caecum was observed where there was no associated weight change. Another finding that had been previously reported in male mice was cystitis of the urinary bladder in animals dosed at 6295 mg/kg bw/day (1995).
Overall, it can be concluded that administration of glyphosate via the diet over 13 weeks resulted in both rodent species in low toxicity, with the rat being the more sensitive species. The lowest NOAEL was found to be 79 and 90 mg/kg bw/day for male and female rats respectively (1996).

1. Information on the study

Data point	CA 5.3.2/001
Report author	
Report year	1996 (Study Report)
Report title	First Revision to Glyphosate Acid: 90 Day Oral Feeding Study in Rats
Report No	/P/4599
Document No	Not reported
Guidelines followed in study	No guideline statement, but in accordance with OECD 408 (1998), OPPTS 870.3100 (1998), 2001/59/EC B.26 (2001)
Deviations from current test guideline (OECD 408, 2018)	No pre-dose ophthalmology, no reticulocyte count, T3, T4, TSH, less blood clinical chemistry parameters evaluated, thyroids not weighed, no blood hormones measured. Deviations from the current version of OECD 408 (2018) are basically due to the fact that the study was aligned to an older version of the OECD test guideline 408.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a
Data point	CA 5.3.2/002
Report author	
Report year	1996 (Appendix to Study Report)
Report title	First Revision to Glyphosate Acid: 90 Day Oral Feeding Study in Rats - Appendix

Report No	[redacted]/P/1599
Document No	Not reported
Guidelines followed in study	No guideline statement, but in accordance with OECD 408 (1998), OPPTS 870.3100 (1998), 2001/59/EC B.26 (2001)
Deviations from current test guideline (OECD 408, 2018)	No pre-dose ophthalmology, no reticulocyte count, T3, T4, TSH, less blood clinical chemistry parameters evaluated, thyroids not weighed, no blood hormones measured. Deviations from the current version of OECD 408 (2018) are basically due to the fact that the study was aligned to an older version of the OECD test guideline 408.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

In a sub-chronic toxicity study, groups of twelve male and twelve female Alpk:AP (now known as Alpk:APfSD) Wistar-derived rats were fed diets containing 0 (control), 1000, 5000 or 20000 ppm glyphosate acid for 90 consecutive days (equivalent to 0, 81.33, 413.5, or 1612 mg/kg bw/day in males and 0, 90.42, 446.9 or 1821 mg/kg bw/day in females).

Clinical observations, body weights, food consumption and blood biochemistry parameters were measured and at the end of the scheduled period, the animals were killed and subjected to a full examination *post mortem*. Cardiac blood samples were taken for clinical pathology, selected organs were weighed and specified tissues were taken for subsequent histopathology examination.

Males fed 20000 ppm glyphosate acid showed small reductions in body weight gain, food consumption and food utilisation efficiency compared to controls. There were associated minor reductions in plasma urea, total protein and triglycerides. Increased levels of plasma ALT and ALP were seen in both male and female rats at the top dose and are considered to indicate an altered liver metabolism but there was no evidence of histological change in this organ.

Effects in rats fed 5000 ppm glyphosate acid were confined to marginal increases in plasma ALT levels in females and in both plasma ALT and ALP levels in males. The magnitude and isolated nature of these effects leads them to be considered of no biological significance.

Glyphosate acid when fed to rats at a level of 20000 ppm resulted in reduced growth (males only) and associated changes in clinical chemistry. The latter also provided limited evidence for an altered liver metabolism which was not associated with any histopathological change. Toxicologically significant changes were confined to the 20000 ppm glyphosate acid dose level and occurred mainly in male rats.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification: Glyphosate acid
Description: White solid

Lot/Batch #:	P15
Purity:	97.4 %
Stability of test compound:	Not reported

2. Vehicle and/or positive control: Plain diet / none

3. Test animals:

Species:	Rat
Strain:	Alpk:APfSD
Source:	[redacted]
Age:	36 – 38 days
Sex:	Male and female
Weight at dosing:	♂ 98 – 170 g; ♀ 96 – 140 g
Acclimation period:	Approximately 1 week
Diet/Food:	CTI diet, *ad libitum,* (except during collection of urine samples)
Water:	Mains water, *ad libitum,* (except during collection of urine samples)
Housing:	4/cage, sexes separately in stainless steel cages 34.0 × 37.5 × 20.3 cm giving a floor area of 1275cm^2
Environmental conditions:	Temperature: 21 ± 2 °C Humidity: 36 – 60 % Air changes: ≥15/hour 12 hours light/dark cycle

B: Study design and methods

In life dates: 1986-02-25 to 1986-05-

Animal assignment and treatment:
The study was divided into six single-sex replicates (randomised blocks). Each replicate consisted of four cages, one per treatment group. The animals were randomly allocated to cages.
The study consisted of one control and three treatment groups each containing twelve male and twelve female rats.

Table 5.3.2-2: First Revision to Glyphosate Acid: 90 Day Feeding Study in Rats ([redacted], 1996): Study design

Test group	Dietary concentration [ppm]	Males	Females
Control	0	12	12
Low	1000	12	12
Mid	5000	12	12
High	20000	12	12

The experimental diets were made in 60 kg batches by adding the appropriate amount of glyphosate acid to the diet using dry premixes.

Samples from all dietary levels (including controls) were taken from both batches prepared for the study and analysed quantitatively for glyphosate acid. The homogeneity of glyphosate acid in CT1 diet was

determined by analysing samples from the low and high dose levels from the first batch of diet. The chemical stability of glyphosate acid in diet was determined at the highest and lowest dose levels at 1, 4, 6 and 10 weeks after preparation. Analysis was by high performance liquid chromatography (HPLC).

Mortality
Each animal was checked for mortality or signs of morbidity at least once daily during the treatment period.

Clinical observations
A check for clinical signs of toxicity was made once daily on all animals. In addition, a detailed clinical examination was performed at least once before of the beginning of the treatment period and then once a week until the end of the study.

Body weight
The body weight of each animal was recorded immediately before feeding of the experimental diets commenced and then on the same day, where practicable, of each subsequent week until termination. The body weight determination was done on the same day on which the detailed clinical examination was performed.

Food consumption and utilisation
Food consumption was recorded continuously throughout the study for each cage of rats and calculated as a weekly mean (g food/rat/day) for each cage. The food utilisation value per cage was calculated as the body weight gained by the rats in the cage per 100 g of food eaten.

Ophthalmoscopic examination
The eyes of all animals from the control group and the 20000 ppm glyphosate acid dose level group were examined in the week prior to termination, using an indirect ophthalmoscope and a mydriate to dilate the pupil.

Haematology and clinical chemistry
At termination, all surviving rats were bled by cardiac puncture and the blood samples were collected both in tubes containing EDTA as anticoagulant and also in tubes containing 0.11 M trisodium citrate. These samples were submitted for haematological examination and the following parameters measured: haemoglobin, haematocrit, red blood cell count, mean cell volume (MCV), mean cell haemoglobin concentration (MCHC), mean cell haemoglobin (MCH), kaolin-cephalin times, thrombocytes, leucocytes, differential white cell count, red blood cell morphology, prothrombin time.

For clinical chemistry analysis blood samples were collected by tail vein bleeding at week 4 of the study and by cardiac puncture at termination (week 13). The blood was collected in lithium heparinised tubes and the following parameters measured: glucose, urea, total protein, albumin, total cholesterol, triglycerides, alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

Urinalysis
Urine samples were collected over an 18 hour (approximately) period from all rats during week 13 (the week prior to termination). During urine collection, the rats were individually housed in metabolism cages and denied access to food and water. The following parameters were measured: volume, pH, specific gravity, proteins, glucose, ketones, and urobilinogen.

Sacrifice and pathology
On completion of the treatment period, all surviving animals were sacrificed and subjected to a gross pathological examination. Any macroscopic findings were recorded.
The following organ weights were determined: adrenals, brain, epididymides, heart, kidneys, liver, and testes. Paired organs were weight together.

Tissue samples were taken from the following organs and preserved in buffered formalin: all gross lesions, adrenals, aorta, bone marrow (femur), brain, caecum, colon, duodenum, epididymides, eyes (stored),

Harderian gland (stored), heart, ileum, jejunum, kidneys, larynx (stored), liver, lungs, lymph nodes (cervical and mesenteric), mammary gland, ovaries, oviducts, pancreas, pituitary gland, prostrate, rectum, salivary glands, seminal vesicles, spinal cord, sciatic nerve, skin, spleen, stomach, testes, thymus, thyroid gland, trachea, urinary bladder, uterus (with cervix), voluntary muscle and nasal cavity.

Following fixation, all tissues from the control and 20000 ppm glyphosate acid groups (except those stored) were processed by standard methods, embedded in paraffin wax, sectioned at 5µm, stained with haematoxylin and eosin and examined by light microscopy. Liver, kidney, adrenals, lungs and abnormal tissues from animals fed 1000 ppm or 5000 ppm glyphosate acid were also processed to blocks and were examined histologically.

Statistics
All data were evaluated using analysis of variance (body weight gain from start of study, final body weight, haematology, clinical chemistry – blood and urine, total food consumption and utilisation, organ weights) and covariance (organ weights on terminal body weights) for each specified parameter using the GLM procedure in SAS (1982).

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities.

B. CLINICAL OBSERVATIONS
The incidence of clinical findings was low and none was unequivocally related to treatment. There was a low incidence of diarrhoea (during the second week of the study) in the group receiving 20000 ppm glyphosate acid. The faeces of both sexes at this dose level were observed to be paler than those of control or other test groups.

C. BODY WEIGHT
No relevant differences in the mean body weight gain were noted between controls and animals given 1000 or 5000 ppm.
Body weight gain was reduced in male rats fed 20000 ppm glyphosate acid from the first week of the study. The body weights continued to diverge from control values as the study progressed, and final body weights were approximately 8 % lower than those of controls (see Table 5.3.2-3 below)

Table 5.3.2-3: First Revision to Glyphosate Acid: 90 Day Feeding Study in Rats ([redacted], 1996): Intergroup comparison of body weight gain – selected time points from start of study

	Mean cumulative body weight gain [g]							
	Initial	**Week 1**	**Week 2**	**Week 4**	**Week 7**	**Week 10**	**Week 13**	**Final weight**
Dose [ppm]	**Males**							
0	135	51.8	104.0	185.5	254.6	305.1	333.3	468.3
1000	↑140.3	↑54.3	↑106.3	↑187.5	↓253.4	↓304.9	↓327.0	↓467.3
5000	↑136.3	51.8	↓103.4	↑186.1	↑255.1	↑306.3	↓331.9	468.3
20000	↓134.5	**↓45.1****	**↓94.0***	**↓166.9****	**↓226.0****	**↓272.00** **	**↓295.8****	**↓430.3****
	Females							
0	121.3	26.6	47.3	81.6	112.8	130.8	143.3	264.6
1000	↑122.2	↑27.7	↑51.4	↑82.7	↑113.1	↑132.1	↑146.0	↑268.2
5000	121.3	↓25.9	↑50.2	↑82.9	↓110.0	↓129.5	↓138.4	↓259.8
20000	↓118.6	↓24.3	**↑53.5***	↑83.3	↑115.1	↑132.7	↓142.5	↓261.1

* Statistically significant from controls, $p < 0.05$ (Student´s t-test, 2-sided);
** Statistically significant from controls, $p < 0.01$ (Student´s t-test, 2-sided)

D. FOOD CONSUMPTION AND TEST SUBSTANCE INTAKE

The food consumption of males fed 20000 ppm glyphosate acid was reduced from the fifth week of the study compared to control values but the reduction was small and did not attain statistical significance in any week. The food utilisation efficiency of males at this dose level was reduced throughout the study. The food consumption and food utilisation efficiency of males fed 1000 or 5000 ppm glyphosate acid and of females at all dose levels were similar to those of controls.

Table 5.3.2-4: First Revision to Glyphosate Acid: 90 Day Feeding Study in Rats ([redacted], 1996): Intergroup comparison of food consumption [g/rat/day] – selected time points from start of study

	Dietary concentration of glyphosate acid [ppm]							
	Males				Females			
Weeks	0	1000	5000	20000	0	1000	5000	20000
1	22.9	↑23.9	↑23.7	↓22.6	17.8	↑17.9	↓17.5	17.8
5	27.2	↓26.6	↑27.3	↓25.5	19.0	19.0	↓18.2	↓18.5
9	28.5	↓28.3	28.5	↓26.9	19.9	↑20.0	↓19.5	19.9
13	24.9	↓23.7	↓24.3	↓22.8	17.6	↑18.0	↓17.4	17.6

* Statistically significant from controls, $p < 0.05$ (Student´s t-test, 2-sided)

Table 5.3.2-5: First Revision to Glyphosate Acid: 90 Day Feeding Study in Rats ([redacted], 1996): Intergroup comparison of food utilisation [g growth/100 g food] – selected time points from start of study

	Dietary concentration of glyphosate acid [ppm]							
	Males				Females			
Weeks	0	1000	5000	20000	0	1000	5000	20000
1-4	25.15	↓24.85	↓24.99	**↓22.89***	15.73	↓15.42	↑15.78	↑15.91
5-8	11.25	↓11.09	↑11.36	↓10.31	7.49	↓6.74	↓6.71	↓7.29
9-13	6.45	↓5.76	↓6.05	↓5.88	3.08	↑3.95	↑3.10	↓2.96
Overall (1-13)	13.59	↓13.30	↓13.44	**↓12.54***	8.28	↑8.34	↓8.14	↓8.25

* Statistically significant from controls, $p < 0.05$ (Student´s t-test, 2-sided)

Calculated mean test compound intakes are presented in the following table.

Table 5.3.2-6: First Revision to Glyphosate Acid: 90 Day Feeding Study in Rats ([redacted], 1996): Overall mean test compound intake

	Males				Females			
Dietary concentration of glyphosate acid [ppm]	0	1000	5000	20000	0	1000	5000	20000
Achieved intake [mg/kg bw/day]	0	81.33	413.5	1612	0	90.42	446.9	1821

E. OPHTHALMOSCOPIC EXAMINATION

There were no test substance-related ophthalmological findings at the end of the treatment period. The small incidence of findings recorded was within the normal background incidence for rats of this age and strain.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

There were no treatment-related effects noted in any dose group.

Blood clinical chemistry

The plasma activities of alanine transaminase (ALT) and alkaline phosphatase (ALP) were increased in both sexes fed 20000 ppm glyphosate acid throughout the study. Plasma aspartate transaminase activity was increased in females fed 20000 ppm glyphosate acid at week 4 only.

Plasma ALT activity was also increased in males receiving 5000 ppm glyphosate acid at weeks 4 and 13 and in females at week 4 only.

The plasma ALP activities of males receiving 5000 or 1000 ppm glyphosate acid were marginally increased. These increases were not dose-related and for the 1000 ppm glyphosate acid group were attributed to the high values in 3 out of 12 males. These marginal differences from the control group are considered to be of doubtful significance and not to be treatment-related.

Plasma urea levels were marginally decreased in both sexes at week 13 and in males at week 4 in the 20000 ppm glyphosate acid group.

Males receiving glyphosate acid showed marginal reductions in plasma glucose levels at week 4 but not at week 13. Females at 20000 ppm glyphosate acid showed a slight increase in this parameter at week 13 only.

Plasma cholesterol levels were unaffected by treatment with glyphosate acid. Plasma triglyceride levels were slightly reduced in males receiving 20000 ppm glyphosate acid at both weeks 4 and 13, the effect being greater at week 13.

Both males and females receiving glyphosate acid showed marginal reductions in plasma albumin and total protein. The changes were not consistent, showed no dose-response relationship and are therefore considered to be of dubious significance.

Table 5.3.2-7: First Revision to Glyphosate Acid: 90 Day Feeding Study in Rats ([redacted], 1996): Intergroup comparison of selected clinical chemistry parameters

Parameter	Week	Dietary concentration of glyphosate acid [ppm]							
		Males				Females			
		0	1000	5000	20000	0	1000	5000	20000
ALT [mU/mL]	4	61.0	↑66.8	↑**76.0****	↑**83.8****	47.3	↑50.8	↑**57.7***	↑**73.8****
	13	51.9	↑52.3	↑**62.3***	↑**65.2****	45.0	↑45.2	↑46.2	↑**55.0****
ALP [mU/mL]	4	273	↑**326****	↑**320***	↑**411****	188	↑199	↑212	↑**309****
	13	148	↑159	↑**176***	↑**215****	91	↑94	↑99	↑**140****
ASAT [mU/mL]	4	62.8	↑67.0	↑69.1	↑68.5	56.0	↑57.0	↑57.5	↑**64.8****
	13	52.7	↓52.3	↑56.4	↓52.4	53.1	↓49.8	↑52.8	↓52.6
Urea [mg/100 mL]	4	47.0	↓45.8	↓44.6	↓**43.6***	45.8	↓45.3	↑46.8	↓44.2
	13	41.9	↓39.9	↓40.0	↓**37.7***	40.6	↓40.1	↑42.1	↓**35.9***
Glucose [mg/100 mL]	4	143	↓**133***	↓**132***	↓**128****	141	↑146	↑142	↓136
	13	191	↓186	↑208	↑197	182	↑183	↑183	↑**208****
Triglycerides [mg/100 mL]	4	151	↓145	↓147	↓136	80	↓75	↑88	↑84
	13	153	↑157	↓144	↓**120****	72	↑74	↑77	↑77
Albumin [g/100 mL]	4	4.68	↓4.67	↓4.64	↓4.67	4.63	↓4.57	↓**4.49***	↓4.58
	13	4.81	↓**4.60***	↑4.82	↓**4.62***	4.54	↓4.42	↓4.42	↓4.42
Total Protein [g/100 mL]	4	6.13	↑6.15	↓6.12	6.13	5.84	↑5.88	↓5.80	↑5.87
	13	6.53	↓**6.22****	↓6.43	↓**6.06****	5.84	↓5.79	↓5.82	↓5.69

*Statistically significant from controls, $p < 0.05$ (Student´s t-test, 2-sided);
**Statistically significant from controls, $p < 0.01$ (Student´s t-test, 2-sided)

G. URINALYSIS

There were no treatment-related findings.

H. NECROPSY

Organ weights
Absolute heart weight of top dose males was reduced compared to controls but the reduction reflected the reduced body weight. There were no other differences in organ weights which were considered to be related to treatment.

Table 5.3.2-8: First Revision to Glyphosate Acid: 90 Day Feeding Study in Rats [redacted] 1996): Intergroup comparison of heart weights [grams]

	Dietary concentration of glyphosate acid [ppm]							
	Males				Females			
Parameter	0	1000	5000	20000	0	1000	5000	20000
Absolute	1.310	↑1.331	↓1.296	**↓1.197***	0.881	↑0.889	↑0.892	↑0.910
Adjusted for body weight	1.283	↑1.308	↓1.269	↓1.274	0.878	↓0.877	↑0.901	↑0.915

* Statistically significant from controls, $p < 0.05$ (Student´s t-test, 2-sided)

Gross pathology
A small number of lesions were observed, none of which was related to treatment.

Histopathology
There were no histopathological findings related to treatment. The incidence of findings was low and, with one exception, of a type commonly found in rats of this strain and age. An uterine leiomyosarcoma was seen in a female fed 5000 ppm glyphosate acid. Whilst the occurrence of a malignant tumour of smooth muscle in the uterus of a young rat is unusual, this isolated finding in an intermediate dose group is considered not to be related to treatment.

III. CONCLUSIONS

Males fed 20000 ppm glyphosate acid showed small reductions in body weight gain, food consumption and food utilisation efficiency compared to controls. There were associated minor reductions in plasma urea, total protein and triglycerides. Increased levels of plasma ALT and ALP were seen in both male and female rats at the top dose and are considered to indicate an altered liver metabolism but there was no evidence of histological change in this organ.

Effects in rats fed 5000 ppm glyphosate acid were confined to marginal increases in plasma ALT levels in females and in both plasma ALT and ALP levels in males. The magnitude and isolated nature of these effects leads them to be considered of no biological significance.

Glyphosate acid when fed to rats at a level of 20000 ppm resulted in reduced growth (males only) and associated changes in clinical chemistry. The latter also provided limited evidence for an altered liver metabolism which was not associated with any histopathological change.

Toxicologically significant changes were confined to the 20000 ppm glyphosate acid dose level and occurred mainly in male rats. The minor changes in clinical chemistry seen at 5000 ppm glyphosate acid were considered biologically insignificant and this was, therefore, judged to be the no-effect level for glyphosate acid in this study.

3. Assessment and conclusion

Assessment and conclusion by applicant:
In this study, groups of twelve male and twelve female Alpk:AP (now known as Alpk:APfSD) Wistar-derived rats were fed diets containing 0 (control), 1000, 5000 or 20000 ppm glyphosate acid for 90 consecutive days in accordance with OECD 408 (1998) and in compliance with GLP (no certificate of the competent authority was provided).
Glyphosate acid when fed to rats at a level of 20000 ppm resulted in reduced growth (males only) and associated changes in clinical chemistry. The latter also provided limited evidence for an altered liver metabolism which was not associated with any histopathological change.
Toxicologically significant changes were confined to the 20000 ppm glyphosate acid dose level and occurred mainly in male rats. The minor changes in clinical chemistry seen at 5000 ppm glyphosate acid were considered biologically insignificant and this was, therefore, judged to be the NOAEL for glyphosate acid in this study (equivalent to 413.5 mg/kg bw/day for males and 446.9 mg/kg bw/day for females).

Assessment and conclusion by RMS:
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1. Information on the study

Data point	CA 5.3.2/003
Report author	[redacted]
Report year	1996
Report title	Technical Glyphosate: Ninety Day Sub-Chronic Oral (Dietary) Toxicity Study in the Rat
Report No	434/016
Document No	Not reported
Guidelines followed in study	JMMAF 59 NohSan No. 4200; data from the study report is equivalent to OECD 408.
Deviations from current test guideline (OECD 408, 2018)	Reticulocytes not counted; cholesterol not measured, no blood hormones (T3, T4 and TSH) measured; thymus, uterus, epididymis, prostate and seminal vesicles not weighed with testes; epididymis, coagulating glands not examined microscopically and spinal cord only examined at one level. Deviations from the current version of OECD 408 (2018) are basically due to the fact that the study was aligned to an older version of the OECD test guideline 408.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary
The test material was administered by dietary admixture to three groups, each of ten male and ten female

Sprague Dawley (CD) strain rats, for ninety consecutive days, at dietary concentrations of 1000, 10000 or 50000 ppm (equivalent to an estimated mean achieved dose level of 79, 730 or 3706 mg/kg bw/day for males and 90, 844 or 4188 mg/kg bw/day for females). A further group of ten males and ten females was exposed to basal laboratory diet to serve as a control.
Clinical signs, body weight, food and water consumption were monitored during the study. Haematology, blood chemistry and urinalysis were evaluated for all animals at the end of the study. Ophthalmoscopic examination was also performed. All animals were subjected to a gross necropsy examination and a comprehensive histopathological evaluation of tissues was performed.

At 1000 ppm no treatment-related effects were noted in any of the investigation conducted.

In the mid dose group statistically significant reduction in plasma calcium concentration and an increase in alkaline phosphatase was observed in both sexes. Histopathology revealed a mucosal atrophy of the caecum in this group. No other treatment-related findings were observed in this dose group.

Animals treated with 50000 ppm showed soft faeces/diarrhoea from Day 4 which continued throughout the study period. In addition, body weight gain, food intake and food efficiency in animals of both sexes in the high-dose group was reduced over the first four weeks of treatment when compared with controls. Body weight development, food consumption and efficiency recovered in females and were comparable with the control group by the end of the treatment period. In males body weight gain showed only a partial recovery, and an adverse effect on dietary intake was still apparent during the remaining treatment period. Animals of both sexes treated with 50000 ppm showed a statistically significant reduction in plasma calcium concentration and creatinine levels, as well as an increase in alkaline phosphatase and inorganic phosphorous in comparison with controls. Reductions in total protein and albumin were observed only in high-dose females. Urinalysis revealed increased levels of haemoglobin when compared with controls. Microscopic examination of sediment revealed unidentified particulate matter in the samples obtained from males treated with 50000 ppm. At necropsy high dose animals of both sexes showed an enlarged and fluid-filled caecum, as well as statistically increased liver and kidney weights. Microscopic examination of the caecum revealed changes identified as mucosal atrophy for animals of both sexes treated with 50000 or 10000 ppm. No treatment-related histopathological changes were detected in the 1000 ppm dose group.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Technical Glyphosate
Description:	White powder
Lot/Batch #:	H95D 161 A
Purity:	95.3 %
Stability of test compound:	No data given in the report

2. Vehicle and/or positive control: Plain diet

3. Test animals:

Species:	Rats
Strain:	Sprague-Dawley (CD)
Source:	[redacted]
Age:	6 – 7 weeks
Sex:	Male and female
Weight at dosing:	♂ 175 – 218 g; ♀ 145 – 195 g

Acclimation period:	7 days
Diet/Food:	Rat and Mouse SQC Ground Diet No.1 (Special Diets Services Limited, Witham, Essex, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	In groups of up to four by sex in polypropylene grid-floor cages.
Environmental conditions:	Temperature: 21 ± 2 °C Humidity: 55 ± 15 % Air changes: 15/hour 12 hours light/dark cycle

B: Study design and methods

In life dates: 1995-08-11 to 1996-01-30

Animal assignment and treatment:
In a 90 day feeding study groups of 10 Sprague-Dawley rats per sex received daily dietary doses of 0, 1000, 10000 or 50000 ppm (equivalent to mean achieved dose levels of 0, 79, 730 or 3706 mg/kg bw/day for males and 90, 844, or 4188 mg/kg bw/day for females) technical glyphosate in the diet.
Test diets were prepared prior to start of treatment and then twice during the three month study period by mixing a known amount of the test substance with a small amount of basal diet and blending for 19 minutes. This pre-mix was then added to larger amount of basal diet and blended for further 30 minutes. The stability and homogeneity of the test material in diet were determined. Samples of each dietary admixture were analysed for homogeneity and achieved concentration.

Clinical observations
A check for clinical signs of toxicity, ill health and behavioural changes was made once daily on all animals. All observations were recorded.

Body weight
Individual body weights were recorded on Day 0 (prior to treatment) and at weekly intervals thereafter. Body weights were also determined at necropsy.

Food consumption and compound intake
Food consumption was recorded once weekly for each cage group throughout the study.

Water consumption
Water intake was observed daily, for each cage group, by visual inspection of the water bottles for any overt changes.

Ophthalmoscopic examination
The eyes of all control and high dose animals were examined before administration of the test and control diets and before termination of treatment (during Week 12). Examinations included observation of the anterior structures of the eye, pupillary and corneal blink reflex and, following pupil dilation with 0.5 % Tropicamide solution ("Mydriacyl" - Alcon Laboratories Ltd., Watford, Hertfordshire, UK), detailed examination of the internal structure of each eye using a direct ophthalmoscope.

Haematology and clinical chemistry
Haematological and blood chemical investigation were performed on all animals from each test and control group at the end of the study (Day 90).

Urinalysis
Analytical investigations of the urine were performed on all animals during Week 12. Urine samples were

collected overnight by housing the rats in metabolism cages. Animals were maintained under conditions of normal hydration during collection but without access to food.

Sacrifice and pathology
All animals sacrificed at scheduled termination were subjected to a gross pathological examination. Any macroscopic findings were recorded.

The following organ weights were determined: adrenals, brain, gonads, heart, kidneys, liver, pituitary and spleen.

Tissue samples were taken from the following organs and preserved in buffered formalin: Adrenals, aorta (thoracic), bone & bone marrow (sternum and femur (incl. stifle joint)), brain (at three levels), caecum, colon, duodenum, eyes, gross lesions, heart, ileum, jejunum, kidneys, liver, lungs, mammary gland, lymph nodes (cervical and mesenteric), muscle (skeletal), oesophagus, ovaries, pancreas, pituitary, prostrate, rectum, salivary glands, sciatic nerve, seminal vesicles, skin (hind limb), spinal cord (cervical), spleen, stomach, testes, thymus, thyroid/parathyroid, tongue, trachea, urinary bladder, uterus and vagina.

Statistics
Absolute and relative organ weights, haematological and blood chemical data were analysed by one way analysis of variance incorporating 'F-max' test for homogeneity of variance. Data showing heterogeneous variances were analysed using Kruskal-Wallis non-parametric analysis of variance and Mann Whitney U-Test.

The levels of probability chosen as significant were $p < 0.001$***, $p < 0.01$** and $p < 0.05$*.

Histopathology data were analysed using the following methods to determine significant differences between control and treatment groups for the individual sexes:

1. Chi squared analysis for differences in the incidence of lesions occurring with an overall frequency of 1 or greater.

2. Kruskal-Wallis one way non-parametric analysis of variance for the comparison of severity grades for the more frequently observed graded conditions.

The levels of probability chosen as significant were $p < 0.001$***, $p < 0.01$**, $p < 0.05$* and $p < 0.1^{(*)}$.

II. RESULTS AND DISCUSSION

A. MORTALITY
No deaths occurred during the study.

B. CLINICAL OBSERVATIONS
Animals of both sexes treated with 50000 ppm showed soft faeces and diarrhoea from Day 4 which continued throughout the study period.
The remaining observable sign of generalised fur loss was noted in one male and two females treated with 10000 and 1000 ppm respectively. This is a commonly reported incidental finding in laboratory maintained rats that, in the absence of any dose-related response, is of no toxicological significance and unrelated to treatment with the test material.

C. BODY WEIGHT
Animals of both sexes treated with 50000 ppm showed a reduction in body weight gain over the first four weeks of treatment when compared with controls (see table below). Female body weight development recovered as the study progressed and was comparable with the control group by the end of the treatment period. Male individuals showed only a partial recovery with body weight gain remaining slightly lower

than the control group values during the subsequent weeks of treatment.

Body weight development was unaffected by treatment with the test material at the remaining dose levels.

Table 5.3.2-9: Technical Glyphosate: Ninety Day Sub-Chronic Oral (Dietary) Toxicity Study in the Rat ([redacted] 1996): Group mean weekly body weights and standard deviations (SD)

Dietary concentration [ppm]		Body weight [g] at Day						
		0	7	14	21	28	35	42
Males								
0	mean	206	269	315	354	382	411	444
	SD	8	12	17	24	33	38	45
1000	mean	↓199	↓260	↓309	↓350	↓377	↓400	↓427
	SD	11	14	19	21	24	26	30
10000	mean	↓200	↓257	↓303	↓338	↓364	↓393	↓414
	SD	12	12	15	21	25	30	35
50000	mean	↓198	↓215	↓247	↓268	↓283	↓306	↓329
	SD	8	8	15	21	26	31	33
Females								
0	mean	173	197	214	232	243	256	269
	SD	9	11	12	15	16	18	20
1000	mean	173	↑199	↑218	↑238	↑249	↑261	↑272
	SD	10	13	14	16	16	17	18
10000	mean	↓166	↓184	↓201	↓217	↓226	↓237	↓246
	SD	14	18	21	25	24	26	27
50000	mean	173	↓183	↓197	↓214	↓219	↓231	↓240
	SD	11	12	14	15	14	18	21

Dietary concentration [ppm]		Body weight [g] at Day						
		49	56	63	70	77	84	90
Males								
0	mean	457	488	508	523	537	536	551
	SD	44	49	52	55	58	56	58
1000	mean	↓446	↓470	↓485	↓497	↓513	↓516	↓528
	SD	31	32	32	35	37	36	37
10000	mean	↓429	↓454	↓470	↓483	↓494	↓495	↓506
	SD	35	38	38	38	40	39	43
50000	mean	↓335	↓356	↓369	↓382	↓394	↓395	↓408
	SD	38	41	43	43	44	42	44
Females								
0	mean	276	284	291	295	306	304	307
	SD	19	20	21	24	25	25	27
1000	mean	↑280	↑286	↑292	↑300	↑308	304	↑313
	SD	19	18	18	19	21	20	20
10000	mean	↓256	↓262	↓267	↓272	↓277	↓276	↓282
	SD	27	27	27	27	29	28	29
50000	mean	↓246	↓251	↓260	↓265	↓271	↓267	↓273
	SD	21	20	23	23	26	22	25

D. FOOD CONSUMPTION

Animals of both sexes treated with 50000 ppm showed a reduction in both dietary intake and food efficiency over the first four weeks of treatment when compared with controls (see Table 5.3.2-10 below). Female food consumption and efficiency recovered as the study progressed and was comparable with control values by the end of the treatment period. Male food consumption however, remained adversely affected during the subsequent weeks of treatment. A similar prolonged effect on food efficiency was not evident during the same period as male body weight gain demonstrated a partial recovery over the corresponding weeks.

Dietary intake and food efficiency were unaffected by treatment with the test material at the remaining dose levels and were comparable with controls.

Table 5.3.2-10: Technical Glyphosate: Ninety Day Sub-Chronic Oral (Dietary) Toxicity Study in the Rat ([redacted] 1996): Group mean weekly food consumption

Dietary concentration [ppm]	Mean food consumption [g/rat/week]						
	1	2	3	4	5	6	7
Males							
0	201	199	204	212	208	218	208
1000	↓200	↑205	↑213	↓211	↓205	↓210	↑211
	(0)	(3)	(4)	(0)	(-1)	(-4)	(1)
10000	↓187	↓193	↓199	↓204	↓202	↓198	↓201
	(-7)	(-3)	(-2)	(-4)	(-3)	(-9)	(-3)
50000	↓122	↓183	↓178	↓177	↓183	↓182	↓168
	(-39)	(-8)	(-13)	(-17)	(-12)	(-17)	(-19)
Females							
0	140	131	171	153	149	149	152
1000	↑143	↑146	↓152	↑156	↑158	↑163	↑157
	(2)	(11)	(-11)	(2)	(6)	(9)	(3)
10000	↓123	↑135	↓142	↓144	↓143	↓140	↓143
	(-12)	(3)	(-17)	(-6)	(-4)	(-6)	(-6)
50000	↓128	↑143	↓131	↓148	↑167	↑157	↓148
	(-9)	(9)	(-23)	(-3)	(12)	(5)	(-3)

Dietary concentration [ppm]	Mean food consumption [g/rat/week]					
	8	9	10	11	12	13*
Males						
0	222	224	223	214	192	185
1000	↓217	↓204	↓219	214	↓191	↓180
	(-2)	(-9)	(-2)	(0)	(-1)	(-3)
10000	↓205	↓211	↓211	↓201	↓185	↓179
	(-8)	(-6)	(-5)	(-6)	(-4)	(-3)
50000	↓187	↓189	↓193	↓188	↓174	↓171
	(-16)	(-16)	(-13)	(-12)	(-9)	(-8)
Females						
0	152	151	147	155	139	128
1000	↑159	↑152	↑154	↑161	↑141	↑137
	(5)	(1)	(5)	(4)	(1)	(7)
10000	↓146	↓143	↓143	↓142	↓133	↑131
	(-4)	(-5)	(-3)	(-8)	(-4)	(2)
50000	↓151	151	↑151	↑161	139	↑139
	(-1)	(0)	(3)	(4)	(0)	(9)

( ) % change compared to control group;
* Week 13 comprises six days only

E. WATER CONSUMPTION

There were no treatment-related effects on water consumption for either sex noted during the study.

F. OPHTHALMOSCOPIC EXAMINATION

No treatment-related ocular effects for either sex were detected during the study.

G. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

No treatment-related effects were detected in the haematological parameters measured.

Blood chemistry

Animals of both sexes treated with 50000 or 10000 ppm showed a statistically significant reduction in plasma calcium concentration and an increase in alkaline phosphatase (AP) when compared with controls (see table below). A statistically significant increase in inorganic phosphorus and reduction in plasma creatinine were also evident amongst animals of both sexes treated with 50000 ppm whilst females at this dose level showed statistically significant reductions in total plasma protein and albumin in comparison with controls.

There were no further treatment-related effects.

Table 5.3.2-11: Technical Glyphosate: Ninety Day Sub-Chronic Oral (Dietary) Toxicity Study in the Rat ([redacted] 1996): Group mean blood chemical values and standard deviations (SD)

Dietary concentration [ppm]		Ca^{2+} [mmol/L]	AP [IU/L]	P [mmol/L]	Creatinine [mg/dL]	Total protein [g/dL]	Albumin [g/dL]
Males							
0	mean	2.74	373	2.23	0.61	7.02	3.37
	SD	0.06	101	0.22	0.03	0.47	0.16
1000	mean	↑2.77	↑404	↓2.22	↑0.62	↑7.20	↑3.40
	SD	0.07	115	0.16	0.05	0.20	0.06
10000	mean	**↓2.66***	**↑514***	↑2.32	↓0.59	↓6.78	↓3.30
	SD	0.09	106	0.28	0.04	0.68	0.21
50000	mean	**↓2.64***	**↑597*****	**↑2.46***	**↓0.57***	↓6.63	↓3.27
	SD	0.10	150	0.22	0.04	0.63	0.19
Females							
0	mean	2.78	230	1.70	0.69	7.63	3.90
	SD	0.11	38	0.33	0.07	0.45	0.23
1000	mean	↓2.76	↑261	↓1.65	0.69	↑7.64	↓3.87
	SD	0.05	71	0.21	0.04	0.29	0.13
10000	mean	**↓2.70***	**↑408*****	↑1.76	↓0.65	↓7.41	↓3.82
	SD	0.07	123	0.23	0.04	0.45	0.20
50000	mean	**↓2.56*****	**↑358****	**↑2.12*****	**↓0.61****	**↓6.86****	**↓3.47*****
	SD	0.10	90	0.15	0.05	0.82	0.39

* Significantly different from control group ($p < 0.05$);
** Significantly different from control group ($p < 0.01$);
*** Significantly different from control group ($p < 0.001$)

H. URINALYSIS

Animals of both sexes treated with 50000 ppm showed an increased level of haemoglobin in the urine when compared with controls (see table below). Microscopic examination of sediment revealed unidentified particulate matter in the samples obtained from males treated at 50000 ppm. This probably represents external contamination, possibly of faecal origin.

There were no treatment-related changes detected at the remaining dose levels.

Table 5.3.2-12: Technical Glyphosate: Ninety Day Sub-Chronic Oral (Dietary) Toxicity Study in the Rat ([redacted] 1996): Urinalysis findings

Dietary concentration [ppm]	Blood (haemoglobin)							
	Males				Females			
	-	+	++	+++	-	+	++	+++
0	8	0	1	1	10	0	0	—
1000	10	0	0	0	10	0	0	—
10000	7	2	1	0	10	0	0	—
50000	1	5	2	2	4	3	3	—

– negative;
+ ca. 5-10 × 10^6 ery/L;
++ ca. 50 × 10^6 ery/L;
+++ ca. 250 × 10^6 ery/L

I. NECROPSY

Organ weights

Animals of both sexes treated with 50000 ppm showed statistically significant increases in both relative liver and kidney weight when compared with controls (see table below).

There were no further direct effects of treatment.

Table 5.3.2-13: Technical Glyphosate: Ninety Day Sub-Chronic Oral (Dietary) Toxicity Study in the Rat ([redacted] 1996): Group mean organ weights and standard variations

Dietary concentration [ppm]		Relative organ weight [%]			
		Liver		Kidney	
		♂	♀	♂	♀
0	mean	2.9749	2.9734	0.5861	0.6516
	SD	0.2629	0.1558	0.0575	0.0523
1000	mean	↓2.8868	↓2.9093	↑0.5901	↓0.6257
	SD	0.2652	0.2146	0.0804	0.0375
10000	mean	↓2.8853	↑2.9801	↑0.6070	↓0.6454
	SD	0.3758	0.1556	0.0552	0.0532
50000	mean	**↑3.2433***	**↑3.1989***	**↑0.6963*****	**↑0.7180***
	SD	0.2452	0.2098	0.0436	0.0707

* Significantly different from control group ($p < 0.05$);
*** Significantly different from control group ($p < 0.001$)

Necropsy

Macroscopic abnormalities were detected in the 50000 ppm dose group with all animals showing an enlarged and fluid-filled caecum whilst one female treated with 50000 ppm showed gaseous distension of the stomach at terminal necropsy.

There were no treatment-related macroscopic abnormalities detected at 10000 or 1000 ppm.

Table 5.3.2-14: Technical Glyphosate: Ninety Day Sub-Chronic Oral (Dietary) Toxicity Study in the Rat ([redacted] 1996): Summary of necropsy findings

Finding	Dietary concentration [ppm]							
	Males				Females			
	0	1000	10000	50000	0	1000	10000	50000
Caecum: enlarged with fluid contents	0/10	0/10	0/10	10/10	0/10	1/10	0/10	10/10

Histopathology
Treatment-related changes were observed in the caecum. Atrophy, characterised by flattening of the intestinal mucosa, was observed for five rats of both sexes dosed at 50000 ppm ($p < 0.05$ for male rats) and for one male and two female rats receiving 10000 ppm of the test material. The aetiology of this change is uncertain and may represent no more than a stretch atrophy of the mucosa resulting from caecal distension. There were no further treatment-related changes.

Table 5.3.2-15: Technical Glyphosate: Ninety Day Sub-Chronic Oral (Dietary) Toxicity Study in the Rat ([redacted]., 1996): Summary incidence of histopathological findings

Finding	Dietary concentration [ppm]							
	Males				Females			
	0	1000	10000	50000	0	1000	10000	50000
Caecum: Mucosal atrophy	0/10	0/10	1/10	5/10	2/10	0/10	2/10	5/10

III. CONCLUSIONS

At 1000 ppm no treatment-related effects were noted in any of the investigation conducted.
In the mid dose group statistically significant reduction in plasma calcium concentration and an increase in alkaline phosphatase was observed in both sexes. Histopathology revealed a mucosal atrophy of the caecum in this group. No other treatment-related findings were observed in this dose group.

Animals treated with 50000 ppm showed soft faeces/diarrhoea from Day 4 which continued throughout the study period. In addition, body weight gain, food intake and food efficiency in animals of both sexes in the high-dose group was reduced over the first four weeks of treatment when compared with controls. Body weight development, food consumption and efficiency recovered in females and were comparable with the control group by the end of the treatment period. In males body weight gain showed only a partial recovery, and an adverse effect on dietary intake was still apparent during the remaining treatment period. Animals of both sexes treated with 50000 ppm showed a statistically significant reduction in plasma calcium concentration and creatinine levels, as well as an increase in alkaline phosphatase and inorganic phosphorous in comparison with controls. Reductions in total protein and albumin were observed only in high-dose females. Urinalysis revealed increased levels of haemoglobin when compared with controls. Microscopic examination of sediment revealed unidentified particulate matter in the samples obtained from males treated with 50000 ppm. At necropsy high dose animals of both sexes showed an enlarged and fluid-filled caecum, as well as statistically increased liver and kidney weights. Microscopic examination of the caecum revealed changes identified as mucosal atrophy for animals of both sexes treated with 50000 or 10000 ppm. No treatment-related histopathological changes were detected in the 1000 ppm dose group.

Dietary administration of the test material, technical glyphosate, to rats for a period of 90 consecutive days at concentrations of up to 50000 ppm, resulted in treatment-related changes at 50000 and 10000 ppm. No such effects were demonstrated in the 1000 ppm treatment group and the "No Observed Effect Level" was, therefore, considered to be 1000 ppm.

3. Assessment and conclusion

Assessment and conclusion by applicant:
In this study, glyphosate technical was administered via the diet to three groups, each of ten male and ten female Sprague Dawley (CD) strain rats, for 90 consecutive days, at dietary concentrations of 1000, 10000 or 50000 ppm (equivalent to mean achieved dose level of 79, 730 or 3706 mg/kg bw/day for males and 90, 844 or 4188 mg/kg bw/day for females). Dietary administration of the test material, technical glyphosate, to rats for a period of 90 consecutive days at concentrations of up to 50000 ppm, resulted in treatment-related changes at 50000 and 10000 ppm. No such effects were demonstrated in the 1000 ppm treatment group and the NOAEL was, therefore, considered to be 1000 ppm (equivalent to 79 mg/kg bw/day for males, and 90 mg/kg bw/day for females).

Assessment and conclusion by RMS:
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1. Information on the study

Data point	CA 5.3.2/004
Report author	[redacted]
Report year	1995
Report title	HR-001: 13-week Subchronic Oral Toxicity Study in Rats
Report No	[redacted] 94-0138
Document No	Not reported
Guidelines followed in study	Japan MAFF Guidelines 59 NohSan No. 4200, 1985; U.S. EPA FIFRA Guidelines Subdivision F, 1984; OECD 408 (1981)
Deviations from current test guideline (OECD 408, 2018)	Reticulocytes not counted, clotting not evaluated, total cholesterol but not HDL and LDL measured, urea not measured, no blood hormones (T3, T4 and TSH) measured; organ weights limited to brain, liver, kidneys, testes, adrenals and cecum. Deviations from the current version of OECD 408 (2018) are basically due to the fact that the study was aligned to an older version of the OECD test guideline 408.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

A sub-chronic oral toxicity study of HR-001 was conducted in Sprague-Dawley (Crj:CD) rats of both sexes. The test substance was administered to the rats (12 animals/group/sex) by incorporating it into the basal diet at dose levels of 0, 3000, 10000 or 30000 ppm (equivalent to 0, 168.4, 569 or 1735 mg/kg bw/day for males and 0, 195.2, 637 or 1892 mg/kg bw/day for females) for a period of 13 weeks (91 days).

30000 ppm group: Body weights of males and females were slightly lower than in the control throughout

the treatment period and statistically significant decreases were sporadically observed. The averaged food efficiency in males and females during the treatment period was slightly lower than that in the control. Females showed a significant increase in alkaline phosphatase (ALP) activity. Distention of the caecum was observed in 9/12 males and 7/12 females with statistical significance. Both sexes showed significant increases in absolute and relative weights of the caecum (containing contents). Histologically, there were no abnormalities related to treatment in any tissues including the caecum.

10000 ppm group: At necropsy, 3 males showed distention of the caecum. Organ weight analysis revealed a statistically significant increase (females) or an increasing trend (males) in both absolute and relative weights of the caecum.

3000 ppm group: There were no abnormalities attributable to the treatment in either sex.

I. MATERIALS AND METHODS

A: Materials

1. Test material:	Glyphosate technical
Identification:	HR-001
Description:	White crystal
Lot/Batch #:	940908-1 941209 T-941209
Purity:	95.68 % 95.0 % 97.56 %
Stability of test compound:	12/12/1994 19/12/1994 26/12/1994
2. Vehicle and/or positive control:	Plain diet / none
3. Test animals:	
Species:	Rat
Strain:	Sprague-Dawley Crj:CD
Source:	[redacted]
Age:	5 weeks
Sex:	Male and female
Weight at dosing:	♂ 136 – 150 g; ♀ 109 – 121 g
Acclimation period:	1 week
Diet/Food:	MF Mash (Oriental Yeast Co., Ltd.)
Water:	Filtered and sterilised tap water, *ad libitum*
Housing:	3/cage, sexes separately in stainless steel cages 31.0 × 44.0 × 20.3 cm
Environmental conditions:	Temperature: 24 ± 2 °C Humidity: 55 ± 15 % Air changes: 15/hour 12 hours light/dark cycle

B: Study design and methods

In life dates: 1994-12-06 to 1995-03-22

Animal assignment and treatment:
The test substance was incorporated into the basal effect diet and administered on a continuous basis in the basal diet to groups of 24 Sprague-Dawley rats (12 males + 12 females) for a period of 13 weeks. Dietary concentrations were 0, 3000, 10000 or 30000 ppm (equivalent to 0, 168.4, 569 or 1735 mg/kg bw/day for males and 0, 195.2, 637 or 1892 mg/kg bw/day for females).

Table 5.3.2-16: HR-001: 13-week Subchronic Oral Toxicity Study in Rats ([redacted] 1995): Study design

Test group	Dietary concentration [ppm]	Achieved concentration [mg/kg bw/day]	Males	Females
Control	0	♂: 0; ♀: 0	12	12
Low	3000	♂: 168.4; ♀: 195.2	12	12
Mid	10000	♂: 569; ♀: 637	12	12
High	30000	♂: 1735; ♀: 1892	12	12

Chemical analysis for homogeneity and concentration of the test substance in the diet were performed on samples (about 50 g each) of each does level taken from top, middle and bottom portions of the mixer at the first diet preparation. The control diet was also sampled (50 g each) and analysed to confirm that there was no contamination with the test substance. Concentrations of the test substance in test diets at all dose levels were monitored on the same amount of samples (50 g each) every 3 weeks during the study.

Mortality
Each animal was checked for mortality or signs of morbidity at least once daily during the treatment period.

Clinical observations
Cage-side observation was performed daily on all animals to detect moribund or dead animals and abnormal clinical signs, and all findings were recorded. In addition, a detailed examination including palpation for masses was performed at least once a week.

Body weight
Body weights of all animals were recorded at initiation of treatment and weekly during the study. Group mean body weight was calculated for each dose group at each measurement. Final body weight was recorded for all animals before necropsy.

Food consumption and utilisation
Food consumption for each cage was measured weekly over a period of 3 consecutive days. Mean daily food consumption per animal in each cage was calculated by dividing the food consumption by the number of animals per cage and by the number of days for measurement. Group mean food consumption (g/rat/day) was calculated at each measurement from the mean daily food consumption per animal in each cage.

Group mean chemical intake (mg/kg bw/day) was calculated from nominal dietary concentrations of the test substance, food consumption and body weight.

Group mean food efficiency for each dose group was calculated weekly from the ratio of the group body weight gain to group mean food consumption and expressed as percentage. Overall group mean efficiency throughout the treatment period was also calculated for all dose groups.

Ophthalmoscopic examination
Ophthalmological examinations including observation with a halogen ophthalmoscope were performed on all animals during acclimatization period and on all surviving animals in the control and the highest dose groups from the main group at week 13.

The following parameters were determined: eyeball, cornea, anterior chamber, pupil, iris, lens/vitreous body and fundus.

Haematology and clinical chemistry
After 13 weeks of treatment, all surviving animals were subjected to haematological examinations. The animals were laparotomised under anaesthesia following overnight fasting, and blood samples were withdrawn from the posterior vena cava using heparinised syringes. A part of each sample was poured into a cup treated with EDTA and subjected to the examinations.

The following parameters were determined with a fully automated haematology analyser: Haematocrit (Ht), haemoglobin (Hb), erythrocyte count (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count (PLT), total leukocyte count (WBC) and differential leukocyte count.

After 13 weeks of treatment, all surviving animals were subjected to blood biochemical examinations. Plasma samples obtained from the heparinised blood were used for examinations.

The following parameters were determined: Alkaline phosphatase (ALP), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), γ-glutamyl transpeptidase (GGTP), creatine phosphokinase (CPK), creatinine (Creat.), blood urea nitrogen (BUN), total protein (TP), albumin (Alb), globulin (Glob.), albumin/globulin ratio (A/G ratio), glucose (Gluc.), total cholesterol (T. Chol.), triglyceride (TG), total bilirubin (T. Bil.), calcium (Ca), inorganic phosphorus (P), sodium (Na), potassium (K) and chloride (Cl).

Urinalysis
At 13 weeks of treatment, all surviving animals were subjected to urinalysis. Fresh urine samples were collected by pressing the lumbodorsal region of the animals. Specific gravity was determined with a handy refractometer. Glucose, ketones, occult blood, pH, protein, and urobilinogen were semi-quantitatively analysed by Uro-labstix. Then animals were housed individually in metabolic cages overnight, and urine samples collected were examined for volume and appearance. Urinary sediments were also examined microscopically on these samples.

Sacrifice and pathology
Clinical pathology evaluations were also conducted. Selected organs were weighed at the scheduled necropsy. Histopathological examinations were performed on selected tissues from all animals.

The following parameters were determined: Brain, spinal cord, sciatic nerve, pituitary, thyroids with parathyroids, thymus, adrenals, spleen, bone with marrow, tibio-femoral joint, lymph nodes, heart, aorta, pharynx, buccal mucosa of oral cavity, salivary glands, oesophagus, stomach, liver, pancreas, duodenum, jejunum, ileum, cecum, colon, rectum, head, larynx, trachea, lung, kidneys, urinary bladder, testes, prostate, seminal vesicles, epididymides, coagulating glands, ovaries, uterus, vagina, harderian glands, eyes, skeletal muscle, skin, mammary gland and all gross lesions.

Statistics
All data were evaluated using variance analysis (body weight, food consumption, urine specific gravity, urine volume, haematological parameters, blood chemical parameters, and organ weights).

Data on clinical sign, mortality, ophthalmology, necropsy, and histopathology were evaluated by Fisher's exact probability.

II. RESULTS AND DISCUSSION

A. MORTALITY
No deaths were noted in the control and treated groups of either sex.

B. CLINICAL OBSERVATIONS

There were no abnormalities related to the treatment in clinical signs in the treated groups of either sex. In the 30000 ppm group, one female showed a poor general condition including emaciation and decreased spontaneous motor activity. The poor general condition seemed to be caused by elongated incisor, malocclusion, or hepatorenal genetic lesions revealed by histopathology. Thus, it was not considered to be treatment related.

C. BODY WEIGHT

In the 30000 ppm group, body weights of males and females were slightly lower (about 5 - 10 % decrease in males and 5 % in females) than those in the control throughout the treatment period. Statistically significant decreases in their body weights were sporadically observed during the treatment period (weeks 3, 4 and 11 in males and weeks 10 and 11 in females) when compared to the control. It should be noted that the calorific value of the diet would have been lower due to the incorporation, 3 % by weight, of HR-001 (Glyphosate).

In the 10000 and 3000 ppm groups, body weight changes in males and females were comparable to the control throughout the treatment period.

Table 5.3.2-17: HR-001: 13-week Subchronic Oral Toxicity Study in Rats ([redacted] 1995): Group mean weekly body weights (selected weeks) and standard deviations (SD)

Dietary concentration [ppm]	Body weight [g] at week						
		0	3	4	10	11	13
Males							
0	mean	142	314	351	503	519	541
	SD	4	23	30	47	48	50
3000	mean	142	↓310	↓348	↓502	↓516	↓540
	SD	4	20	24	45	49	53
10000	mean	142	↓311	↑352	↓501	↓517	541
	SD	4	21	29	41	45	48
30000	mean	142	↓**291***	↓**323***	↓462	↓**468***	↓497
	SD	4	19	30	50	53	53
Females							
0	mean	113	196	216	298	304	310
	SD	3	10	12	15	19	22
3000	mean	113	↑201	↑225	↑306	↑316	↑325
	SD	3	12	15	25	28	32
10000	mean	113	↑201	↑220	↓291	↓298	↓306
	SD	3	11	12	16	17	17
30000	mean	113	↓194	↓212	↓**278***	↓**282***	↓290
	SD	3	11	13	18	19	19

* Significantly different from control group ($p < 0.05$)

D. FOOD CONSUMPTION AND TEST SUBSTANCE INTAKE

In the 30000 ppm group, males and females showed significant decreases in food consumption at week 1 which were 9 and 14 % lower than that of the control, respectively. However, their food consumption was comparable to the control at week 2 and thereafter.

In the 10000 ppm group no significant change was observed while in the 3000 ppm group, significant changes were sporadically observed during the treatment period in females during the weeks 6 and 7. The food consumption recovered from the week 8 up to the end of the study.

The overall food consumption by males and females was comparable to the control and there were no abnormalities considered to be treatment related.

In the 30000 ppm group, the averaged food efficiency for males and females during the treatment period was 6 % and 5 % less than that for control, respectively. This in part would have been accounted for by the 3 % by weigh inclusion of HR-001 in the diet. Food efficiency for males and females was comparable to control in the 10000 and 3000 ppm groups.

Table 5.3.2-18: HR-001: 13-week Subchronic Oral Toxicity Study in Rats ([redacted] 1995): Average food efficiency during the treatment period [body weight gain/food consumption × 100]

Dose level [ppm]	Average food efficiency [%]	
	Males	Females
0	20.7	14.6
3000	20.9	14.8
10000	20.7	14.0
30000	19.5	13.9

The overall group mean chemical intakes averaged, calculated from food consumption and nominal concentrations of the test substance, through the treatment period, are summarised in the table below.

Table 5.3.2-19: HR-001: 13-week Subchronic Oral Toxicity Study in Rats ([redacted], 1995): Chemical Intake [mg/kg bw/day]

Dose [ppm]	Chemical Intake [mg/kg bw/day]	
	Males	Females
3000	168.4	195.2
10000	569	637
30000	1735	1892

E. OPHTHALMOSCOPIC EXAMINATION

In the ophthalmological examination performed on all animals before the start of the treatment and on the animals of the control and 30000 ppm groups at 13 weeks of treatment, no abnormalities were observed in either sex.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

There were no abnormalities in any group of either sex.

Blood clinical chemistry

In the 30000 ppm group, females showed a significant increase in alkaline phosphatase (ALP) activity and a significant decrease in albumin (Alb). There were no abnormalities in males.

In the 10000 and 3000 ppm groups, there were no abnormalities in either sex.

Table 5.3.2-20: HR-001: 13-week Subchronic Oral Toxicity Study in Rats ([redacted] 1995): Intergroup comparison of selected week 13 clinical chemistry parameters

Parameter		Dietary concentration [ppm]							
		Males				Females			
		0	3000	10000	30000	0	3000	10000	30000
ALP	mean	89	↓84	↑103	↑106	38	↓37	↑48	**↑69****
[U/L]	SD	19	20	20	13	8	9	11	37
Alb	mean	3.15	↓3.12	↓3.08	↓3.14	3.78	↑3.82	↓3.70	**↓3.40***
[g/dL]	SD	0.12	0.09	0.18	0.15	0.23	0.36	0.29	0.35

* Statistically significant from controls ($p < 0.05$);
** Statistically significant from controls ($p < 0.01$)

G. URINALYSIS

In the 30000 ppm group, urine pH in males and females was significantly lower than that in the control. Urine protein showed a significant decrease in males and a decreasing trend in females. In addition, females showed a significantly higher urine volume than that of the control, but males showed a decreasing trend in urine volume as compared with the control.

In the 10000 ppm group, urine, pH and protein in males were lower than those in the control. In females, no statistically significant change was observed in any parameter.

In the 3000 ppm group, no statistically significant changes were observed in either sex.

H. NECROPSY

Organ weights

In the 30000 ppm group, both sexes showed significant increases in absolute and relative weights of the caecum (containing contents). In addition, females in this group also showed significant increases in relative weights of the brain and liver.

In the 10000 ppm group, the absolute and relative weight of the caecum showed a statistically significant increase in males and increasing trend in females.

In the 3000 ppm group, there were no abnormalities attributable to the treatment in either sex.

Table 5.3.2-21: HR-001: 13-week Subchronic Oral Toxicity Study in Rats ([redacted], 1995): Intergroup comparison of selected organ weights – absolute and relative to body weight

Organ		Dietary concentration [ppm]							
		Males				Females			
		0	3000	10000	30000	0	3000	10000	30000
Caecum	Absolute [g]	2.823 ± 0.794	↑3.187 ± 0.609	3.383 ± 1.081	**5.854** ± 2.053**	2.367 ± 0.582	2.586 ± 0.462	**3.546** ± 0.959*	**5.268** ± 1.189**
	Relative [%]	0.55 ± 0.16	0.62 ± 0.13	0.84 ± 0.20	**1.22** ± 0.41**	0.79 ± 0.17	0.84 ± 0.17	**1.22** ± 0.32*	**1.92** ± 0.41**
Brain	Absolute [g]	2.166 ± 0.088	2.122 ± 0.077	2.174 ± 0.078	2.125 ± 0.094	1.975 ± 0.106	1.999 ± 0.077	2.013 ± 0.059	1.965 ± 0.061
	Relative [%]	0.42 ± 0.04	0.41 ± 0.03	0.42 ± 0.03	0.45 ± 0.04	0.66 ± 0.06	0.65 ± 0.06	0.70 ± 0.05	**0.72** ± 0.04*
Liver	Absolute [g]	13.11 ± 1.48	12.69 ± 1.25	14.05 ± 2.01	12.96 ± 1.99	7.14 ± 0.44	7.38 ± 0.66	7.11 ± 0.59	7.77 ± 2.72
	Relative [%]	2.53 ± 0.16	2.45 ± 0.11	2.70 ± 0.30	2.73 ± 0.24	2.40 ± 0.19	2.37 ± 0.08	2.45 ± 0.18	**2.85** ± 1.06*

* Statistically significant from controls ($p < 0.05$);
** Statistically significant from controls ($p < 0.01$)

Gross pathology

In the 30000 ppm group, distention of the caecum was observed in 9/12 males and 7/12 females with statistical significance. There were no other macroscopic abnormalities attributable to the treatment.

In the 10000 ppm group, 3/12 males showed distention of the caecum, but there were no macroscopic abnormalities in females.

In the 3000 ppm group, there were no macroscopic abnormalities attributable to the treatment in either sex.

Histopathology
Although histopathological examinations revealed various histological changes in each treatment group of both sexes, treatment-related changes were not observed. One male in the 10000 ppm group and one female in the 30000 ppm group showed renal lesion (polycystic kidney) and hepatic lesions (bile ductal proliferation and cholangiectasis). It is generally regarded that these lesions were caused by genetic disorder and were not considered to be treatment-related.

III. CONCLUSIONS

30000 ppm group: Body weights of males and females were slightly lower than in the control throughout the treatment period and statistically significant decreases were sporadically observed. The averaged food efficiency in males and females during the treatment period was slightly lower than that in the control. Females showed a significant increase in alkaline phosphatase (ALP) activity. Distention of the caecum was observed in 9/12 males and 7/12 females with statistical significance. Both sexes showed significant increases in absolute and relative weights of the caecum (containing contents). Histologically, there were no abnormalities related to treatment in any tissues including the caecum.

10000 ppm group: At necropsy, 3 males showed distention of the caecum. Organ weight analysis revealed a statistically significant increase (females) or an increasing trend (males) in both absolute and relative weights of the caecum.

3000 ppm group: There were no abnormalities attributable to the treatment in either sex.

Based on these results, the no-adverse-effect level, minimum toxic level, and sure toxic level of HR-001 in Sprague-Dawley (Crj:CD) rats under the conditions of the present study were determined as follows:

	Males	Females
No-adverse-effect level (NOAEL)	3000 ppm (168.4 mg/kg bw/day)	3000 ppm (195.2 mg/kg bw/day)
Minimum toxic level	10000 ppm (569 mg/kg bw/day)	10000 ppm (637 mg/kg bw/day)
Sure toxic level	30000 ppm (1735 mg/kg bw/day)	30000 ppm (1892 mg/kg bw/day)

3. Assessment and conclusion

Assessment and conclusion by applicant:
In this study, groups of male and female Sprague-Dawley (Crj:CD) rats were administered glyphosate technical via the diet at dose levels of 0, 3000, 10000 or 30000 ppm (equivalent to 0, 168.4, 569 or 1735 mg/kg bw/day for males and 0, 195.2, 637 or 1892 mg/kg bw/day for females) for a period of 13 weeks.

Under the experimental conditions of the study, the NOAEL is considered to be 3000 ppm (equivalent to 168.4 and 195.2 mg/kg bw/day for males and females, respectively).

Assessment and conclusion by RMS:
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1. Information on the study

Data point	CA 5.3.2/005
Report author	[redacted]
Report year	1993 (Vol. 1, Study Report)
Report title	90 Day Range Feeding Study of Glyphosate in Rats (Vol. 1)
Report No	011-0001
Document No	Not reported
Guidelines followed in study	No guideline stated, but in accordance with OECD 408 (1981)
Deviations from current test guideline (OECD 408, 2018)	Reticulocytes not counted; blood clotting not evaluated; total cholesterol but not HDL and LDL, BUN, T3, T4 and TSH evaluated; no blood hormones measured; prostate, uterus, thymus, pituitary, thyroids, spleen not weighed; seminal vesicles and coagulating glands were not examined microscopically. Deviations from the current version of OECD 408 (2018) are basically due to the fact that the study was aligned to an older (1981) version of the OECD test guideline 408.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a
Data point	CA 5.3.2/006
Report author	[redacted]
Report year	1993 (Vol. 2, Pathology Report)
Report title	90 Day Range Feeding Study of Glyphosate in Rats (Vol. 2)
Report No	011-0001
Document No	Not reported
Guidelines followed in study	No guideline stated, but in accordance with OECD 408 (1981)
Deviations from current test guideline (OECD 408, 2018)	Reticulocytes not counted; blood clotting not evaluated; total cholesterol but not HDL and LDL, BUN, T3, T4 and TSH evaluated; no blood hormones measured; prostate, uterus, thymus, pituitary, thyroids, spleen not weighed; seminal vesicles and coagulating glands were not examined microscopically. Deviations from the current version of OECD 408 (2018) are basically due to the fact that the study was aligned to an older (1981) version of the OECD test guideline 408.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

Data point	CA 5.3.2/007
Report author	[redacted]
Report year	1993 (Vol. 3, Histopathology Report)
Report title	90 Day Range Feeding Study of Glyphosate in Rats (Vol. 3)
Report No	011-0001
Document No	Not reported
Guidelines followed in study	No guideline stated, but in accordance with OECD 408 (1981)
Deviations from current test guideline (OECD 408, 2018)	Reticulocytes not counted; blood clotting not evaluated; total cholesterol but not HDL and LDL, BUN, T3, T4 and TSH evaluated; no blood hormones measured; prostate, uterus, thymus, pituitary, thyroids, spleen not weighed; seminal vesicles and coagulating glands were not examined microscopically. Deviations from the current version of OECD 408 (2018) are basically due to the fact that the study was aligned to an older (1981) version of the OECD test guideline 408.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

In a subchronic toxicity study, groups of ten male and ten female Sprague-Dawley rats were fed diets containing 0 (control), 2000, 6000 or 20000 ppm glyphosate acid (equivalent to 0, 125.2, 371.9 or 1262.1 mg/kg bw/day for males and 0, 156.3, 481.2 or 1686.5 mg/kg bw/day for females) for 13 consecutive weeks.

Clinical observations were done daily. Body weights and food consumption was assessed weekly. Haematological, blood biochemistry parameters, as well as urine analysis were conducted prior to start of treatment and at termination. At the end of the scheduled period, the animals were killed and subjected to a full examination post mortem, and selected organs were weighed and specified tissues were taken for subsequent histopathology examination.

There were no mortalities in any of the dose groups. The only treatment-related clinical finding was diarrhoea in all high-dose males and in 9 high-dose females. Mean body weights were comparable between all groups. The mean body weight gains were significantly different between groups at several time points, but the total mean body weight gain was similar in all dose groups. There were no differences in food consumption. There were no treatment-related findings in the haematological and clinical chemistry parameters. Urinalysis showed marginal increases in the mean scores for ketones, blood, protein and red blood cell counts in mid- and high-dose males, which were of no biological relevance. In mid- and high-dose females, microscopy revealed an increased number of rats with 1 red blood cell per high power field. Together with the appearance of blood in these groups this change seems to be treatment-related. The gross changes noted at necropsy were few and inconclusive with respect to association with exposure to the test article. The most prevalent abnormality was the non-treatment related swollen, reddened sublingual salivary glands. The mean weight of the adrenal glands in the high and low dose male groups was significantly decreased. The group mean weight of the spleen was significantly increased for the high dose female group when the spleen weights were compared as a percent of body weight. Gross and microscopic examinations did not identify tissue changes which were related to treatment. These findings are in agreement with

clinical chemistry, hematologic and gravimetric data collected during the conduct of this study.

The results indicate that under the conditions of this investigation, dietary levels of up to 20000 ppm glyphosate (equivalent to 1262.1 mg/kg bw/day for males and 1686.5 mg/kg bw/day for females) were tolerated without significant pathophysiologic alterations.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate acid
Description:	Greyish-white or yellowish-white crystalline powder
Lot/Batch #:	46540992
Purity:	97.5 %
Stability of test compound:	1994-10-01

2. Vehicle and/or positive control: Plain diet / none

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley (Crl:CD®BR VAF/Plus®)
Source:	[redacted]
Age:	approx. 5 weeks
Sex:	Male and female
Weight at dosing:	♂ 208.2 – 249.9 g; ♀ 159.8 – 202 g
Acclimation period:	14 days
Diet/Food:	Purina certified Laboratory Rodent Chow 5002, *ad libitum,* (except during collection of urine samples)
Water:	Tap water, *ad libitum*
Housing:	Individually in stainless steel cages
Environmental conditions:	Temperature: 18 – 26 °C Humidity: 55 ± 15 % Air changes: ≥10/hour 12 hours light/dark cycle

B: Study design and methods

In life dates: 1993-02-17 to 1993-05-21

Animal assignment and treatment:
In a 90-day oral toxicity study groups of 10 Sprague-Dawley per sex received daily doses of 0, 2000, 6000 or 20000 ppm (equivalent to 125.2, 371.9 or 1262.1 mg/kg bw/day for males and 156.3, 481.2 or 1686.5 mg/kg bw/day for females) in the diet. The test diets were prepared weekly and stored at room temperature. Samples of the control and test substance diets were analysed for stability and homogeneity. Dietary preparations were analysed for achieved concentrations weekly during the first 4 weeks, and then every fourth week thereafter.

Table 5.3.2-22: 90 Day Range Feeding Study of Glyphosate in Rats ([redacted], 1993): Study design

Test group	Dietary concentration [ppm]	Achieved dose level [mg/kg bw/day]	Males	Females
Control	0	♂: 0; ♀: 0	10	10
Low	2000	♂: 125.2; ♀: 156.3	10	10
Mid	6000	♂: 371.9; ♀: 481.2	10	10
High	20000	♂: 1262.1; ♀: 1686.5	10	10

The experimental diets were prepared weekly by adding the appropriate amount of glyphosate acid to the diet.

The homogeneity of glyphosate in the diet was determined by replicate samples from each dose level. Achieved concentrations were determined by analysing samples from each dose level during weeks 1 to 4, and every fourth week thereafter. The chemical stability of glyphosate acid in diet at room temperature was determined for all dose levels after preparation. Analysis was done by high performance liquid chromatography (HPLC).

Mortality
Each animal was checked for mortality or signs of morbidity twice daily during the treatment period.

Clinical observations
A check for clinical signs of toxicity was made once daily on all animals.

Body weight
The body weight of each animal was recorded on Day 1 before start of treatment and weekly thereafter.

Food consumption and utilisation
Individual food consumption was recorded weekly.

Ophthalmoscopic examination
The eyes of all animals from all dose groups were examined prior to initiation and just prior to termination. The examination included macroscopic and ophthalmoscopic examinations of the anterior portion of the eye, the optic media, and the ocular fundus.

Haematology and clinical chemistry
Prior to initiation and at termination, blood samples from all rats were taken by puncture of the retro-orbital sinus after anesthetisation. These samples were submitted for haematological and clinical chemistry examination. The following haematological parameters were measured: Haemoglobin, haematocrit, red blood cell count, mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), thrombocytes, leucocytes and a blood smear examination (including differential white cell count).

For clinical chemistry analysis the following parameters were measured: Glucose, urea, total protein, albumin, globulin, albumin/globulin ratio, total cholesterol, creatinine, calcium, phosphorous, sodium, potassium, chloride, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamic transferase.

Urinalysis
Urine samples were collected overnight from all rats prior to start of treatment and at termination. During urine collection, the rats were individually housed in metabolism cages and denied access to food. The following parameters were measured: Volume, pH, specific gravity, nitrites, blood, leukocytes, protein, glucose, ketones, bilirubin and urobilinogen.

Sacrifice and pathology
After 13 weeks of consecutive treatment, all surviving animals were sacrificed and subjected to a gross pathological examination. Any macroscopic findings were recorded.
The following organ weights were determined: Adrenals, brain, epididymides, kidneys, liver, seminal vesicles and testes or ovaries. Organ-to-body weight and organ-to-brain weights were determined.

Tissue samples were taken from the following organs and preserved in buffered formalin: All gross lesions, adrenals, aorta, bone marrow (sternum), brain, caecum, colon, duodenum, epididymides, eyes, femur (incl. articular surface), heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes (mandibular and mesenteric), mammary gland, nasal turbinate, nasal cavity, oesophagus, ovaries, pancreas, pituitary gland, prostrate, rectum, salivary glands (sublingual), spinal cord, sciatic nerve, skeletal muscle, skin, spleen, stomach, testes, thymus, thyroid/parathyroid, trachea and urinary bladder. All tissue sampled from the control and high dose group were examined histopathologically. From the mid- and low- dose only tissues from lungs, liver, kidneys and gross lesions were subjected to histopathological evaluation.

Statistics
Mean and standard deviation were calculated for all quantitative data. Comparisons with controls were done using ANOVA with a post hoc Dunnett´s t Test or Duncan´s multiple range test. A 95 % confidence level ($p < 0.05$) was used to determine statistically significant differences between control and treated groups.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities.

B. CLINICAL OBSERVATIONS
The only obvious treatment-related finding was diarrhoea observed in 10/10 males and 9/10 females in the high-dose group. Diarrhoea was also observed in one male of the low dose group.

Table 5.3.2-23: 90 Day Range Feeding Study of Glyphosate in Rats ([redacted], 1993): Intergroup comparison of clinical findings

	Dietary concentration of glyphosate acid [ppm]							
	Males				Females			
Finding	0	2000	6000	20000	0	2000	6000	20000
Diarrhoea	0/10	1/10	0/10	10/10	0/10	0/10	0/10	9/10

C. BODY WEIGHT
Mean body weights were not significantly different between test substance and control animals. The mean body weight gains were however significantly different from controls at several intervals (see table below). The total mean body weights over the entire study period were not statistically different.

Table 5.3.2-24: 90 Day Range Feeding Study of Glyphosate in Rats ([redacted], 1993): Intergroup comparison of body weights and body weight gain – selected time points from start of study

	Mean body weight or body weight gain [g]					
Time point	Initial body weight	Final body weight	Weight gain Day 43	Weight gain Day 50	Weight gain Day 85	Total weight gain
Dose [ppm]	Males					
0	224.6	510.3	-3.4	39.4	12.6	285.7
2000	↑231.0	↑530.8	↑9.1	↓29.3	↓9.2	↑299.7
6000	↑229.0	↑526.0	↑**17.1***	↓28.6	↓11.1	↑297.0
20000	224.6	↑512.9	↑**21.1****	↓**19.3****	↓**1.9***	↑288.4

Table 5.3.2-24: 90 Day Range Feeding Study of Glyphosate in Rats ([redacted], 1993): Intergroup comparison of body weights and body weight gain – selected time points from start of study

Time point	Mean body weight or body weight gain [g]					
	Initial body weight	Final body weight	Weight gain Day 43	Weight gain Day 50	Weight gain Day 85	Total weight gain
	Females					
0	178.3	321.9	6.9	16.6	6.1	143.7
2000	↑179.7	↑331.3	↑7.5	↓14.7	↑6.2	↑151.6
6000	↓176.0	↓316.1	↑7.8	↓15.7	↓2.4	↓140.1
20000	↓176.9	↓309.0	↑7.8	**↓4.8***	↓2.8	↓132.0

* Statistically significant from controls, $p < 0.05$ (Student´s t-test, 2-sided);
** Statistically significant from controls, $p < 0.01$ (Student´s t-test, 2-sided)

D. FOOD CONSUMPTION AND TEST SUBSTANCE INTAKE

There were no treatment-related effects. The mean food consumption in treatment groups was not significantly different from controls.

Calculated mean test compound intakes are presented in the following table.

Table 5.3.2-25: 90 Day Range Feeding Study of Glyphosate in Rats ([redacted], 1993): Overall mean test compound intake [mg/kg bw/day]

	Dietary concentration of glyphosate acid [ppm]							
	Males				Females			
	0	2000	6000	20000	0	2000	6000	20000
Achieved dose [mg/kg bw/day]	0	125.2	371.9	1262.1	0	156.3	481.2	1686.5

E. OPHTHALMOSCOPIC EXAMINATION

There were no test substance-related ophthalmological findings at the end of the treatment period.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

There were no treatment-related differences noted in any dose group.

Blood clinical chemistry

At termination, low dose males had a significantly decreased mean potassium (K^+) concentration as compared to control animals. Since this finding was restricted to the low dose group, it was not considered to be treatment-related.

High dose males showed a significantly lower ALT value at termination than control males. However, because of the minor degree of change, the absence of a dose-response at the other dose levels this finding was considered to be of no biological relevance. The increased total bilirubin value in high-dose males at termination is also considered to be of no biological relevance.

Table 5.3.2-26: 90 Day Range Feeding Study of Glyphosate in Rats ([redacted], 1993): Intergroup comparison of selected clinical chemistry parameters pre-dose and at termination

Parameter	Dietary concentration of glyphosate acid [ppm]							
	Males				Females			
	0	2000	6000	20000	0	2000	6000	20000
ALT [U/L]	48	↓35	↓41	**↓32***	42	↓39	↓35	↓30
Total bilirubin	0.3	0.3	↑0.5	**↑0.6***	0.2	↑0.3	↑0.3	↑0.3

Table 5.3.2-26: 90 Day Range Feeding Study of Glyphosate in Rats ([redacted], 1993): Intergroup comparison of selected clinical chemistry parameters pre-dose and at termination

	Dietary concentration of glyphosate acid [ppm]							
	Males				Females			
Parameter	0	2000	6000	20000	0	2000	6000	20000
[mg/dL]								
Potassium [mmol/L]	6.9	↓**5.8****	↓6.2	↓6.8	5.8	↑6.2	↓5.7	↑6.2
Potassium [mmol/L] (pre-dose)	5.2	↓5.1	↓4.8	5.2	5.2	↓**4.6***	↓**4.4****	↓4.7

* Statistically significant from controls, $p < 0.05$;
** Statistically significant from controls, $p < 0.01$

G. URINALYSIS

At termination there were marginal increases in the mean scores for ketones, blood, protein and red blood cells (RBCs) in the mid- and high-dose males. It has to be noted that the unusual appearance of statistical differences among zeros (ketones) or 1 (protein) was the result of rounding (see table below). The number of affected rats and their respective scores are shown in the Table 5.3.2-27 below. The lower protein value observed in high-dose males was statistically different from control, but still within the normal range. The presence of blood and RBCs is minimally elevated in all treated groups when compared to control animals. However, the observation of a few RBCs is common in male rats and this mild degree cannot be attributed unequivocally to the test substance. In mid- and high-dose females the number of rats with 1 RBC/high power microscope field was also increased. Together with the appearance of blood in these groups this change seems to be treatment-related. In addition, there were no indications for microscopic haematuria found during the histopathological examinations. Therefore, the changes in urine analysis parameters in the mid-dose group are considered not to be adverse effects.

Table 5.3.2-27: 90 Day Range Feeding Study of Glyphosate in Rats ([redacted], 1993): Urinalysis at termination – group mean values for selected parameters

	Dietary concentration of glyphosate acid [ppm]							
	Males				Females			
Parameter	0	2000	6000	20000	0	2000	6000	20000
Ketones [mg/dL]	0	0	**0***	0	0	0	0	0
Blood [mg/dL]	0	2	1	**2****	0	0	1	1
Protein [mg/dL]	1	1	1	**1***	0	0	0	0
RBC [cells/hpf]	0	1	1	**2****	0	0	**1****	1

* Statistically significant from controls, $p < 0.05$;
** Statistically significant from controls, $p < 0.01$

Table 5.3.2-28: 90 Day Range Feeding Study of Glyphosate in Rats ([redacted] 1993): Urinalysis at termination – affected animals for selected parameters

	Dietary concentration of glyphosate acid [ppm]							
	Males				Females			
Parameter	0	2000	6000	20000	0	2000	6000	20000
Ketones (Score 0)	10/10	10/10	6/10	9/10	10/10	10/10	10/10	10/10
Ketones (Score 1)	0/10	0/10	4/10	1/10	0/10	0/10	0/10	0/10
Blood (score 0)	8/10	3/10	3/10	2/10	8/10	8/10	5/10	7/10
Blood (score 1)	2/10	3/10	3/10	3/10	2/10	2/10	2/10	0/10
Blood (score 2)	0/10	2/10	2/10	1/10	0/10	0/10	2/10	3/10
Blood (score 3)	0/10	0/10	1/10	0/10	0/10	0/10	0/10	0/10
Blood (score 4)	0/10	2/10	1/10	4/10	0/10	0/10	1/10	0/10
Protein (score 0)	1/10	3/10	1/10	6/10	9/10	7/10	7/10	10/10

Table 5.3.2-28: 90 Day Range Feeding Study of Glyphosate in Rats ([redacted], 1993): Urinalysis at termination – affected animals for selected parameters

Parameter	Dietary concentration of glyphosate acid [ppm]							
	Males				Females			
	0	2000	6000	20000	0	2000	6000	20000
Protein (score 1)	5/10	6/10	7/10	3/10	1/10	2/10	3/10	0/10
Protein (score 2)	4/10	1/10	2/10	1/10	0/10	1/10	0/10	0/10
RBC (score 1)	3/10	7/10	8/10	6/10	2/10	2/10	8/10	6/10
RBC (score 2)	-	-	-	1/10	-	-	1/10	-
RBC (score 3)	-	-	-	0/10	-	-	-	-
RBC (score 4)	-	-	-	2/10	-	-	-	-

- No finding

H. NECROPSY

Organ weights

The mean adrenal weights in high- and low-dose males were significantly decreased, whereas the relative adrenal weights were only significantly decreased in high dose males. In females of the high-dose group relative spleen weights were significantly increased when compared to controls.

Table 5.3.2-29: 90 Day Range Feeding Study of Glyphosate in Rats ([redacted], 1993): Results from absolute and relative organ weight determination

	Dietary concentration of glyphosate acid [ppm]							
	Males				Females			
	0	2000	6000	20000	0	2000	6000	20000
Mean adrenal weight [g]	0.070 ± 0.009	↓**0.059* ± 0.008**	↓0.061 ± 0.014	↓**0.052* * ± 0.006**	0.093 ± 0.013	↓0.080 ± 0.008	↓0.086 ± 0.022	↓0.080 ± 0.010
Relative adrenal weight [% bw]	0.014 ± 0.003	↓0.012 ± 0.002	↓0.012 ± 0.003	↓**0.011* * ± 0.001**	0.031 ± 0.004	↓0.026 ± 0.004	↓0.029 ± 0.008	↓0.028 ± 0.002
Spleen weight [g]	0.785 ± 0.103	↑0.786 ± 0.093	↑0.808 ± 0.121	↓0.752 ± 0.090	0.548 ± 0.071	↑0.598 ± 0.066	↑0.589 ± 0.103	↑0.648 ± 0.030
Relative spleen weight [% bw]	0.157 ± 0.026	↓0.154 ± 0.018	↑0.160 ± 0.024	↓0.152 ± 0.015	0.180 ± 0.017	↑0.192 ± 0.029	↑0.198 ± 0.026	↑**0.223* * ± 0.035**

* Statistically significant from controls, $p < 0.05$;
** Statistically significant from controls, $p < 0.01$

Gross pathology

A few gross lesions were noted at necropsy in all dose groups. Swollen, reddened sublingual salivary glands were observed in one control male and in one treated male and female of some test substance groups (see table Table 5.3.2-30). Therefore, this finding is considered not to be related to treatment.

Table 5.3.2-30: 90 Day Range Feeding Study of Glyphosate in Rats ([redacted], 1993): Summary of necropsy findings

Finding	Dietary concentration of glyphosate acid [ppm]							
	Males				Females			
	0	2000	6000	20000	0	2000	6000	20000
Sublingual salivary glands: enlarged and/or reddened	1/10	0/10	1/10	1/10	0/10	1/10	0/10	1/10

Histopathology
There were no histopathological findings related to treatment. The incidence of findings was low and of a type commonly found in rats of this strain and age.

III. CONCLUSIONS

There were no mortalities in any of the dose groups. The only treatment-related clinical finding was diarrhoea in all high-dose males and in 9 high-dose females. Mean body weights were comparable between all groups. The mean body weight gains were significantly different between groups at several time points, but the total mean body weight gain was similar in all dose groups. There were no differences in food consumption. There were no treatment-related findings in the haematological and clinical chemistry parameters. Urinalysis showed marginal increases in the mean scores for ketones, blood, protein and red blood cell counts in mid- and high-dose males, which were of no biological relevance. In mid- and high-dose females, microscopy revealed an increased number of rats with 1 red blood cell per high power field. Together with the appearance of blood in these groups this change seems to be treatment-related. The gross changes noted at necropsy were few and inconclusive with respect to association with exposure to the test article. The most prevalent abnormality was the non-treatment related swollen, reddened sublingual salivary glands. The mean weight of the adrenal glands in the high and low dose male groups was significantly decreased. The group mean weight of the spleen was significantly increased for the high dose female group when the spleen weights were compared as a percent of body weight. Gross and microscopic examinations did not identify tissue changes which were related to treatment. These findings are in agreement with clinical chemistry, hematologic and gravimetric data collected during the conduct of this study.

The results indicate that under the conditions of this investigation, dietary levels of up to 20000 ppm glyphosate were tolerated without significant pathophysiologic alterations.

Based on the results of this study, the Maximum Tolerated Dose (MTD) was reported to be 20000 ppm glyphosate and the No Observable Adverse Effects Level (NOAEL) was reported to be 2000 ppm glyphosate.

3. Assessment and conclusion

Assessment and conclusion by applicant:
In this sub-chronic toxicity study, groups of ten male and ten female Sprague-Dawley rats were fed diets containing 0, 2000, 6000 or 20000 ppm glyphosate acid (equivalent to 0, 125.2, 371.9 or 1262.1 mg/kg bw/day for males and 0, 156.3, 481.2 or 1686.5 mg/kg bw/day for females) for 13 consecutive weeks.

The results indicate that under the conditions of this investigation, dietary levels of up to 20000 ppm glyphosate (equivalent to 1262.1 mg/kg bw/day for males and 1686.5 mg/kg bw/day for females) were tolerated without significant pathophysiologic alterations.

Based on the results of this study, the Maximum Tolerated Dose (MTD) was 20000 ppm glyphosate (equivalent to 1262.1 mg/kg bw/day for males and 1686.5 mg/kg bw/day for females).
There were no consistent effects at the dose levels of 6000 and 20000 ppm. At the highest dose of 20000 ppm there were statistically significant effects on organ weights, i.e. reduced adrenal weights in males and increased spleen weights in females. Therefore, the No Observable Adverse Effect Level (NOAEL) was set at 6000 ppm glyphosate (equivalent to 371.9 mg/kg bw/day for males and 481.2 mg/kg bw/day for females).

Assessment and conclusion by RMS:
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1. Information on the study

Data point:	CA 5.3.2/008
Report author	[redacted]
Report year	1992 (study report)
Report title	90–day Oral Toxicity Study with Glyphosate Technical in Wistar Rats (FSG 03090 h/05 March 1990)
Report No	ES.882.90 OR
Document No	Not reported
Guidelines followed in study	OECD 408 (1981)
Deviations from current test guideline (OECD 408, 2018)	The following organs were not noted in the gross pathology evaluation: aorta, cervix, epididymides, mammary gland, peripheral nerve, prostate, salivary glands, skeletal muscle and bone, skin, spinal cord, thymus, vagina. The following organs were not weighed: testes, epididymides, prostate and seminal vesicles with coagulating glands, thymus, heart, brain, and spleen. Thyroid hormone levels (i.e., T4, T3, and TSH) were not measured. It was not noted if an ophthalmological examination was conducted. Sensory reactivity to different stimuli was not evaluated. The rationale for dose selection was not provided. Deviations from the current version of OECD 408 (2018) are basically due to the fact that the study was aligned to an older version of the OECD test guideline 408.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a
Data point:	CA 5.3.2/009
Report author	[redacted]
Report year	1992 (appendix to study report)
Report title	90–day Oral Toxicity Study with Glyphosate Technical in Wistar Rats (FSG 03090 h/05 March 1990) (Appendix)
Report No	ES.882.90 OR
Document No	Not reported
Guidelines followed in study	OECD 408 (1981)
Deviations from current test guideline (OECD 408, 2018)	The following organs were not noted in the gross pathology evaluation: aorta, cervix, epididymides, mammary gland, peripheral nerve, prostate, salivary glands, skeletal muscle and bone, skin, spinal cord, thymus, vagina. The following organs were not weighed: testes, epididymides, prostate and seminal vesicles with coagulating glands, thymus, heart, brain, and spleen. Thyroid hormone levels (i.e., T4, T3, and TSH) were not measured. It was not noted if an ophthalmological examination was conducted. Sensory reactivity to different stimuli was not evaluated. The rationale for dose selection was not provided. Deviations from the current version of OECD 408 (2018) are basically due to the fact that the study was aligned to an older version of the OECD test guideline 408.

Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a
Data point:	CA 5.3.2/010
Report author	[redacted]
Report year	June 1994 (1st amendment); November 1994 (2nd amendment)
Report title	Amendments to Final Report. 90–day Oral Toxicity Study in Wistar Rats
Report No	ES.882.90 OR
Document No	Not reported
Guidelines followed in study	OECD 408 (1981)
Deviations from current test guideline (OECD 408, 2018)	The following organs were not noted in the gross pathology evaluation: aorta, cervix, epididymides, mammary gland, peripheral nerve, prostate, salivary glands, skeletal muscle and bone, skin, spinal cord, thymus, vagina. The following organs were not weighed: testes, epididymides, prostate and seminal vesicles with coagulating glands, thymus, heart, brain, and spleen. Thyroid hormone levels (i.e., T4, T3, and TSH) were not measured. It was not noted if an ophthalmological examination was conducted. Sensory reactivity to different stimuli was not evaluated. The rationale for dose selection was not provided. Deviations from the current version of OECD 408 (2018) are basically due to the fact that the study was aligned to an older version of the OECD test guideline 408.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

In a 90–day toxicity study, groups of 10 male and 10 female Wistar rats were administered technical glyphosate for 90 consecutive days via the diet at concentrations of 0, 200, 2000, or 20000 ppm. These dose levels were equivalent to 0, 14.0, 147.3, or 1358.6 mg/kg bw/day for males and 0, 18.6, 195.7, or 2012.4 mg/kg bw/day for females, respectively. A high-dose recovery group was administered 20000 ppm (equivalent to 1482.6 mg/bw/day for males and 1878.3 mg/kg bw/day for females) for 13 weeks and then were followed for 4 weeks without treatment before being sacrificed.

General clinical observations were done daily. Body weights and food consumption were assessed in weekly intervals. Haematological and blood biochemistry parameters were evaluated at sacrifice. At the end of the scheduled period, the animals were killed and subjected to a post-mortem examination and selected organs were weighed and tissues were taken for subsequent histopathology examination.

There was no mortality in any of the study groups during treatment or recovery period. In general, there were no clinical signs of toxicity observed in any of the treatment groups. While significant changes were observed in body weight gains, haematology parameters, and clinical chemistry parameters, these changes were considered to be incidental in nature. At 20000 ppm, decreased body weight gains were observed in female rats; body weight changes were not observed in males. Significant changes were observed in haematology values for males (decreased eosinophils at the high dose, increased mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration at the mid dose and high-dose recovery group, decreased red blood cells at the mid and low dose, and decreased haematocrit at the low dose). There were no significant changes to haematology parameters for female rats at any dose level.

With regards to clinical chemistry parameters, there was an increase in alkaline phosphatase activity (high dose) and a decrease in albumin levels (low dose) and total bilirubin (high-dose recovery group) in males. For females, there was a decrease in creatinine (all dose levels) and calcium concentrations (all doses except the recovery group), while there was an increase in glucose levels (high dose). There also was decrease in glucose levels for the high-dose recovery group when compared with the high dose.

There were no notable intergroup differences in organ weights. No gross pathology or histopathology findings attributed to administration of glyphosate were recorded.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Technical Glyphosate
Description:	Solid, odourless white coloured crystals
Lot/Batch #:	FSG 03090 h/05 March 1990
Purity:	96.8 %
Stability of test compound:	Fairly stable for 30 days under ambient temperature and stored in polyethylene lined stainless steel drums

2. Vehicle and/or positive control: Plain diet / none

3. Test animals:

Species:	Rat
Strain:	Wistar
Source:	[redacted]
Age:	8 weeks
Sex:	Male and female
Weight at dosing:	♂ group means 163 ± 17.1 g; ♀ group means 132 ± 14.6 g
Acclimation period:	At least one week
Diet/Food:	Standard "Gold Mohur" brand powdered rat feed , *ad libitum,*
Water:	Deep borewell water passed through activation charcoal filter and exposed to UV rays, *ad libitum*
Housing:	Groups of 3-5 rats/ sex in steam sterilized standard polypropylene rat cages with stainless steel top grill
Environmental conditions:	Temperature: 23 ± 2 °C Humidity: 40 – 70 % Air changes: 10 - 15/hour 12 hours light/dark cycle

B: Study design and methods

In life dates: May 1991 to September 1991

Animal assignment and treatment:
Groups of ten male and ten female Wistar rats were administered technical glyphosate for 90 consecutive days via the diet at concentrations calculated to achieve dose levels of 0 (control), 20, 2000, or 20000 ppm.

Table 5.3.2-31: 90–day Oral Toxicity Study with Glyphosate Technical in Wistar Rats (FSG 03090 h/05 March 1990) ([redacted] 1992): Animal assignment

Test group	Dose Level (ppm)	Males	Females
Control	0	10	10
Low	200	10	10
Intermediate	2000	10	10
High	20000	10	10
High Recovery	20000	10	10

The required amount of finely ground test compound was weighed and mixed manually with 1.0 kg powdered rat feed to prepare the premix. The premix was added in portions to the remaining quantity of feed and mixed. To the control group feed, the powdered rat feed was mixed for 20 minutes. Prepared feed was sampled at different levels for assaying the test compound concentration.

All test groups received diet specifically prepared for the group ad libitum. Animals were treated for seven days a week for thirteen weeks. The recovery group received powdered normal rat feed for four weeks following thirteen weeks of treatment.

Mortality
Each animal was checked for mortality or signs of morbidity once daily during the treatment period.

Clinical observations
General clinical observations were done once daily. Cage side observations included changes in skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous system, somatomotor activity and behaviour pattern.

Body weight
Individual body weights were recorded at the end of each week.

Food consumption and utilisation
Daily feed consumption per cage measured during the last two days of the week.

Ophthalmoscopic examination
Dedicated eye examinations were not performed.

Haematology and clinical chemistry
One day prior to sacrifice, blood smears from surviving rats were made by tail clipping and the blood smears were stained by Wright's stain. At the end of the study all the surviving animals were fasted overnight (water allowed) and blood was collected from abdominal aorta under ether anaesthesia.

For haematology, differential leucocyte counts were done manually. Fractions of blood were taken for coagulation time. For haematology and plasma separation, blood was heparinized. The following haematological parameters were measured: white blood cell (WBC) count, red blood cell (RCB) count,

haemoglobin (Hb), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC).

For clinical chemistry analysis the following parameters were measured: glucose (GLU), total bilirubin (TOT BIL), creatinine (CREAT), glutamic pyruvic transaminase, glutamic oxaloacetic transaminase, enzymatic blood urea nitrogen, albumin (ALB), alkaline phosphatase (ALP), total protein, sodium, potassium, and calcium (Ca^{2+}).

Urinalysis
Not performed.

Sacrifice and pathology
At the end of the study, rats were fasted overnight and sacrificed by total blood collection under either anaesthesia. After sacrifice, a detailed gross necropsy was conducted. The following organs were collected and preserved from every animal: adrenals (both), gonads (both), kidneys (both), and liver. The relative organ weights were determined as a percentage of body weight. Tissue samples from brain, lungs, heart, eye (Bouin's fluid), spleen, lymph nodes, (mesenteric) oesophagus, stomach, small and large intestines, salivary gland, liver, pancreas, kidneys (both), urinary bladder, testes/ovaries (both), seminal vesicles/uterus, pituitary, thyroid and adrenals (both) were preserved in neutral buffered formalin and 5 µm sections were stained. The tissues sections were studied for histopathological changes. All the tissues from all animals in control and high-dose group and all lesions were subjected to detailed histopathological studies.

Statistics
Body weight and feed consumption were compared using the Bartlett's test for homogeneity of variance and one-way classification analysis of variance (ANOVA) and Dunnet's multiple pairwise comparison. The clinical laboratory analysis data and organ weight data from the control group were compared with the data of treated groups by the Bartlett's test for homogeneity of variance followed by ANOVA, where the variances proved to be heterogeneous, the data was transformed using the appropriate transformation. If ANOVA of homogeneous data was significant, Dunnet's pairwise comparison procedure was used to compare the treated group with the control group individually. All analyses were evaluated at 5 % probability level.

II. RESULTS AND DISCUSSION

A. MORTALITY
There was no mortality in any of the study groups during treatment or recovery period.

B. CLINICAL OBSERVATIONS
In general there were no clinical signs of toxicity observed in any of the treatment groups. However, respiratory effects (i.e., nasal discharge) was reported in treatment groups; this effect appeared to reduce during the recovery period.

C. BODY WEIGHT
For males, body weight gains were comparable between treatment groups and control animals and there were no statistically significant differences. For females, initial body weights (week 0) were lower in treated than control groups and in the low-dose group this achieved statistical significance; however, body weights of low-dose animals were comparable to control animals in later weeks. High-dose animals also showed significantly lower body weight gains when compared to control animals from Week 3 through Week 6, as well as week 10. Similar results were not observed in the high-dose recovery group, which casts some doubt on the significance of these high dose changes. Results are presented in the following table:

Table 5.3.2-32: 90–day Oral Toxicity Study with Glyphosate Technical in Wistar Rats (FSG 03090 h/05 March 1990) ([redacted] 1992): Intergroup comparison of mean body weights and body weight gain

Body weight gain [g ± standard deviation]	Mean dietary concentration of glyphosate [ppm]									
	Males					Females				
	0	200	2000	20000	20000 (recovery group)	0	200	2000	20000	20000 (recovery group)
Week 0	166 ± 11.5	154 ± 22.0	163 ± 21.6	168 ± 22.8	166 ± 7.8	144 ± 18.1	**121 ± 15.0*** ↓	132 ± 8.6	129 ± 13.8	136 ± 17.5
Week 1	194 ± 14.3	185 ± 23.5	183 ± 22.6	194 ± 22.9	192 ± 9.3	152 ± 17.2	140 ± 12.6	142 ± 7.8	140 ± 13.7	152 ± 18.9
Week 2	223 ± 17.2	215 ± 25.4	209 ± 23.2	221 ± 26.6	218 ± 14.3	165 ± 16.6	153 ± 14.0	155 ± 6.3	150 ± 12.5	171 ± 23.8
Week 3	245 ± 17.4	234 ± 21.5	238 ± 25.9	237 ± 26.5	232 ± 17.8	178 ± 16.4	171 ± 13.3	166 ± 7.4	**161 ± 13.1*** ↓	181 ± 23.9
Week 4	256 ± 18.4	242 ± 20.9	247 ± 23.7	246 ± 26.5	252 ± 15.7	178 ± 16.5	179 ± 14.5	173 ± 6.4	165 ± 11.9	186 ± 24.9
Week 5	256 ± 17.2	257 ± 19.7	260 ± 25.5	260 ± 27.2	262 ± 15.8	181 ± 16.1	179 ± 16.4	173 ± 7.0	**164 ± 14.1*** ↓	191 ± 31.6
Week 6	274 ± 18.9	268 ± 22.7	266 ± 24.2	266 ± 27.8	268 ± 17.7	187 ± 16.6	182 ± 13.1	177 ± 8.3	**168 ± 16.1*** ↓	193 ± 31.1
Week 7	295 ± 17.9	280 ± 24.0	284 ± 23.2	282 ± 29.0	286 ± 20.7	196 ± 19.1	193 ± 16.9	185 ± 8.6	181 ± 22.6	200 ± 29.3
Week 8	300 ± 18.5	284 ± 25.4	290 ± 22.4	287 ± 29.4	288 ± 19.2	202 ± 17.3	194 ± 18.6	189 ± 8.2	186 ± 24.7	203 ± 26.1
Week 9	307 ± 20.6	292 ± 26.3	294 ± 20.4	295 ± 32.0	292 ± 20.6	204 ± 15.5	197 ± 16.7	192 ± 6.8	185 ± 21.9	202 ± 25.2
Week 10	313 ± 19.3	300 ± 28.5	297 ± 21.0	302 ± 33.7	302 ± 20.3	212 ± 14.7	204 ± 19.9	198 ± 8.8	**184 ± 22.2*** ↓	205 ± 26.5
Week 11	321 ± 21.7	300 ± 30.5	297 ± 15.3	301 ± 32.2	304 ± 19.8	212 ± 15.6	203 ± 19.6	199 ± 6.9	189 ± 22.5	204 ± 25.1
Week 12	321 ± 24.7	306 ± 28.8	305 ± 19.6	307 ± 30.4	310 ± 27.1	213 ± 16.3	205 ± 21.4	205 ± 8.4	194 ± 22.8	202 ± 24.1
Week 13	322 ± 23.2	315 ± 24.1	313 ± 20.2	313 ± 36.5	315 ± 30.7	213 ± 14.8	205 ± 22.2	208 ± 10.6	199 ± 21.7	209 ± 20.5

* Significant at P = 0.05 over control group value
↓ Decreased

D. FOOD CONSUMPTION AND TEST SUBSTANCE INTAKE

All groups receiving Glyphosate performed similarly to their respective controls. Test compound intakes are presented in the following table:

Table 5.3.2-33: 90–day Oral Toxicity Study with Glyphosate Technical in Wistar Rats (FSG 03090 h/05 March 1990) ([redacted] 1992): Test compound intake

	Mean dietary concentration of glyphosate [ppm]									
	Males					Females				
	0	200	2000	20000	20000 (recovery group)	0	200	2000	20000	20000 (recovery group)
Dose [mg/kg bw/day]	0	14.0	147.3	1358.6	1482.6	0	18.6	195.7	2012.4	1878.3

E. OPHTHALMOSCOPIC EXAMINATION

Ophthalmoscopic eye examinations were not performed.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

There were no major treatment-related changes apparent in both sexes. Male rats showed incidental significant changes, including decreased eosinophils at the high dose, increased MCH and MCHC in the mid and high dose recovery groups, decreased RBC at the mid and low dose, and decreased HCT at the low dose. There were no significant changes to haematology parameters for female rats at any dose level.

Table 5.3.2-34: 90–day Oral Toxicity Study with Glyphosate Technical in Wistar Rats (FSG 03090 h/05 March 1990) ([redacted] 1992): Intergroup comparison of selected haematology parameters (mean ± SD) in male rats

Parameter	Males				
	Dose level [ppm]				
	0	200	2000	20000	20000 (recovery group)
RBC [10^6/mm^3]	9.26 ± 0.36	**8.72 ± 0.37***↓	**8.38 ± 0.59***↓	8.96 ± 0.47	8.48 ± 0.69
HCT [%]	37.9 ± 1.97	35.1 ± 1.87	35.7 ± 2.24	37.6 ± 3.12	39.6 ± 1.74
MCH pg [10^{-12}]	11.9 ± 0.57	**13.6 ± 1.32***↑	**13.1 ± 0.93***↑	12.7 ± 1.01	**18.9 ± 1.34****↑
MCHC [%]	29.2 ± 2.09	**34.1 ± 3.89***↑	30.9 ± 1.67	30.5 ± 3.06	**39.7 ± 2.24****↑
Eosinophils [%]	3 ± 1.49	2 ± 2.22	2 ± 1.83	1 ± 1.05	2 ± 2.84

* Significant at P = 0.05 over control group value
** Significant difference at P = 0.05 between the high-dose group and the high-dose recovery group
↓ Decreased; ↑ Increased

Blood clinical chemistry

For males, there was a significant increase in ALP activity at the high dose that remained high (although not statistically significant) even at the end of the recovery period. Low-dose males exhibited a significant decrease in albumin levels. High-dose recovery males exhibited a significant decrease in total bilirubin.

For females, a significant decrease in creatinine concentrations was observed at all dose levels. Calcium concentrations were low in all treatment groups except the high-dose recovery group that showed levels that were similar to control animals. Decreases in calcium concentrations reached significance at the mid dose, with the low- and high-dose groups showing a non-significant decrease. A significant increase in

glucose levels also were observed at the high dose, while a significant decrease in glucose levels was observed in the high-dose recovery group when compared with the high dose.

Table 5.3.2-35: 90–day Oral Toxicity Study with Glyphosate Technical in Wistar Rats (FSG 03090 h/05 March 1990) ([redacted]., 1992): Intergroup comparison of selected clinical chemistry parameters (mean ± SD) in male rats

	Males				
	Dose level [ppm]				
Parameter	**0**	**200**	**2000**	**20000**	**20000 (recovery group)**
ALP [IU/L]	105 ± 32.1	121 ± 31.1	106 ± 29.5	**157 ± 45.7*↑**	150 ± 55.7
ALB [g/dL]	3.4 ± 0.11	**3.3 ± 0.09*↓**	3.3 ± 0.16	3.4 ± 0.08	3.5 ± 0.18
TOT BIL [mg/dL]	0.51 ± 0.20	0.45 ± 0.20	0.47 ± 0.22	0.71 ± 0.23	**0.35 ± 0.10**↓**

* Significant at P = 0.05 over control group value
** Significant difference at P = 0.05 between the high-dose group and the high-dose recovery group
↓ Decreased; ↑ Increased

Table 5.3.2-36: 90–day Oral Toxicity Study with Glyphosate Technical in Wistar Rats (FSG 03090 h/05 March 1990) ([redacted]., 1992): Intergroup comparison of selected clinical chemistry parameters (mean ± SD) in female rats

	Females				
	Dose level [ppm]				
Parameter	**0**	**200**	**2000**	**20000**	**20000 (recovery group)**
GLU [mg/dL]	114 ± 25.1	124 ± 16.0	124 ± 20.3	**141 ± 20.6*↑**	**110 ± 13.3**↓**
CREAT [mg/dL]	0.63 ± 0.06	**0.55 ± 0.06*↓**	**0.56 ± 0.04*↓**	**0.56 ± 0.03*↓**	**0.50 ± 0.06*↓**
Ca^{2+} [mg/dL]	10.0 ± 0.65	9.4 ± 0.51	**9.2 ± 0.48*↓**	9.6 ± 0.50	10.2 ± 1.05

* Significant at P = 0.05 over control group value
** Significant difference at P = 0.05 between the high-dose group and the high-dose recovery group
↓ Decreased; ↑ Increased

G. URINALYSIS

Not performed

H. NECROPSY

Organ weights

There were no intergroup differences in either sex.

Gross pathology

Gross pathological observations were in general evenly distributed across all treatment groups. A few gross lesions were observed in the liver and lung; however, these changes were not considered treatment related.

Table 5.3.2-37: 90–day Oral Toxicity Study with Glyphosate Technical in Wistar Rats (FSG 03090 h/05 March 1990) ([redacted] 1992): Summary incidence of gross pathological findings

Findings	Dose level [ppm]									
	Males					Females				
	0	200	2000	20000	20000 (recovery group)	0	200	2000	20000	20000 (recovery group)
Liver										
Focal thickening of capsule	1/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Yellow discolouration of papillary process	0/10	0/10	0/10	1/10	0/10	0/10	0/10	0/10	0/10	0/10
Pale	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	2/10	0/10
Lungs										
Petechiae	1/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Consolidation	0/10	0/10	0/10	0/10	2/10	1/10	0/10	0/10	0/10	0/10
Emphysema	0/10	0/10	0/10	0/10	1/10	1/10	0/10	0/10	0/10	0/10
Echymoses	0/10	0/10	0/10	0/10	1/10	0/10	0/10	0/10	0/10	0/10

Histopathology

There were no treatment-related findings reported during the histopathological evaluation. Hyperplasia of the lymphoid nodules in the submucosa of the duodenum, ileum and colon were observed but these effects were considered to be spontaneous lesions commonly observed in Wistar rats. An instance of focal chronic hepatitis and interstitial lymphoid cell infiltration in the prostate also appeared to be incidental in nature.

Table 5.3.2-38: 90–day Oral Toxicity Study with Glyphosate Technical in Wistar Rats (FSG 03090 h/05 March 1990) ([redacted] 1992): Summary incidence of histopathological findings

Findings	Dose level [ppm]									
	Males					Females				
	0	200	2000	20000	20000 (recovery group)	0	200	2000	20000	20000 (recovery group)
Liver										
Inflammation, chronic	0/10	---	---	1/10	---	0/10	---	---	0/10	---
Bile duct proliferation	3/10	---	---	2/10	---	0/10	---	---	0/10	---
Lungs										
Congestion	8/10	---	---	9/10	1/2	7/10	---	---	6/10	---
Lymphoid hyperplasia	2/10	---	---	4/10	1/2	6/10	---	---	2/10	---
Atelectasis	7/10	---	---	9/10	1/2	7/10	---	---	5/10	---
Broncho-pneumonia	0/10	---	---	0/10	2/2	1/10	---	---	0/10	---
Emphysema, alveolar	2/10	---	---	0/10	1/2	2/10	---	---	0/10	---
Perivascular lymphocytic aggregation	4/10	---	---	4/10	0/2	3/10	---	---	3/10	---

Table 5.3.2-38: 90–day Oral Toxicity Study with Glyphosate Technical in Wistar Rats (FSG 03090 h/05 March 1990) ([redacted] 1992): Summary incidence of histopathological findings

Findings	Dose level [ppm]									
	Males					Females				
	0	200	2000	20000	20000 (recovery group)	0	200	2000	20000	20000 (recovery group)
Kidneys										
Lymphocytic infiltration	1/10	---	1/1	2/10	---	2/10	---	---	0/10	---
Hydro-nephrosis	0/10	---	1/1	0/10	---	0/10	---	---	0/10	---
Duodenum										
Inflammation, acute	0/10	1/?	---	0/10	---	0/10	---	---	0/10	---
Lymphoid hyperplasia	0/10	0/?	---	0/10	---	1/10	---	---	1/10	---
Ileum										
Inflammation, acute	0/10	1/2	---	0/10	---	0/10	---	---	0/10	---
Lymphoid hyperplasia	1/10	1/2	---	2/10	---	0/10	---	---	0/10	---
Colon										
Lymphoid hyperplasia	2/10	---	---	2/10	---	2/10	---	---	1/10	---
Mesenteric Lymph Nodes										
Lymphoid hyperplasia	0/10	1/1	---	0/10	1/1	0/10	---	---	0/10	---
Trachea										
Lymphocytic infiltration	5/10	---	---	3/10	---	3/10	---	---	3/10	---
Lymphoid hyperplasia	0/10	---	---	0/10	---	1/10	---	---	0/10	---

III. CONCLUSIONS

There was no mortality in any of the study groups during treatment or recovery period. In general, there were no clinical signs of toxicity observed in any of the treatment groups. While significant changes were observed in body weight gains, haematology parameters, and clinical chemistry parameters, these changes were considered to be incidental in nature. At 20000 ppm, decreased body weight gains were observed in female rats; body weight changes were not observed in males. Significant changes were observed in haematology values for males (decreased eosinophils at the high dose, increased mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration at the mid dose and high-dose recovery group, decreased red blood cells at the mid and low dose, and decreased haematocrit at the low dose). There were no significant changes to haematology parameters for female rats at any dose level.

With regards to clinical chemistry parameters, there was an increase in alkaline phosphatase activity (high dose) and a decrease in albumin levels (low dose) and total bilirubin (high-dose recovery group) in males. For females, there was a decrease in creatinine (all dose levels) and calcium concentrations (all doses except the recovery group), while there was an increase in glucose levels (high dose). There also was decrease in glucose levels for the high-dose recovery group when compared with the high dose.

There were no notable intergroup differences in organ weights. No gross pathology or histopathology findings attributed to administration of glyphosate were recorded.

According to the 2nd amendment to study report created in November of 1994, the No Observed Effect Level (NOEL) was determined to be 2000 ppm in diet, which is equivalent to 147.3, 195.7, and 171.5 mg/kg bw/day for male, female and combined sex, respectively.

3. Assessment and conclusion

Assessment and conclusion by applicant:
In this 90–day toxicity study, groups of 10 male and 10 female Wistar rats were administered technical glyphosate for 90 consecutive days via the diet at concentrations of 0, 200, 2000, or 20000 ppm. These dose levels were equivalent to 14.0, 147.3, or 1358.6 mg/kg bw/day for males and 18.6, 195.7, or 2012.4 mg/kg bw/day for females, respectively. A high-dose recovery group was administered 20000 ppm (equivalent to 1482.6 mg/bw/day for males and 1878.3 mg/kg bw/day for females) for 13 weeks and then was followed for 4 weeks without treatment before being sacrificed.
Dosing Wistar rats via the diet with glyphosate produced decreased body weight gains in high-dose females. Additionally, there were significant increases in ALP activity in male rats at the high-dose level, as well as significant decreases in creatinine and calcium levels in female rats. The latter effects (creatinine and calcium) did not show a dose-response relationship and were therefore considered to be incidental. Female rats also exhibited increased glucose levels at the high-dose level. There were no treatment-related effects at any dose level with regards to mortality, clinical signs of toxicity, haematology, organ weight and gross and histopathological findings. Based on this information, the no adverse observed effect level is determined to be 2000 ppm in diet corresponding to 147.3 mg/kg bw/day for males and 195.7 mg/kg bw/day for females.

Assessment and conclusion by RMS:
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1. Information on the study

Data point	CA 5.3.2/011
Report author	[redacted]
Report year	1991
Report title	Glyphosate – 13 week dietary toxicity study in rats
Report No	7136
Document No	Not reported
Guidelines followed in study	FIFRA 82-1; OECD 408 (1981)
Deviations from current test guideline (OECD 408, 2018)	Ophthalmoscopy was only performed prior to termination; haematology was performed without determining reticulocyte count and prothrombin time; clinical chemistry was performed without determining HDL, LDL, T4, T3 and TSH; organ weights of the thyroid gland was not determined; histopathology was performed without bone/bone marrow, cervix, coagulating glands, gall bladder, spinal cord and vagina. Deviations from the current version of OECD 408 (2018) are basically due to the fact that the study was aligned to an older version of the OECD test guideline 408.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

This study was designed to give toxicity information over 13 weeks on Glyphosate administered to rats via the diet. The concentrations of the diet were adjusted weekly to achieve dose levels of 0, 30, 300 or 1000 mg/kg bw/day. The group size was 10 animals per sex and dose group.

The animals were examined for mortality, clinical signs, body weight, food and water consumption, ophthalmoscopy, haematology, clinical chemistry, urinalysis, gross pathology, organ weights and histopathology.

There were no mortalities or clinical signs. Body weight, food intake, water consumption, ophthalmoscopy and haematology were unaffected by treatment. Glucose, total protein, albumin and creatinine were slightly and equivocally increased in high dose females. There was a reduction of urinary pH in high dose males. Gross pathology revealed no changes attributable to glyphosate treatment however animals at all dose levels showed increased incidence and severity of "cellular alteration" (deep basophilic staining and enlargement of cytoplasm) in the parotid salivary gland of both sexes at 1000 mg/kg bw/day and at 300 mg/kg bw/day in males. Incidence only was increased at 300 mg/kg bw/day in females and 30 mg/kg bw/day in both sexes.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate
Description:	White powder
Lot/Batch #:	206-Jak-25-1
Purity:	98.6 %
Stability of test compound:	Not reported

2. Vehicle and/or positive control: Diet / none

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	ca 5 – 6 weeks
Sex:	Male and female
Weight at dosing:	♂ 95 – 97 g; ♀ 67 – 68 g
Acclimation period:	9 days
Diet/Food:	SDS Rat and Mouse No. 1 Expanded (Fine Ground) Diet
Water:	Tap water, *ad libitum*
Housing:	2 of one sex per cage in suspended polypropylene cages (42.0 × 27.0 × 20.0 cm) with wire grid tops and bottoms
Environmental conditions:	Temperature: 20 ± 2 °C Humidity: 55 ± 10 % Air changes: 15 – 20/hour 12 hours light/dark cycle

B: Study design and methods

In life dates: 1989-06-22 to 1989-09-21/22

Animal assignment and treatment:
The test material was offered on a continuous basis in the basal diet to groups of 10 Sprague-Dawley rats per sex for a minimum of 90 days. Dietary concentrations were 0, 30, 300 and 1000 mg/kg bw/day.

Table 5.3.2-39: Glyphosate – 13 week dietary toxicity study in rats ([redacted] 1991): Study design

Test group	Dietary concentration [mg/kg bw/day]	Males	Females
Control	0	10	10
Low	30	10	10
Mid	300	10	10
High	1000	10	10

Analysis of the test diet
A 100 g sample of diet from each group/sex was retained immediately after each diet preparation.
In addition, usually 3 x 100 g samples were also taken for routine homogeneity and accuracy assessment from diets prepared for Weeks 1, 6 and 13.
In addition, data proving homogeneity and 21-day stability of glyphosate were generated prior to the commencement of the study.

Mortality
Viability was checked once each morning and once as late as practicable each day.

Clinical observations
All animals were examined for reaction to treatment during the day. The onset, intensity and duration of these signs were recorded.
All animals received a detailed clinical examination once each week.

Body weight
The weight of each animal was recorded once during the week before the start of treatment and once each week thereafter.

Food consumption and water consumption
The quantity of food consumed by each cage of animals was recorded once each week, commencing one week before the start of treatment and once each week thereafter.
Water consumption was measured gravimetrically on a weekly basis commencing one week before the start of treatment until the end of the study.

Ophthalmoscopy examination
The eyes of all animals in the Control and High dose groups were examined using an indirect ophthalmoscope after the application of a mydriatic agent (1 % Mydriacyl). Anterior, lenticular and fundic areas were evaluated. This ophthalmoscopic examination was undertaken pretrial and during Week 13 of treatment.

Haematology and clinical chemistry
Samples were taken from all rats from each group during Week 13 of dosing. Blood samples were collected from the orbital sinus under light ether anaesthesia.

Haematology:
The following parameters were determined: Haematocrit, haemoglobin, total red blood cell count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count, total white blood cell count, differential white blood cell count.

Clinical chemistry
The following parameters were determined: Alkaline phosphatase (ALP), aspartate amino transferase (AST), Alanine aminotransferase (ALT), creatinine (Crea), blood urea nitrogen (BUN), total protein (TP), albumin (Alb), albumin/globulin ratio (AG-R), glucose (Glu), total cholesterol (Chol), total bilirubin (T.Bi), sodium (Na), potassium (K), chloride (Cl), calcium (Ca), inorganic phosphorus (P), plasma cholinesterase, RBC cholinesterase and brain cholinesterase (ca. 0.5 g of brain was removed from each animal at necropsy and deep frozen (-20 °C) until assayed).

Urinalysis
Urine samples were collected from animals in metabolism cages. The samples were collected over a 4 h period of food and water deprivation. The following parameters were measured: Specific gravity, glucose, ketones, blood pigments, pH, protein, volume, bilirubin and urobilinogen.

Sacrifice and pathology
All animals were killed and necropsied. Method of killing was by carbon dioxide asphyxiation followed by exsanguination. The gross dissection and necropsy were performed under the supervision of a pathologist.

Organ weights
The following organs were weighed: Adrenals, brain, heart, kidneys, liver, lungs, ovaries (with fallopian tubes), pituitary, prostate, salivary glands (submaxillary, parotid and sublingual), spleen, testes (with epididymides), thymus and uterus.

Histopathology
The following tissues were processed and examined histopathologically from all Control and High dose animals. Only one eye was processed and examined. In addition all other animals received histopathological examination of liver, kidneys and lungs.

Examination of parotid salivary glands was subsequently extended to include all Low and Intermediate dose group males and females.

The following tissues were investigated: Adrenals, aortic arch, bladder, brain, eyes, heart, intestine (duodenum, jejunum, ileum, caecum, colon), kidneys, liver, lungs (perfused), mammary gland, mesenteric lymph node, muscle, oesophagus, ovaries (with fallopian tubes), pancreas, parotid salivary gland, pituitary, prostate, sciatic nerve, seminal vesicles, skin, spleen, stomach, sublingual salivary gland, submaxillary salivary gland, submandibular lymph node, testes (with epididymides), thymus, thyroid, tongue, trachea and uterus.

Statistics
Haematology, clinical chemistry, organ weight and body weight data were statistically analysed for homogeneity of variance using the F-max test. If the group variances appeared homogeneous a parametric

ANOVA was used and pairwise comparisons made via Student's t-test using Fisher's F-protected LSD. If the variances were heterogeneous, log or square root transformations were used in an attempt to stabilise the variances. If the variances remained heterogeneous, then a non-parametric test such as a Kruskal-Wallis ANOVA was used. Organ weights were also analysed conditional on body weight (i.e. analysis of covariance).

Histopathology data were analysed using Fisher's Exact Probability test.

II. RESULTS AND DISCUSSION

A. ACHIEVED DOSAGES AND ANALYSIS OF THE FORMULATED DIETS

The group mean achieved dosages were generally in close agreement with the nominal values.

The majority of diets prepared for Weeks 1, 6 and 13 were seen to be within acceptable limits (±10 %) for accuracy of concentration and homogeneity.

In Week 1, the concentration for Intermediate Dose Group (females) was -11.0 % and the coefficient of variation was 25.4 %. A repeat analysis for this group from diets prepared for Week 2 showed an acceptable level of concentration (-0.7 %) and coefficient of variation (4.2 %). At Week 13 the concentration and coefficient of variation for Low Dose Group (males) were -12.5 % and 21.6 % respectively. No repeat analysis was carried out.

B. MORTALITY

There were no animals found dead or killed *in extremis* in any group during the treatment period.

C. CLINICAL OBSERVATIONS

There were no clinical signs in the control and treated groups that were considered to be due to administration of Glyphosate.

D. BODY WEIGHT

There were no notable intergroup differences in either sex.

E. FOOD CONSUMPTION AND WATER CONSUMPTION

There were no notable intergroup differences in total food or water consumed in either sex at any time.

F. OPHTHALMOSCOPIC EXAMINATION

There were no notable findings in either sex.

G. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

The only finding which showed statistical significance was an increase (300 %) in eosinophils in the male Intermediate dose group. This increase was not reflected in other WBC parameters and due to the lack of an effect in the High dose group was considered to be a chance effect. No notable intergroup differences were found for females.

Table 5.3.2-40: Glyphosate – 13 week dietary toxicity study in rats ([redacted] 1991): Selected haematological findings (week 13)

Parameter	Dose group [mg/kg bw/day]							
	Males				Females			
	0	30	300	1000	0	30	300	1000
Differential white blood cell count - Eosinophils [$\times 10^9$/L]	0.05 ± 0.07	↓0.04 ± 0.10	↑**0.20**** **± 0.14**	↑0.10 ± 0.11	0.06 ± 0.08	↓0.02 ± 0.04	↓0.02 ± 0.04	↑0.07 ± 0.07

** Statistically significant compared to control ($p \leq 0.01$)

Blood clinical chemistry

Alkaline phosphatase was increased (28 %) in the male Intermediate dose group but this was considered to be a chance effect due to the lack of an effect in the High dose group and the high degree of variation seen in the individual values.

The female High dose group showed slight increases in glucose (11 %), total protein (9 %), albumin (9 %) and creatinine (8 %).

There were no other notable intergroup differences.

Table 5.3.2-41: Glyphosate – 13 week dietary toxicity study in rats ([redacted] 1991): Selected clinical chemistry findings (week 13)

Parameter	Dose group [mg/kg bw/day]							
	Males				Females			
	0	30	300	1000	0	30	300	1000
Alkaline phosphatase [iu/L]	317 ± 88	↓299 ± 57	**↑405* ± 102**	↑361 ± 48	222 ± 57	↑242 ± 72	↑255 ± 96	↑391 ± 100
Glucose [mmol/L]	7.36 ± 0.75	↓6.94 ± 0.68	↓6.85 ± 0.98	↓6.92 ± 0.93	7.29 ± 0.67	↑7.42 ± 0.50	↓7.14 ± 0.69	**↑8.10* ± 0.91**
Total protein [g/L]	67 ± 3	↓65 ± 1	67 ± 4	↓65 ± 3	67 ± 3	↑68 ± 3	↑69 ± 3	**↑73*** ± 3**
Albumin [g/L]	32 ± 1	32 ± 1	32 ± 2	32 ± 1	35 ± 2	35 ± 2	↑36 ± 2	**↑38* ± 2**
Creatinine [µmol/L]	48 ± 3	↓47 ± 3	↓47 ± 2	↓47 ± 2	52 ± 2	↑53 ± 2	↑53 ± 2	**↑56** ± 3**
Brain cholinesterase activity [iu/g]	13707 ± 2809	**↑17615** ± 3098**	**↑16527* ± 2455**	↑14715 ± 3533	15837 ± 4021	↓15388 ± 2955	↓13213 ± 3812	↓13588 ± 3266

* Statistically significant compared to control (p ≤ 0.05);
** Statistically significant compared to control (p ≤ 0.01);
*** Statistically significant compared to control (p≤ 0.001)

H. URINALYSIS

The pH was reduced in the male High dose group when compared with Controls.
These were no notable intergroup differences for females.

Table 5.3.2-42: Glyphosate – 13 week dietary toxicity study in rats ([redacted] 1991): Selected urinalysis findings (week 13)

Parameter	Dose group [mg/kg bw/day]							
	Males				Females			
	0	30	300	1000	0	30	300	1000
pH	8.8 ± 0.4	↓8.3 ± 0.8	↓8.6 ± 0.6	↓6.3 ± 0.7	8.1 ± 0.9	↑8.5 ± 0.5	↑8.2 ± 0.6	↓7.1 ± 1.1

I. NECROPSY

Organ weights

There were no notable intergroup differences in either sex.

Table 5.3.2-43: Glyphosate – 13 week dietary toxicity study in rats ([redacted] 1991): Selected absolute organ weights (week 13)

Parameter	Dose group [mg/kg bw/day]							
	Males				Females			
	0	30	300	1000	0	30	300	1000
Salivary gland [g]	1.412 ± 0.153	↓1.325 ± 0.127	↓1.403 ± 0.443	↑1.448 ± 0.299	0.846 ± 0.091	↓0.843 ± 0.118	↓0.831 ± 0.108	↑0.872 ± 0.187

Gross pathology
There were no findings which could be related to treatment with glyphosate.

Histopathology
The most notable findings were seen in the parotid salivary glands, where there was an increase in the incidence and severity of 'cellular alteration' (deep basophilic staining and enlargement of cytoplasm) in both sexes at 1000 and at 300 mg/kg bw/day in males. Incidence only was increased at 300 mg/kg bw/day in females and 30 mg/kg bw/day in both sexes.

The incidence was statistically significant in both sexes of the Intermediate and High dose groups and in the female Low dose group. The finding was present in 7/10 male and 8/10 female Low dose group rats and 10/10 male and 9/10 female Intermediate and High dose rats. Three male Control rats and 2 female Control rats also had the finding (graded as very mild). The severity of the lesion tended to increase with increasing dose of glyphosate and in High dose males 5/10 animals affected were graded as severe, which reached statistical significance at the $p \leq 0.05$ level.

There were also a number of background changes usually seen in rats of this age and strain at the performing laboratory, considered to be unrelated to treatment with glyphosate.

Table 5.3.2-44: Glyphosate – 13 week dietary toxicity study in rats ([redacted] 1991): Selected histopathological findings

Parameter		Dose group [mg/kg bw/day]							
		Males				Females			
		0	**30**	**300**	**1000**	**0**	**30**	**300**	**1000**
Salivary gland	No abnormalities detected	7	↓3	**↓0****	**↓0****	8	**↓2***	**↓1****	**↓1****
	Parotid: cellular alteration (very mild)	3	↑7	↑6	↓0	2	↑7	↑7	↓1
	Parotid: cellular alteration (mild)	0	0	↑3	↑2	0	↑1	↑2	↑4
	Parotid: cellular alteration (moderate)	0	0	↑1	↑3	0	0	0	↑3
	Parotid: cellular alteration (severe)	0	0	0	**↑5***	0	0	0	↑1
	Total incidence of finding	3	↑7	**↑10****	**↑10****	2	**↑8***	**↑9****	**↑9****
Mammary gland	No abnormalities detected	7	-	-	8	10	-	-	9
	Secretion present	3	-	-	2	0	-	-	0
	Adenocarcinoma [M]	0	-	-	0	0	-	-	1

* Statistically significant compared to control ($p \leq 0.05$);
** Statistically significant compared to control ($p \leq 0.01$)

III. CONCLUSIONS

There were no mortalities or clinical signs. Body weight, food intake, water consumption, ophthalmoscopy and haematology were unaffected by treatment. Glucose, total protein, albumin and creatinine were slightly and equivocally increased in high dose females. There was a reduction of urinary pH in high dose males. Gross pathology revealed no changes attributable to glyphosate treatment however animals at all dose levels showed increased incidence and severity of "cellular alteration" (deep basophilic staining and enlargement of cytoplasm) in the parotid salivary gland of both sexes at 1000 mg/kg bw/day and at 300 mg/kg bw/day in males. Incidence only was increased at 300 mg/kg bw/day in females and 30 mg/kg bw/day in both sexes.

There was not a NOEL for parotid salivary gland changes, for all other parameters the NOEL was 300 mg/kg bw/day.

3. Assessment and conclusion

Assessment and conclusion by applicant:
In this study, the test item glyphosate was administered to Sprague-Dawley rats via the diet at dose levels of 0, 30, 300 or 1000 mg/kg bw/day for 13 weeks according to OECD 408 (1981) and in compliance with GLP (no certificate of the competent authority was provided).

Dosing Sprague-Dawley rats via the diet for 13 weeks with up to and including 1000 mg/kg bw/day glyphosate produced increased incidence and severity of 'cellular alteration' (deep basophilic staining and enlargement of cytoplasm) in the parotid salivary gland of both sexes at 1000 mg/kg bw/day and at 300 mg/kg bw/day in males. Incidence only was increased at 300 mg/kg bw/day in females and 30 mg/kg bw/day in both sexes. In addition, there were slight equivocal increases in glucose, total protein, albumin and creatinine in females receiving 1000 mg/kg bw/day and reduced urinary pH in males receiving 1000 mg/kg bw/day.

A clear NOAEL could not be established in this study since the number of animals showing cellular alteration in the parotid salivary glands was markedly increased in all treated groups in both sexes following a dose-related pattern. However, this finding was not accompanied by a significant increase in salivary gland weight. Moderate and severe histological alteration was confined to the male and female rats of the high-dose groups. The other treatment related effects like clinical chemistry and urinalysis changes were also noted at the top dose level only. Therefore, it appears reasonable to establish the NOAEL in this study at 300 mg/kg bw/day.

Assessment and conclusion by RMS:
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1. Information on the study

Data point	CA 5.3.2/012
Report author	[redacted]
Report year	1990
Report title	Glyphosate Technical: 90 Day Oral Toxicity Study in the Rat
Report No	AGC-900914
Document No	Not reported
Guidelines followed in study	OECD 408 (1981)
Deviations from current test guideline (OECD 408, 2018)	Ophthalmoscopy was only performed at the end of the treatment period; no sensory reactivity was examined; haematology was performed without determining reticulocyte count; clinical chemistry was performed without determining cholesterol, HDL, LDL, blood urea nitrogen, T4, T3 and TSH; organ weights of the brain, epididymides, heart, ovaries, pituitary gland, prostate (seminal vesicles and coagulating glands), spleen, thyroid gland, thymus and the uterus were not determined; histopathology was performed without bone + bone marrow, coagulating glands, gall bladder and vagina. Deviations from the current version of OECD 408 (2018) are basically due to the fact that the study was aligned to an older version of the OECD test guideline 408.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes

Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

Groups of male and female CD rats were dosed with glyphosate technical over a 90 to 92 day period. The test chemical was administered in the diet at levels of 0, 2000, 5000 and 7500 ppm (equivalent to 0, 129.1, 320.7 and 482.1 mg/kg bw/day for males and 0, 174.3, 441.6 and 647.3 mg/kg bw/day for females). All concentrations of test compound included in the diet were readily consumed by the animals.

Animals were observed for mortality, clinical signs, body weight, food consumption, test substance intake, ophthalmoscopy, haematology, clinical chemistry, organ weights, gross necropsy and histopathology.

No deaths occurred in the control or any of the test groups throughout the study. Observations on the animals showed no compound-related or dose-related adverse effects - either at the weekly clinical examinations or the ophthalmological examination. There was no compound related adverse effect on growth. Gross necropsy at the end of the dosing period showed background abnormalities only. There was no adverse effect on organ weights or on organ weight/body weight ratios. Studies carried out on the terminal bleeds on the following haematology parameters - white blood cell counts, red blood cell counts, haemoglobin concentration, haematocrit, platelets, neutrophils, lymphocytes, monocytes, eosinophils and basophils - showed no compound-related adverse effects.

Measurement of the coagulation responses - prothrombin time and activated partial thromboplastin time - showed no compound-related adverse effect.

Clinical chemistry analyses showed some group(s) differing significantly from the control for one or other parameter. However, a progressive effect of dose on response was not seen for any parameter. Overall no compound-related adverse effect was seen.

The range of histopathology findings in the study animals was such as would be expected within a normal group of rats of this age range. The animals receiving glyphosate at the various dose levels could not be distinguished on the basis of the histopathology findings. There was no evidence of specific target organ cytotoxicity attributable to administration of the test substance at any dose level.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate technical
Description:	Yellowish (transparent)
Lot/Batch #:	0190 A
Purity:	98.1 %
Stability of test compound:	Stable at room temperature in a dry vermin proof room

2. Vehicle and/or positive control:

Diet / none

3. Test animals:

Species: Rat

Strain:	CD
Source:	[redacted]
Age:	Ca. 6 – 7 weeks
Sex:	Male and female
Weight at dosing:	♂ 233 – 310 g; ♀ 161 – 216 g
Acclimation period:	Approx. 3 – 4 weeks
Diet/Food:	Special quality control powdered diet (SDS Ltd, Witham, Essex, U.K.).
Water:	Bottled mains tap water, *ad libitum*
Housing:	Individually in flat bottomed polypropylene cages with stainless steel lids
Environmental conditions:	Temperature: 19 – 28 °C Humidity: 33 – 70 % Air changes: Not reported 12 hours light/dark cycle

B: Study design and methods

In life dates: 1990-03-21 to 1990-06-22

Animal assignment and treatment:
The test material was offered on a continuous basis in the basal diet to groups of 10 Sprague-Dawley CD rats per sex for a minimum of 90 days. Dietary target concentrations were 0, 2000, 5000 and 7500 ppm (equivalent to 0, 129.1, 320.7 or 482.1 mg/kg bw/day for males and 0, 174.3, 441.6 or 647.3 mg/kg bw/day for females).

Table 5.3.2-45: Glyphosate Technical: 90 Day Oral Toxicity Study in the Rat ([redacted], 1990): Study design

Test group	Dietary concentration [ppm]	Test substance intake [mg/kg bw/day]	Males	Females
Control	0	♂: 0; ♀: 0	10	10
Low	2000	♂: 129.1; ♀: 174.3	10	10
Mid	5000	♂: 320.7; ♀: 441.6	10	10
High	7500	♂: 464.7; ♀: 647.3	10	10

Analysis of the test diet
Premixes of glyphosate in powdered diet were prepared in a stainless steel commercial food processor (Robot Coupe). Each pre-mix was diluted with untreated diet to give the correct final concentration. Mixing of the pre-mix with untreated diet was carried out by using a drum hoop mixer (Engelsmann, Germany).

Samples from each batch of diet containing glyphosate were assayed in The National Food Centre laboratory by HPLC following suitable extraction procedures.

Mortality
Animals were checked twice daily for mortality and moribundity.

Clinical observations
Each animal in each group was observed daily for toxic responses to the administered dose. In addition all cages were checked at the start and end of each day.

A full clinical examination was carried out on each animal at weekly intervals.

Body weight
For each animal, body weight was measured before dosing (Day 0), weekly thereafter and at terminal sacrifice.

Food consumption
Diet consumption was monitored at weekly intervals.

Ophthalmoscopy
The eyes of each animal in each group were subjected to full ophthalmological examination at the end of the dose period (Day 83 for males and Day 84 for females). Animals were subjected to ophthalmoscopy in the order of initial random selection. 15 to 30 min before examination the eyes of each animal were treated with one drop of mydriatic (Trademark Mydriacyl, Alcon). Examination was carried out using an indirect ophthalmoscope (Fisons All-Purpose).

Haematology and clinical chemistry
Samples were taken by cardiac puncture under anaesthesia from animals of each group/sex prior to termination.

Haematology:
The following parameters were determined: Haematocrit, haemoglobin, total red blood cell count, prothrombin time, activated partial thromboplastin time, platelet count, total white blood cell count and differential white blood cell count.

Clinical chemistry
The following parameters were determined: Gamma glutamyl transpeptidase, aspartate amino transferase (AST), alanine aminotransferase (ALT), creatinine (Crea), total protein (TP), albumin (Alb), glucose (Glu), total bilirubin (T.Bi), sodium (Na), potassium (K), chloride (Cl), calcium (Ca) and inorganic phosphorus (P).

Sacrifice and pathology
All animals were subjected to a full gross necropsy which included examination of the external surface of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents.

Organ weights
The following organs were weighed: Adrenals, kidneys, liver and testes.

Histopathology
Histopathological examinations of the following organs and tissues were made on sections stained with haematoxylin and eosin: Adrenals, aorta, brain, epididymides, eyes, heart, intestine (duodenum, jejunum, ileum, caecum, colon), harderian glands, kidneys, lacrimal glands, liver, lungs, lymph node, mammary gland, muscle, oesophagus, ovaries, pancreas, pituitary, prostate, rectum, salivary gland, sciatic nerve, seminal vesicles, skin, spinal cord, spleen, sternum, stomach, testes, thymus, thyroid, trachea, urinary bladder and uterus (with cervix and fallopian tubes).

Additionally, kidneys, liver and heart were examined from mid dose animals.

Statistics
Data was analysed using the General Linear Model ANOVA of SAS. Initial analysis for the effect of dose

group on a given parameter was made by using ANOVA; this was followed by Duncan's Multiple Range Test. This test identifies dose-groups which are not statistically different.

The level of significance for comparison between means was $p \leq 0.05$.

As the above tests showed no differences or minor differences between groups, with no evidence of any dose-related effect, no further tests were carried out.

II. RESULTS AND DISCUSSION

A. ACHIEVED DOSAGES AND ANALYSIS OF THE FORMULATED DIETS

Each dose level of each batch of glyphosate/diet mixture was assayed following preparation to establish concentration and homogeneity of active substance. Each batch was sampled at weekly intervals for analysis of glyphosate content. Diet samples were analysed to assess the stability of glyphosate in the diet. Due to a delay in assay development at the testing facility (The National Food Centre) the diet samples were not assayed at the required time. Final analysis revealed that the glyphosate/diet samples were stable for a period in excess of the feeding period.

Please refer to **Error! Reference source not found.**table above (Table 5.3.2-45) for details on the achieved dosages which have been re-calculated based on food consumption and body weight data (see tables below).

B. MORTALITY

There were no animals found dead or killed *in extremis* in any group during the treatment period.

C. CLINICAL OBSERVATIONS

No compound-related effects were seen in any animal for the dose groups throughout the test period.

One male rat in the 2000 ppm dose level received a cut on its right shoulder during the second week of the study. This was due to the rat scratching against the grid in the bottom of the cage. The grid was replaced and the cut had healed totally within nine days.

One male rat in the 7500 ppm dose group was noticed as somewhat unwell on study day 82. On study day 85 this animal was observed to have grossly overgrown Incisors; the roof of the mouth was infected; the eyes appeared bloody and he had obvious signs of weight loss. The incisors were trimmed and Improvement was obvious within 48 hours. Weight gain recommenced and the rat was eating normally at the termination of the study.

One male rat and one female rat escaped from their respective cages on study day 59. Both rats were recaptured and returned to their cages. All cages were secured and there were no further escapees. Body weight data confirmed that the female rat was pregnant. This rat was not removed from the study and littered on study day 80. Clinical chemistry, coagulation, body weight, haematology and organ weights were recorded, but the data is not included in the statistics for this study.

D. BODY WEIGHT

While there are slight differences in group mean body weights the differences did not reach statistical significance for male or female animals at any time during the 90 day dosing period. There is no evidence that the compound had an adverse effect on growth for either sex.

Table 5.3.2-46: Glyphosate Technical: 90 Day Oral Toxicity Study in the Rat ([redacted], 1990): Summary of mean body weights [g]

	Dose group [ppm]							
	Males				Females			
	0	2000	5000	7500	0	2000	5000	7500
Week 0	268.20 ± 22.69	270.00 ± 18.73	269.70 ± 10.17	267.60 ± 17.37	198.40 ± 6.40	194.80 ± 14.21	191.30 ± 15.36	193.10 ± 11.20
Week 1	318.30 ± 25.79	323.00 ± 22.02	322.00 ± 14.69	318.30 ± 18.60	214.00 ± 7.59	209.50 ± 17.85	205.80 ± 13.85	204.60 ± 13.83
Week 2	364.30 ± 32.06	365.20 ± 20.49	368.90 ± 17.07	357.60 ± 23.58	236.20 ± 10.11	233.90 ± 25.99	228.80 ± 15.82	224.80 ± 16.23
Week 3	403.40 ± 37.67	405.20 ± 26.86	408.30 ± 20.49	393.40 ± 29.12	252.80 ± 10.40	251.40 ± 21.87	247.70 ± 19.25	240.80 ± 17.55
Week 4	438.90 ± 40.98	436.90 ± 28.39	443.80 ± 25.35	426.70 ± 37.97	266.70 ± 8.74	262.60 ± 21.22	258.70 ± 23.61	253.60 ± 19.35
Week 5	462.60 ± 42.23	458.60 ± 36.34	462.90 ± 27.65	449.00 ± 35.24	267.70 ± 10.93	270.40 ± 23.32	267.70 ± 25.41	263.70 ± 25.70
Week 6	491.30 ± 46.56	488.70 ± 36.48	493.30 ± 24.73	476.50 ± 40.89	287.90 ± 12.78	277.50 ± 21.56	271.90 ± 33.02	272.40 ± 23.20
Week 7	511.90 ± 52.04	497.90 ± 40.42	509.50 ± 26.76	487.60 ± 32.34	293.90 ± 14.83	286.80 ± 20.70	288.50 ± 32.30	278.50 ± 23.13
Week 8	533.00 ± 56.25	518.20 ± 42.85	524.60 ± 29.85	506.90 ± 41.70	299.70 ± 14.49	294.70 ± 24.59	290.30 ± 31.32	283.70 ± 24.24
Week 9	547.00 ± 56.01	525.80 ± 42.80	536.90 ± 29.37	516.90 ± 42.64	310.56 ± 12.84	302.20 ± 27.33	296.80 ± 31.57	288.90 ± 23.52
Week 10	563.30 ± 65.58	549.10 ± 40.39	556.60 ± 29.86	537.20 ± 44.60	316.44 ± 17.77	308.50 ± 23.45	301.90 ± 30.98	294.80 ± 21.53
Week 11	583.90 ± 58.85	565.70 ± 40.46	572.10 ± 31.60	551.90 ± 47.16	326.11 ± 21.54	317.90 ± 24.79	309.70 ± 33.92	300.10 ± 21.53
Week 12	593.80 ± 65.84	572.70 ± 42.32	580.20 ± 32.91	555.80 ± 56.89	333.56 ± 19.33	320.60 ± 27.93	313.30 ± 38.63	305.10 ± 23.25
Day 90	612.30 ± 59.80	587.90 ± 46.26	597.20 ± 32.53	572.90 ± 55.67	339.00 ± 18.21	329.60 ± 27.96	324.50 ± 37.86	310.60 ± 24.65

E. FOOD CONSUMPTION

There is an indication of very slight decreased diet consumption for the high dose groups for both sexes.

Table 5.3.2-47: Glyphosate Technical: 90 Day Oral Toxicity Study in the Rat ([redacted], 1990): Summary of food consumption data [g/rat/week]

	Dose group [ppm]							
	Males				Females			
	0	2000	5000	7500	0	2000	5000	7500
Week 1*	201.99 ± 18.73	↑203.55 ± 21.14	↑203.88 ± 15.37	↑202.03 ± 13.87	245.51 ± 10.13	↓236.24 ± 17.94	↓239.99 ± 9.41	↓238.39 ± 13.59
Week 2	216.90 ± 21.67	↓215.07 ± 19.62	↑219.98 ± 14.57	↓206.07 ± 18.25	158.76 ± 11.35	↑162.20 ± 23.25	↓157.51 ± 14.41	↑162.49 ± 30.05
Week 3	220.91 ± 25.79	↑224.12 ± 26.02	↑222.68 ± 13.21	↓217.33 ± 22.00	160.57 ± 15.51	↑162.49 ± 20.20	↓157.60 ± 16.11	↓155.12 ± 10.56
Week 4	217.44 ± 26.71	↓215.43 ± 26.96	↑219.73 ± 19.12	↓212.70 ± 28.79	163.03 ± 15.24	↓162.39 ± 8.75	↓162.38 ± 16.01	↓154.75 ± 9.17
Week 5	208.44 ± 19.84	↑212.13 ± 23.79	↑214.24 ± 14.95	↑215.73 ± 24.32	148.61 ± 15.41	↓147.44 ± 11.36	↑151.05 ± 16.40	↓145.06 ± 11.28
Week 6	214.91 ± 22.26	↑218.33 ± 27.11	↑217.65 ± 17.34	↓213.03 ± 25.50	155.33 ± 16.37	↓150.97 ± 10.31	↓154.85 ± 15.28	↓148.03 ± 11.18

Table 5.3.2-47: Glyphosate Technical: 90 Day Oral Toxicity Study in the Rat ([redacted], 1990): Summary of food consumption data [g/rat/week]

	Dose group [ppm]							
	Males				Females			
	0	2000	5000	7500	0	2000	5000	7500
Week 7	215.76 ± 28.97	↓208.55 ± 27.26	↑216.24 ± 17.22	↓203.22 ± 17.42	152.70 ± 18.83	↓148.49 ± 15.39	↑154.71 ± 21.90	↓147.44 ± 12.92
Week 8	224.01 ± 26.42	↓216.23 ± 25.08	↓212.56 ± 17.17	↓209.93 ± 24.54	151.43 ± 15.77	↓149.53 ± 17.10	↓146.32 ± 14.25	↓137.81 ± 9.51
Week 9	215.13 ± 18.61	↓209.21 ± 33.61	↓208.44 ± 16.67	↓202.62 ± 21.44	156.08 ± 13.84	↑158.13 ± 13.39	↓150.56 ± 10.72	↓144.35 ± 9.70
Week 10	227.52 ± 25.15	↓225.76 ± 23.07	↓217.89 ± 17.69	↓216.18 ± 25.08	162.39 ± 18.70	↓157.39 ± 10.40	↓152.52 ± 11.91	↓144.64 ± 6.60
Week 11	228.34 ± 23.36	↓222.43 ± 23.61	↓224.36 ± 15.65	↓215.50 ± 30.29	172.89 ± 33.06	↓159.77 ± 12.79	↓160.95 ± 28.90	↓144.55 ± 6.20
Week 12	215.02 ± 20.61	↓213.30 ± 27.63	↓212.75 ± 17.33	↓193.32 ± 37.77	164.82 ± 24.12	↓150.27 ± 16.78	↓147.08 ± 17.79	↓135.45 ± 15.10
Day 90	179.17 ± 18.38	↓169.80 ± 22.47	↓176.10 ± 18.17	↓169.76 ± 20.22	126.74 ± 17.48	↓122.00 ± 11.12	↓123.36 ± 12.27	↓113.42 ± 7.52

* Week 1 female food intake figures as reported by the author appear out of expected range.

F. OPHTHALMOSCOPY

For most animals no abnormalities were recorded. The findings are those one would expect in this strain of rats at this age. There is no evidence of a compound related adverse effect.

Observations noted were:
Males: One control animal had vacuoles at 6 o'clock in the cornea of the left eye. One animal of the low dose group had vacuoles in the central region of the cornea of the right eye and on animal of the low dose group at 12 o'clock in the cornea of the left eye. One animal of the mid dose group showed corneal vacuoles at 3 o'clock in the right eye and one animal of the mid dose group had a pinhead posterior polar opacity in the lens of the right eye. One animal of the high dose group had bloody tears in the left eye (Adnexa) and one animal of the high dose group had nasal quadrant keratitis in the cornea of the right eye.

Females: Two animals of the control group showed increased luminescence in the right and left eye respectively (early retinal atrophy). One animal of the low dose group had a pinhead posterior opacity in the lens of both eyes, another animal of the low dose group showed increased luminescence in both eyes (early retinal atrophy) and one animal of the low dose group showed increased luminescence in the right eye (early retinal atrophy). One animal of the high dose group had conjunctivitis in the left eye.

G. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

Males: For the parameter RBC the Duncan test showed a statistically significant difference between the high dose group and the other three dose groups. The mean value for the high dose group was lower than the means for the other three dose groups. The mean value for all dose groups was within the normal range ($4.41 – 8.01 \times 10^6/mm^3$) for rats of this age and strain (Data supplied by [redacted]).

For the parameter Haematocrit there was a statistically significant difference between the high dose group and the other three dosage groups. All dose groups including the control had a mean value slightly below the range (40.44 – 45.1 %) normally measured in male rats of this strain and age at Biological Laboratories (Ballina).

For the parameter Monocytes the Duncan test shows a significant statistical difference between the low

dose group and the other three dose groups. The control, intermediate and high dose groups were statistically similar. There was no evidence of a treatment-related effect.

Females: No statistical differences were seen for White Blood Cells (WBC), Haemoglobin (HGB), Platelets (PLT), Neutrophils (NP), Lymphocytes (LC), Eosinophils (EP) and Basophils (BP).
For the parameter Red Blood Cells (RBC) in the female animals the low dose group was significantly different from the control. The mean values for the intermediate and high dose group were similar to the control. All values were close to the normal range (4.42 – 6.70 × 10^6/mm^3) for rats of that age and strain.

(Data supplied by [redacted]). There was no progressive effect of treatment observed.

For the parameter Haematocrit the mean values for the low dose group was significantly different from the control. The mean values for the intermediate and high dose group were statistically similar to the control group using the Duncan test. All dose groups including the control group had a mean slightly below the range (37.5 – 47.3 %) normally measured in rats of this strain and age at Biological Laboratories.

For the parameter Monocytes the low dose group is significantly different from the control using the Duncan test. The intermediate and high dose levels are statistically similar to the control. ANOVA revealed no treatment related effect. There was no progressive effect of treatment observed.

All other haematological parameters were within the range of the control animals.

There was no statistical difference between the control and the dosage groups for either sex for the parameters Prothrombin time and Activated Partial Thromboplastin Time.

Table 5.3.2-48: Glyphosate Technical: 90 Day Oral Toxicity Study in the Rat ([redacted], 1990): Selected haematological findings

Parameter	Dose group [ppm]							
	Males				Females			
	0	2000	5000	7500	0	2000	5000	7500
Red blood cell count [$x10^6/mm^3$]	7.65 ± 0.49	↓7.54 ± 0.77	↓7.56 ± 0.49	**↓6.91* ± 0.57**	6.16 ± 0.67	**↑7.00** ± 0.63**	↑6.69 ± 0.42	↑6.32 ± 0.53
Haematocrit [%]	38.85 ± 2.00	↓38.29 ± 3.76	↑39.10 ± 2.80	**↓35.63* ± 2.52**	33.11 ± 3.58	**↑37.47** ± 3.00**	↑35.78 ± 2.05	↑34.34 ± 2.59
Monocytes [%]	1.30 ± 0.82	**↓0.60* ± 0.84**	↑1.50 ± 0.85	↑2.00 ± 1.49	0.22 ± 0.44	**↑1.20** ± 0.42**	↑0.90 ± 1.20	↑0.80 ± 0.92

* Statistically significant compared to the other groups (ANOVA/Duncan test);
** Statistically significant compared to control (ANOVA/Duncan test)

Blood clinical chemistry
In the case of Ca levels in the male rats the mean value for the intermediate dose group (5000 ppm) was significantly different from the control. The mean value for the low dose group and the high dose group were statistically similar to the control value and there was no evidence of a dose-related effect.
For Na levels in the male rats ANOVA showed a significant effect of treatment. However, examination of the data shows an apparent suppression of the mean Na value for the control animals. The mean Na values for all groups seem somewhat low - only the value for the high dose group (7500 ppm) falls within the normal range (139 – 146 mmol/L) established for animals kept at Biological Laboratories.

Statistical differences were also seen for mean Cl values. The mean value for the high dose group differs significantly from the mean value for the control and low dose groups. The mean chloride level for the control group was just below the lower end of the normal range; (94.5 – 112.0 mmol/L; Biological Laboratories Data), whilst the values for all other groups were within the normal range.
For glucose concentrations ANOVA showed a significant effect of treatment. Reference to the Duncan test

shows the control and low dose groups differing significantly form the medium and high dose groups. However, the values for all groups are within the range established for similar rats at Biological Laboratories.

All other clinical chemistry parameters were in the range of the control group.

In the case of all parameters assayed, there was no statistical difference between the dosage groups and the controls at any dose level for the female rats.

In general there is no evidence of a toxic effect of glyphosate in male and female rats on the Clinical Chemistry parameters measured.

Table 5.3.2-49: Glyphosate Technical: 90 Day Oral Toxicity Study in the Rat ([redacted], 1990): Selected clinical chemistry findings

Parameter	Dose group [ppm]							
	Males				Females			
	0	2000	5000	7500	0	2000	5000	7500
Calcium [mmol/L]	2.34 ± 0.18	↑2.39 ± 0.16	**↑2.50** ± 0.17**	↑2.48 ± 0.09	2.69 ± 0.15	↓2.64 ± 0.18	↓2.61 ± 0.12	↓2.63 ± 0.19
Sodium [mmol/L]	128.20 ± 11.05	**↑128.70* ± 10.20***	**↑135.90* ± 10.16***	↑139.30 ± 6.41	139.63 ± 3.66	↓138.38 ± 6.55	↑141.13 ± 5.00	↑140.00 ± 3.56
Chloride [mmol/L]	93.67 ± 7.70	↑96.56 ± 6.93	↑98.60 ± 3.61	**↑103.00* ± 2.39***	99.00 ± 3.03	↑103.13 ± 8.87	↓100.20 ± 7.09	↓99.44 ± 4.59
Glucose [mmol/L]	7.66 ± 0.68	7.66 ± 0.68	**↑8.41§ ± 0.54**	**↑8.76§ ± 0.96**	8.79 ± 0.93	↓8.52 ± 0.63	↑8.69 ± 0.57	↑8.71 ± 0.84

* Statistically significant compared to control and low dose group (ANOVA/Duncan test);
** Statistically significant compared to control (ANOVA/Duncan test);
§ Statistically significant compared to control and high dose group (ANOVA/Duncan test)

H. NECROPSY

Organ weights/ body weight ratios

The organ weights (liver, kidneys, adrenals, and testes) in male animals showed no statistical differences between the dosage groups and the control group.

In the female animals the group mean value for the liver and the adrenals showed no statistical differences between the dosage groups and the control group. ANOVA showed that the mean weight of the kidneys for the control group was statistical different from the three dose groups. The mean values for the three dose groups were statistically similar. There was no progressive treatment-related effect observed.

Table 5.3.2-50: Glyphosate Technical: 90 Day Oral Toxicity Study in the Rat ([redacted], 1990): Selected organ weight findings (week 13)

Parameter	Dose group [ppm]							
	Males				Females			
	0	2000	5000	7500	0	2000	5000	7500
Kidneys	4.00 ± 0.42	3.97 ± 0.48	4.36 ± 0.44	4.26 ± 0.49	**2.56* ± 0.19**	2.23 ± 0.25	2.27 ± 0.14	2.35 ± 0.15

* Statistically significant compared to the dose groups (ANOVA)

For male animals the statistics showed no treatment-related effect on body weight at death or on the organ weight/body weight ratios for liver, adrenals and testes. An effect of treatment was observed for kidneys with the mean weight for the low dose group differing significantly from the control. The Duncan test showed that for male animals the 2000 ppm dose group was significantly different from the control but the 5000 ppm and 7500 ppm were similar to the control group. There was no indication of a dose-related effect

in the kidney weight/body weight ratios.

For the kidney weight/body weight ratio in the female animals a similar pattern was seen. The Duncan test reveals that the control group was statistically different from the low dose group; however, the control group, intermediate group and the high dose group were statistically similar. No dose-related effect of treatment was observed. For female animals there were no statistical differences between the dose groups for the liver weight/body weight ratio and the adrenal weight/body weight ratio or for body weight at death.

Gross pathology
The few macroscopic abnormalities observed at necropsy are common in rats and were not considered related to administration of the test material.

Histopathology
The range of histopathology findings in the study animals was such as would be expected within a normal group of rats of this age range. The animals receiving glyphosate at the various dose levels could not be distinguished on the basis of the histopathology findings. There was no evidence of specific target organ cytotoxicity attributable to administration of the test substance at any dose level.

III. CONCLUSIONS

No deaths occurred in the control or any of the test groups throughout the study. Observations on the animals showed no compound-related or dose-related adverse effects - either at the weekly clinical examinations or the ophthalmological examination. There was no compound related adverse effect on growth. Gross necropsy at the end of the dosing period showed background abnormalities only. There was no adverse effect on organ weights or on organ weight/body weight ratios. Studies carried out on the terminal bleeds on the following haematology parameters - white blood cell counts, red blood cell counts, haemoglobin concentration, haematocrit, platelets, neutrophils, lymphocytes, monocytes, eosinophils and basophils - showed no compound-related adverse effects.

Measurement of the coagulation responses - prothrombin time and activated partial thromboplastin time - showed no compound-related adverse effect.

Clinical chemistry analyses showed some group(s) differing significantly from the control for one or other parameter. However, a progressive effect of dose on response was not seen for any parameter. Overall no compound-related adverse effect was seen.

The range of histopathology findings in the study animals was such as would be expected within a normal group of rats of this age range. The animals receiving glyphosate at the various dose levels could not be distinguished on the basis of the histopathology findings. There was no evidence of specific target organ cytotoxicity attributable to administration of the test substance at any dose level.

Glyphosate technical appeared to be without adverse effect when fed in the diet to CD rats over a 90 – 92 day period. The no adverse effect level was found to be in excess of 7500 ppm in the diet.

3. Assessment and conclusion

Assessment and conclusion by applicant:
In this study, groups of male and female CD rats were dosed with glyphosate technical over a 90 to 92 day period. The test material was administered in the diet at levels of 0, 2000, 5000 or 7500 ppm (equivalent to 0, 129.1, 320.7 or 482.1 mg/kg bw/day for males and 0, 174.3, 441.6 or 647.3 mg/kg bw/day for females) according to OECD 408 (1981) and in compliance with GLP (no certificate of the competent authority was provided).
Glyphosate technical appears to be without adverse effect when fed in the diet to CD rats over a 90 – 92 days period. The no observed adverse effect level was found to be in excess of 7500 ppm in the diet (equivalent to 482.1 mg/kg bw/day for males and 647.3 mg/kg bw/day for females).

Assessment and conclusion by RMS:
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1. Information on the study

Data point	CA 5.3.2/013
Report author	[redacted]
Report year	1989
Report title	Glyphosate Technical: 90 Day Oral Toxicity study in the Rat
Report No	BY-891002
Document No	Not reported
Guidelines followed in study	OECD 408 (1981); EEC Directive 87/302 EEC (Page 8)
Deviations from current test guideline (OECD 408, 2018)	Haematology was performed without determining reticulocyte count; clinical chemistry was performed without determining cholesterol, HDL, LDL, T4, T3 and TSH. Organ weight of the brain, epididymides, heart, ovaries, prostate with seminal vesicles, spleen, thyroid, thymus, pituitary gland and uterus was not determined; histopathology was performed without bone/bone marrow, coagulating glands, gall bladder, gross lesions, lymph nodes, male mammary glands, seminal vesicles and vagina. Deviations from the current version of OECD 408 (2018) are basically due to the fact that the study was aligned to an older version of the OECD test guideline 408.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability / Reliability	Valid
Category study in AIR 5 dossier (L does)	Category 2a

2. Full summary

Executive Summary
Groups of individually housed male and female rats were dosed with glyphosate technical over a 90 – 92 day period. The chemical was administered in the diet at levels of 0, 2000, 3000, 5000, or 7500 ppm (equivalent to 0, 100, 150, 250 or 375 mg/kg bw/day for males and females). All concentrations of test compound included in the diet were readily consumed by the animals.

Mortality, clinical observations, ophthalmology, body weight and food consumption were recorded during the treatment period. In the final week of dosing ophthalmological examination were made and blood taken for determination of haematological and blood chemistry parameters. At necropsy, organ weights were determined and histopathological examinations were performed on processed tissues.

No deaths occurred during the study. Observations on the live animals showed no compound-related or dose-related adverse effects - either at the weekly clinical examinations or the ophthalmological examinations. There was no compound related adverse effect on growth. Gross necropsy at the end of the dosing period showed no abnormalities. There was no adverse effect on organ weights or on organ weight \ body weight ratios. Studies carried out on the terminal bleeds showed no adverse effects on the following haematology parameters: white blood cell counts, red blood cell counts, haemoglobin concentration, haematocrit and platelets. Investigation of the differential leucocyte count showed some groups differing significantly from the control. However, a progressive effect of dose on response was not seen for any parameter. The occasional minor differences seen are considered not to be compound related.

Measurement of the coagulation responses showed no compound related adverse effect. Clinical chemistry analyses showed some group mean values differing significantly from the control for one or other parameter. However, a progressive effect of dose on response was not seen for any parameter. Overall no compound related adverse effect was seen.

The range of histopathology findings in the study animals was such as would be expected within a normal group of rats of this age range. The animals receiving glyphosate at up to 7500 ppm could not be distinguished on the basis of the histopathology findings. There was no evidence of specific target organ cytotoxicity attributable to administration of the test substance at any dose level.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate technical
Description:	Grey solid
Lot/Batch #:	L1656
Purity:	97.1 %
Stability of test compound:	Stable for the duration of the study

2. Vehicle and/ or positive control: Diet / none

3. Test animals:

Species:	Rat
Strain:	CD
Source:	[redacted]
Age:	Ca. 6 – 7 weeks
Sex:	Male and female
Weight at dosing:	♂ 119 – 242 g; ♀ 116 – 172 g
Acclimation period:	14 days
Diet/Food:	Standard powdered diet
Water:	Bottled mains tap water, *ad libitum*

Housing: Individually in flat bottomed polypropylene cages with stainless steel lids
Environmental conditions: Temperature: 18 – 23 °C
Humidity: 40 – 70 %
Air changes: Not reported
12 hours light / dark cycle

B: Study design and methods

In life dates: 1989-04-12 to 1989-10-02

Animal assignment and treatment
The test material was offered on a continuous basis in the basal diet to groups of 10 CD rats per sex for 90 – 92 days. Dietary concentrations were 0, 2000, 3000, 5000, 7500 and 7500 (satellite group) ppm. The satellite groups were fed untreated for further 5 weeks.

Table 5.3.2-51: Glyphosate Technical: 90 Day Oral Toxicity study in the Rat ([redacted], 1989): Study design

Test group	Dietary concentration [ppm]	Number of animals	
		Males	Females
Control	0	20	20
Control (satellite)	0	10	10
Low	2000	10	10
Intermediate low	3000	10	10
Intermediate high	5000	10	10
High	7500	10	10
High (satellite)	7500	10	10

Analysis of the test diet
Samples from each batch of diet containing glyphosate were assayed in the Sponsor's laboratory by HPLC following suitable extraction procedures.

Mortality
Viability was checked once per day.

Clinical observations
All animals were observed daily for signs of toxicity. In addition all cages were checked at the start and end of each day. A full clinical examination was carried out on each animal at weekly intervals.

Body weight
For each animal body weight was measured before dosing (Day 0), weekly thereafter and at terminal sacrifice.

Food consumption and test substance intake
For the first 21 days of the study food consumption was monitored at 3 day intervals. For the remainder of the study diet consumption was monitored at weekly intervals.

The body weight values of the first three weeks of treatment were only given in 3-Day intervals. Therefore it was not possible to calculate the achieved test substance intake in mg/kg bw/day from these values. For the conversion from ppm to mg/kg bw/day the factor of 20 for elder rats set by Derelanko, M.J. (2014, Handbook of Toxicology) was used to access a worst case scenario.

Ophthalmoscopy examination
The eyes of each animal in each group were subjected to full ophthalmological examination during (Day 58 – males, Day 57 – females) and at the end of the dose period (Day 89 – males, Day 88 – females). 15 – 30 min before examination the eyes of each animal were treated with one drop of mydriatic. Examination was carried out using an indirect ophthalmoscope.

Haematology and clinical chemistry
All animals in the dosage groups were bled in random sequence by cardiac puncture under anaesthesia for haematology, coagulation and clinical chemistry analysis.

Another group of animals of the same age range was selected before the start of the study and bled to establish normal ranges for haematology, coagulation and clinical chemistry parameters.

Haematology:
The following parameters were determined: White blood cell count (WBC), red blood cell count (RBC), haemoglobin concentration (HGB), haematocrit (HCT), platelets (PLT) and differential white blood cell count. Additionally, the plasma from the citrate blood sample was assayed for Prothrombin time manually (Quick's method) and for Activated Partial Thromboplastin Time - also by a manual method.

Clinical chemistry
The following parameters were determined: Calcium, phosphorus, chloride, sodium, potassium, fasting glucose, serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), gamma glutamyl transpeptidase (gGT), urea, albumin, blood creatinine, total serum protein and total bilirubin.

Sacrifice and pathology
All animals were subjected to a full gross necropsy which included examination of the external surface of the body, all orifices and the cranial, thoracic and abdominal cavities and their contents.

Organ weights
The following organs were weighed: Adrenals, kidneys, liver and testes.

Histopathology
The following tissues were processed and examined histopathologically from all Control and High dose animals. In addition, examinations of liver, kidneys and lungs were performed on all groups.

The following tissues were investigated: Adrenals, aorta, brain, eyes, heart, intestine (duodenum, jejunum, ileum, caecum, colon), Harderian glands, kidneys, lacrimal glands, liver, lungs, mammary gland (female), muscle, oesophagus, ovaries (with fallopian tube, cervix), pancreas, pituitary, prostate, rectum, peripheral and sciatic nerve, seminal vesicles, skin, spinal cord, spleen, stomach, salivary glands, testes (with epididymides), thymus, thyroid, trachea, urinary bladder and uterus.

The satellite groups were observed for further 5 weeks after treatment. Delayed effects were not seen during the additional 5 week observation period. At the end of this period animals were subjected to the following termination procedures: Haematology, coagulation, gross necropsy and organ weight measurements.

Statistics
Data was analysed using the General Linear Model <ANOVA> and NPAR1WAY <WILCOXON> procedures of SAS.

Initial analysis for the effect of dose group on a given parameter was made by using ANOVA; this was followed by Duncan's Multiple Range Test. This test identifies dose groups which are not statistically different.

In addition data for gamma glutamyl transpeptidase, total bilirubin, monocytes, eosinophils and basophils

were analysed using the Wilcoxon Rank Sum Test. Where a dose group related effect was seen further Wilcoxon tests were carried out to identify groups differing significantly from the control. The level of significance for comparison between means was $p \leq 0.05$. As the above test showed no differences or minor differences between groups, with no evidence of any dose related effect, the further tests outlined in the protocol were not carried out.

II. RESULTS AND DISCUSSION

A. ACHIEVED DOSAGES

The achieved test substance concentrations are summarised in the following table:

Table 5.3.2-52: Glyphosate Technical: 90 Day Oral Toxicity study in the Rat ([redacted], 1989): Achieved test substance intake

Test group	Dietary concentration [ppm]	Achieved test substance intake [mg/kg bw/day]*	
		Males	Females
Control	0	0	
Control (satellite)	0	0	
Low	2000	100	
Intermediate low	3000	150	
Intermediate high	5000	250	
High	7500	375	
High (satellite)	7500	375	

* Recalculation of dose levels by applying the appropriate diet conversion factor of 20 as published by Derelanko (The Toxicologist's Pocket Handbook, 2nd Ed., 2008)

B. MORTALITY

There were no animals found dead or killed *in extremis* in any group during the treatment period.

C. CLINICAL OBSERVATIONS

No compound related effects were seen in any animal for the dose groups throughout the test period.

Three animals did show injuries of a mechanical nature. One female rat in the 3000 ppm dose group showed a cut on the head. This cut was food-hopper related and the animal showed immediate signs of improvement once the hopper was changed, and the cut had healed completely within eleven days.

One male rat in the 7500 ppm dose group developed a red bald patch on its back. The reason for this was not obvious. The animal made good body weight gains and showed no other adverse effects. Fur had regrown completely by four weeks.

One female rat in the 7500 ppm satellite dose group was reported to have slight hair loss on the head. The bald patch was 0.5 cm², approximately and remained for ca. 11 days when a regain in fur growth was noted. No other obvious abnormalities were seen in this animal.

D. BODY WEIGHT

Throughout the bulk of the feeding period the dosage groups did not differ significantly from the control group. There is no evidence that the compound had an adverse effect on growth for either sex.

E. FOOD CONSUMPTION

There is no evidence of decreased diet consumption for any dose group for either sex.

F. OPHTHALMOSCOPIC EXAMINATION

There were no notable findings in either sex.

G. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

There were no statistical differences between the control and the dosage groups for male and female rats for the parameters WBC, RBC, HGB, HCT and PLT.

In the case of the differential leucocyte count for the male animals no treatment related effect was seen for eosinophils, basophils and neutrophils. For monocytes both ANOVA and the Wilcoxon test showed the 2000 ppm group to be significantly different from all other groups. However, there was no dose-related effect as the control, the 3000, 5000 and 7500 ppm dose groups were not statistically different.
For the parameter lymphocytes the 2000 and 5000 ppm dose groups were significantly elevated with respect to the control. However, the 3000 and 7500 ppm groups were not significantly different and a progressive effect of dose was not seen.

The parameters monocytes, eosinophils and basophils showed no treatment-related effect either on the ANOVA or Wilcoxon tests, for the female animals. For the parameter neutrophils the 3000 ppm group showed a significant decrease with respect to the control. The other dose groups were not different from the control and there was no evidence of a progressive effect.

Table 5.3.2-53: Glyphosate Technical: 90 Day Oral Toxicity study in the Rat ([redacted], 1989): Selected haematological findings (means ± SD)

Parameter	Dose group [ppm]									
	Males					Females				
	0	2000	3000	5000	7500	0	2000	3000	5000	7500
Monocytes [%]	4.25 ± 1.02	**2.9* ± 0.99**	4.10 ± 1.37	4.20 ± 1.03	4.00 ± 0.94	3.32 ± 0.95	3.80 ± 1.48	3.90 ± 1.10	2.90 ± 0.99	3.90 ± 1.20
Lymphocytes [%]	78.60 ± 3.12	**83.00* ± 4.24**	81.30 ± 3.16	**83.80* ± 3.16**	80.10 ± 3.67	78.68 ± 3.32	74.20 ± 6.94	82.60 ± 4.99	82.10 ± 6.51	80.90 ± 4.95
Neutrophils [%]	15.30 ± 3.08	12.70 ± 3.47	13.10 ± 3.85	10.90 ± 2.13	13.40 ± 3.69	16.84 ± 3.48	19.60 ± 6.82	**11.80* ± 3.58**	13.30 ± 5.72	13.70 ± 3.71

* Significant effect of treatment

There were no statistical differences between the control and the dosage groups for either sex for the parameter Prothrombin time.
There were no statistical differences between the control and the dosage groups for female rats for the parameter Activated Partial Thromboplastin Time (APTT). However, in the case of male animals, the 7500 ppm dose group was statistically different from the 2000, 3000 and 5000 ppm dose groups. The mean value for this parameter for CD rats kept at Biological Laboratories is 22.25 seconds with a standard deviation of 3.45. Both the control and 7500 ppm groups in this experiment were significantly different from the mean baseline value. The reason for this anomaly is not clear.

Table 5.3.2-54: Glyphosate Technical: 90 Day Oral Toxicity study in the Rat ([redacted], 1989): Selected clinical chemistry findings (means ± SD)

Parameter	Dose group [ppm]									
	Males					Females				
	0	2000	3000	5000	7500	0	2000	3000	5000	7500
APTT [s]	27.3 ± 5.5	**↓20.2* ± 2.5**	**↓20.5* ± 2.5**	**↓23.3* ± 2.6**	↑27.7 ± 5.6	23.5 ± 9.4	↓20.7 ± 2.0	↓20.4 ± 2.9	↓22.7 ± 3.1	↓20.3 ± 1.6

* Significant effect of treatment

Blood clinical chemistry

In the case of the following parameters - gGT, calcium, total protein, potassium and urea - there was no statistical difference between the dosage groups and the controls at any dose level (Statistical analysis was not performed on gGT values for male rats, due to small sample numbers).

For the parameter ALT all male dose groups were slightly but significantly elevated with respect to the control. However there was no progressive increase with dose level (the 2000, 3000 and 7500 ppm groups were not significantly different from each other). For all dose groups the values seen were within the normal range (39 – 183 u/L) for male CD rats.

For female rats the ALT levels for the 3000 ppm and the 5000 ppm group did not differ significantly from the control group. The 2000 ppm and 7500 ppm animals did not differ significantly from each other but were significantly elevated with respect to the control group. There was no progressive increase with dose level. For all dose groups the values seen were within the range (30 – 262 u/L) for female CD rats of the same age group.

For the parameter AST, there was no significant difference between any of the dose groups and the control for the male animals. For the female animals the 2000 ppm group was significantly elevated. There was no significant difference between the mean levels of all other groups and the mean level for the control. There was no evidence of a progressive increase with dose.

For total bilirubin, the dose groups in the case of the male animals had significantly lower mean levels than the control group. The reason for this result is not clear. For the female animals there was no significant difference between any dose group and the control.

For the parameter total protein, the group mean values did not differ from the control for either sex.

For the parameter albumin, the 5000 ppm dose group had a slightly but significantly lower mean value than the control. The other groups – 2000, 3000 and 7500 ppm - were not statistically different from the control. The albumin levels for all male animals including the control rats were considerably lower than the values for the female animals. For the female rats no dose group had a mean value significantly different from the control group mean value.

For the parameter sodium, the 5000 ppm male group mean value was slightly but significantly lower than the control group level. The levels for the other groups were not statistically different from the control value. There was no evidence of a progressive effect with dose. The group mean values for the female rats were not significantly different from the control value.

In the case of creatinine values the dose groups were not statistically different for female animals. For male animals the values for the 2000, 3000 and 5000 ppm dose groups are significantly lower than the control and 7500 ppm values. There is no evidence of a dose-related effect.

Table 5.3.2-55: 90 Day Oral Toxicity study in the Rat ([redacted], 1989): Selected clinical chemistry findings

Parameter	Dose group [ppm]									
	Males					Females				
	0	2000	3000	5000	7500	0	2000	3000	5000	7500
ALT [u/L]	64.35 ± 8.63	↑**87.56* ± 19.22**	↑**80.56* ± 12.94**	↑79.11 ± 6.47	↑**89.70* ± 11.21**	65.80 ± 11.01	↑**100.90 * ± 45.26**	↑76.29 ± 9.23	↑76.67 ± 9.25	↑**94.33* ± 25.18**
AST [u/L]	150.55 ± 24.65	↑166.44 ± 36.95	↑179.33 ± 32.34	↑150.67 ± 24.15	↓149.40 ± 30.32	123.07 ± 21.62	↑**181.90 * ± 87.72**	↑**145.43 * ± 25.83**	↑133.89 ± 25.98	↑126.67 ± 26.41
Albumin	31.65 ±	↓31.22	↑31.67	↓**29.00***	↑31.80	42.27 ±	↓39.44	↑42.29	↓41.89	↓40.33

Table 5.3.2-55: 90 Day Oral Toxicity study in the Rat ([redacted], 1989): Selected clinical chemistry findings

Parameter	Dose group [ppm]									
	Males					Females				
	0	2000	3000	5000	7500	0	2000	3000	5000	7500
[g/L]	2.21	± 1.64	± 1.12	**± 1.87**	± 1.75	3.47	± 2.60	± 3.15	± 3.95	± 3.87
Total bilirubin [µM/L]	4.45 ± 0.51	**↓1.67* ± 0.50**	**↓1.89* ± 0.33**	**↓1.89* ± 0.60**	**↑3.60* ± 1.26**	4.36 ± 0.50	↓4.22 ± 0.83	↑4.43 ± 0.79	↓4.33 ± 0.50	↓4.11 ± 0.60
Sodium [mM/L]	144.25 ± 8.88	↓142.89 ± 2.32	↓142.33 ± 2.45	**↓137.25 * ± 7.80**	↓143.40 ± 5.56	141.54 ± 3.48	↑144.88 ± 3.48	↑145.71 ± 2.14	↑145.67 ± 2.24	↑141.57 ± 11.01
Creatinine [µM/L]	45.40 ± 3.16	**↓39.89* ± 2.26**	**↓38.63* ± 2.56**	**↓39.88* ± 3.23**	↑45.78 ± 5.52	41.00 ± 4.76	↑44.43 ± 5.91	↓39.67 ± 3.61	↑42.67 ± 3.20	↑45.33 ± 4.08

* Significant effect of treatment

H. NECROPSY

Organ weights

For liver, kidneys, adrenals and testes in male animals, there were no statistical differences between the dosage groups and the control.

In female animals, there were no statistical differences between the dosage groups and the control for kidneys and adrenals. In the 2000 ppm dose group, the mean liver weight is statistically different from the control, but is well within the normal range (Mean ± 2 × SD) for control animals. The 3000, 5000 and 7500 ppm dose groups were not statistically different from the control group.

Table 5.3.2-56: 90 Day Oral Toxicity study in the Rat ([redacted], 1989): Selected organ weights

Organ	Dose group [ppm]									
	Males					Females				
	0	2000	3000	5000	7500	0	2000	3000	5000	7500
Liver [g]	18.24 ± 2.96	↓18.04 ± 3.67	↑18.79 ± 3.43	↓17.51 ± 3.12	↑19.23 ± 3.40	10.26 ± 1.51	**↑11.91* ± 1.28**	↑10.35 ± 1.29	↑10.79 ± 1.46	↑10.40 ± 1.12

* Statistically significant from control ($p \leq 0.05$)

For male animals the statistics showed no treatment related effect on body weight at death or on the organ/body weight ratios examined (liver, kidney, adrenals and testes).

For female animals ANOVA showed no treatment related (i.e. test compound-related) effect on body weight at death or on the organ /body weight ratios.

At the 2000 ppm dose the p-value was 0.064. The Duncan test showed this group to be significantly different from the control. However, there was no progressive effect of treatment. All of the higher dose levels did not significantly change the liver/body weight ratio.

Table 5.3.2-57: 90 Day Oral Toxicity study in the Rat ([redacted], 1989): Selected organ/body weight ratios

Organ	Dose group [ppm]									
	Males					Females				
	0	2000	3000	5000	7500	0	2000	3000	5000	7500
Liver/body weight ratio	0.04258 ± 0.0037	↓0.03994 ± 0.0067	↑0.04335 ± 0.0045	↓0.04185 ± 0.0040	↑0.04297 ± 0.0066	0.04174 ± 0.0054	↑0.04659 ± 0.0043	↑0.04218 ± 0.0328	↑0.04360 ± 0.0039	↑0.04191 ± 0.0033

Gross pathology
In the 2000 ppm dosage group one male rat was reported to have enlarged intestines. The epididymal fat pad appeared red and irritated and an abscess-like growth was found attached to the caecum.

In the 3000 ppm dosage group, one female rat had a distended uterus. A second female in this group had an enlarged uterine wall.

No obvious abnormalities were seen in the other animals for the dosage groups.

Histopathology
The range of histopathological findings in the study animals was within the expected spectrum of background pathology. The dosed animals were not distinguishable from the control animals. There were no histopathological findings suggestive of specific target organ cytotoxicity attributable to administration of the test substance at any dose level.

Satellite group
Satellite animals were fed normal powdered diet for 5 weeks following the end of the live phase of the main study. There was no evidence of a delayed effect during this period. No adverse clinical effects were noted.

No compound related abnormalities were seen at gross necropsy. For the haematology and coagulation parameters the satellite control and 7500 ppm dose groups were statistically similar. The body weight, organ weight and organ weight/body weight ratios were also statistically similar for the two groups. No further investigations were carried out.

III. CONCLUSIONS

No deaths occurred during the study. Observations on the live animals showed no compound-related or dose-related adverse effects - either at the weekly clinical examinations or the ophthalmological examinations. There was no compound related adverse effect on growth. Gross necropsy at the end of the dosing period showed no abnormalities. There was no adverse effect on organ weights or on organ weight \ body weight ratios. Studies carried out on the terminal bleeds showed no adverse effects on the following haematology parameters: white blood cell counts, red blood cell counts, haemoglobin concentration, haematocrit and platelets. Investigation of the differential leucocyte count showed some groups differing significantly from the control. However, a progressive effect of dose on response was not seen for any parameter. The occasional minor differences seen are considered not to be compound related.

Measurement of the coagulation responses showed no compound related adverse effect. Clinical chemistry analyses showed some group mean values differing significantly from the control for one or other parameter. However, a progressive effect of dose on response was not seen for any parameter. Overall no compound related adverse effect was seen.

The range of histopathology findings in the study animals was such as would be expected within a normal group of rats of this age range. The animals receiving glyphosate at up to 7500 ppm could not be distinguished on the basis of the histopathology findings. There was no evidence of specific target organ cytotoxicity attributable to administration of the test substance at any dose level.
The no adverse effect level was greater than 7500 ppm in the diet.

3. Assessment and conclusion

Assessment and conclusion by applicant:
In this study, groups of male and female CD rats were administered glyphosate technical via the diet at dose levels of 0, 2000, 3000, 5000, 7500 or 7500 (satellite group) ppm (equivalent to 100, 150, 250 or 375 mg/kg bw/day for males and females) over a period of 90 – 92 days according to OECD 408 (1981) and in compliance with GLP (no certificate of the competent authority was provided).

There were no treatment-related adverse effects on survival, clinical signs, body weight, haematology, clinical chemistry and histopathology. Therefore, the no observed adverse effect level (NOAEL) was in excess of 7500 ppm in the diet (equivalent to 375 mg/kg bw/day in males and females) under the conditions of this study.

Assessment and conclusion by RMS:
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1. Information on the study

Data point	CA 5.3.2/014
Report author	[redacted]
Report year	1987
Report title	90 Day Study of Glyphosate Administered in Feed to Sprague/Dawley Rats
Report No	[redacted]-7375
Document No	Not reported
Guidelines followed in study	Not reported, but in general compliance to OECD 408 (1981)
Deviations from current test guideline (OECD 408, 2018)	Clinical signs were not recorded daily; no sensory reactivity was examined; haematology was performed without determining prothrombin time; clinical chemistry was performed without determining HDL, LDL, T4, T3 and TSH; organ weights of the adrenals, brain, heart, ovaries, pituitary gland, prostate (seminal vesicles and coagulating glands), spleen, thyroid gland, thymus and the uterus were not determined; histopathology was performed without coagulating glands, gall bladder and vagina. Deviations from the current version of OECD 408 (2018) are basically due to the fact that the study was aligned to an older version of the OECD test guideline 408.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary
Glyphosate (Lot XLG 161) was administered to Sprague-Dawley rats at target levels of 0, 1000, 5000 or

20000 ppm in the feed for approximately three months. Analyses to verify the stability of the test material, both neat and when mixed with the diet, the diet homogeneity, and concentrations of the test material in the diet were performed with satisfactory results. Overall averages of dietary concentrations for the study were 950, 4600 or 19000 ppm for the low, middle and high levels, respectively. Overall averages for consumption of test material at the low, middle and high levels, respectively, were 63, 317 or 1267 mg/kg bw/day for males and 84, 404 or 1623 mg/kg bw/day for females.

The group size was 12 animals per sex and dose group. The animals were examined for mortality (daily), clinical signs, body weight, food consumption (weekly), ophthalmoscopy (before treatment and termination), and haematology, clinical chemistry, urinalysis, gross pathology, organ weights and histopathology at termination.

No evidence of toxicological effects was observed in any parameter examined.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate (T860067)
Description:	White powdery solid
Lot/Batch #:	Lot XLG 161
Purity:	95.21 %
Stability of test compound:	Not reported

2. Vehicle and/or positive control: Diet / none

3. Test animals:

Species:	Albino rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	ca. 6 weeks
Sex:	Male and female
Weight at dosing:	♂ 206 g; ♀ 141 g
Acclimation period:	16 days
Diet/Food:	Ralston Purina RODENT CHOW No. 5002
Water:	Water (sodium zeolite-conditioned St. Louis public water supply), *ad libitum*
Housing:	Individual suspended stainless steel cages, over paper bedding
Environmental conditions:	Temperature: 21 – 23 °C Humidity: 35 – 60 % Air changes: Not reported 12 hours light/dark cycle

B: Study design and methods

In life dates: 1986-11-20 to 1987-02-26

Animal assignment and treatment:
The test material was offered on a continuous basis in the basal diet to groups of 12 Sprague-Dawley rats per sex for a minimum of 90 days. Dietary target concentrations were 0, 1000, 5000 or 20000 ppm. Analysis of the test diets revealed an average dose of 0, 950, 4600 or 19000 ppm which is equivalent to 0, 63, 317 or 1267 mg/kg bw/day for males and 0, 84, 404 or 1623 mg/kg bw/day for females.

Table 5.3.2-58: 90 Day Study of Glyphosate Administered in Feed to Sprague/Dawley Rats ([redacted], 1987): Study design

Test group	Dietary target concentration [ppm]	Average dietary concentration [ppm]	Average achieved dose[mg/kg bw/day]	Males	Females
Control	0	0	♂: 0; ♀: 0	12	12
Low	1000	950	♂: 63; ♀: 84	12	12
Mid	5000	4600	♂: 317; ♀: 404	12	12
High	20000	19000	♂: 1267; ♀: 1623	12	12

Analysis of the test diet
Test Material Stability: Determined prior to and after the in-life portion of the study.

Homogeneity of Diet Mixtures: Analysis of duplicate samples from top, middle, and bottom of mixer for lowest and highest levels (determined once during the study).

Diet Mixture Stability: Analysis of samples kept refrigerated (closed container, 33 days) or at ambient temperature (open container, 6 and 14 days)

Dietary Level Verification: Extraction of diets with water/chloroform; analysis by liquid chromatography with UV/VIS detector; all dietary levels for first 6 weeks, one level/week thereafter

Mortality
Animals were checked twice daily for mortality and moribundity.

Clinical observations
Detailed observations for clinical signs of toxicity were performed weekly.

Body weight
The weight of each animal was recorded once weekly.

Food consumption
The quantity of food consumed by each animal was recorded once each week.

Ophthalmoscopy
Ophthalmoscopic examination was performed by indirect ophthalmoscopy on all animals before treatment and near termination on all surviving animals.

Haematology and clinical chemistry
Samples were taken from the posterior *vena cava* of all anesthetised animals from each group/sex at termination. Food and water was withheld for approximately 18 hours prior to blood collection.

Haematology:
The following parameters were determined: Haematocrit, haemoglobin, total red blood cell count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count, total white blood cell count, differential white blood cell count (thin blood smears on labelled glass slides prepared, stained with Wright's stain, and examined microscopically)

and reticulocyte count (a portion of the EDTA-treated sample mixed with a vital stain (methylene blue), a slide prepared and examined microscopically).

Clinical chemistry
The following parameters were determined: Alkaline phosphatase (ALP), aspartate amino transferase (AST), Alanine aminotransferase (ALT), creatinine (Crea), blood urea nitrogen (BUN), total protein (TP), albumin (Alb), globulin, glucose (Glu), total cholesterol (Chol), total bilirubin (T.Bi), direct bilirubin, sodium (Na), potassium (K), chloride (Cl), calcium (Ca), inorganic phosphorus (P).

Urinalysis
Urine samples were collected from all animals via metabolism trays for approximately eighteen hours. Food and water was withheld during urine collection. The following parameters were measured: Specific gravity, glucose, ketones, blood, pH, protein, urine sediment, bilirubin and urobilinogen.

Sacrifice and pathology
All animals were killed and necropsied. External and internal investigations were performed on opened internal cavities and organs *in situ* and then removed. Hollow organs were opened and examined.

Organ weights
The following organs were weighed: Kidneys, liver and testes (with epididymides).

Histopathology
The following tissues were processed and examined histopathologically from all Control and High dose animals.
The following tissues were investigated: Adrenals, aorta, bone with bone marrow, brain, eyes, heart, intestine (duodenum, jejunum, ileum, caecum, colon), harderian gland, kidneys, liver, lungs, mammary gland, mesenteric lymph node, muscle, nasal turbinates, oesophagus, ovaries, pancreas, pituitary, prostate, rectum, sciatic nerve, seminal vesicles, skin, spinal cord, spleen, stomach, submaxillary salivary gland, submandibular lymph node, testes (with epididymides), thymus, thyroid/parathyroid, trachea, urinary bladder and uterus (with cervix).
Additionally, kidneys, liver and lungs were examined from low and mid dose animals.

Statistics
The following statistical procedures were used to detect statistically significant differences between treated animals and their respective controls:

Dunnett's Multiple Comparison Test (two-tailed): body weights, food consumption, non-categorical clinical pathology data, absolute organ weights.

Mann-Whitney Test with Bonferroni Inequality Procedure: Organ weight/ body weight ratios.

Fisher's Exact Test with Bonferroni Inequality Procedure: Incidence of microscopic lesions.

Other statistical routines used for some data were: Bartlett's Test to evaluate homogeneity of variances, Analysis of Variance to determine if the sample (group) means could be considered as an estimate of a common population and Grubb's Test to detect outliers.

II. RESULTS AND DISCUSSION

A. ACHIEVED DOSAGES AND ANALYSIS OF THE FORMULATED DIETS
Results of analyses for test material stability conducted over a span of time exceeding the length of the study indicated the neat test material was stable. The homogeneity of the diet mixtures was determined to be adequate. The stability of the test material/diet mixtures was demonstrated for the low and high levels, stored in open containers at room temperature for up to 14 days, and stored in closed containers in a

refrigerator for 33 days. Weekly analyses of the test material in the diet were performed on all levels for the first 6 weeks and on one level each week thereafter. Please refer to the table above**Error! Reference source not found.** (Table 5.3.2-58).

B. MORTALITY

There were no animals found dead or killed *in extremis* in any group during the treatment period.

C. CLINICAL OBSERVATIONS

There were no clinical signs in the control and treated groups that were considered to be due to administration of glyphosate.

D. BODY WEIGHT

There were no notable intergroup differences in either sex.

E. FOOD CONSUMPTION

There were no notable intergroup differences in total food consumed in either sex at any time.

F. OPHTHALMOSCOPIC EXAMINATION

There were no notable findings in either sex.

G. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

There were no deviations in haematology values which were attributed to administration of the test material. Statistically significant increases in lymphocytes in low and mid-level males and in WBC count in mid-level males were not a part of a dose-related trend, were within the normal range for rats of this age, and, therefore, were attributed to biological variation.

Table 5.3.2-59: 90 Day Study of Glyphosate Administered in Feed to Sprague/Dawley Rats ([redacted], 1987): Selected haematological findings

Parameter	Dose group [ppm]							
	Males				Females			
	0	950	4600	19000	0	950	4600	19000
Differential white blood cell count – Lymphocytes [10^3/mm^3]	7.49 ± 2.19	↑**9.92* ± 2.34**	↑**11.03** ± 2.04**	↓6.58 ± 1.87	4.35 ± 0.98	↑4.42 ± 1.62	↑4.86 ± 1.71	↓3.78 ± 0.68
White blood cell count [10^3/mm^3]	10.6 ± 2.1	↑12.8 ± 2.3	↑**14.0** ± 2.7**	↓10.3 ± 3.6	5.7 ± 1.2	5.7 ± 1.8	↑6.4 ± 2.0	↓5.5 ± 1.6

* Statistically significant compared to control (Dunnett's test; $p \leq 0.05$);
** Statistically significant compared to control (Dunnett's test; $p \leq 0.01$)

Blood clinical chemistry

Serum inorganic phosphate and potassium were elevated in all treatment groups, and glucose was mildly elevated in mid and high level males when compared to control animals. These elevations did not increase in a dose-related manner and were either within normal ranges (potassium - all levels both sexes, inorganic phosphate - all female groups, glucose - all male groups) or close to the upper limits of normal (inorganic phosphate – male groups). Therefore, these elevations were not considered toxicologically relevant. Elevations in BUN and alkaline phosphatase in high level males were attributed to elevations in one rat from this group and not attributed to the test material. Terminal investigations for this particular rat (M3 007) revealed multiple findings in the kidney, ureter and bladder (bladder distension, numerous calculi in kidney, bladder and ureter, pyelonephritis in the kidney) indicative of urolithiasis and associated bacterial infection. This was considered to be an isolated case unrelated to administration of glyphosate.

Table 5.3.2-60: 90 Day Study of Glyphosate Administered in Feed to Sprague/Dawley Rats ([redacted], 1987): Selected clinical chemistry findings

Parameter	Dose group [ppm]							
	Males				Females			
	0	950	4600	19000	0	950	4600	19000
Calcium [mg/dL]	11.3 ± 0.26	↑11.7 ± 0.24	**↑12.0** ± 0.22**	↑11.4 ± 0.83	11.1 ± 0.27	↑11.3 ± 0.54	11.1 ± 0.31	↓11.0 ± 0.29
Glucose [mg/dL]	175 ± 23.6	↑184 ± 20.4	**↑248** ± 26.4**	**↑205*** ± 36.6	123 ± 20.4	↓120 ± 24.3	↑140 ± 17.3	↓115 ± 14.0
Phosphate [mg/dL]	8.4 ± 0.56	**↑9.4*** ± 0.86	↑9.2 ± 0.61	**↑9.4*** ± 1.3	7.2 ± 0.84	**↑9.2** ± 0.99**	**↑9.1** ± 0.87**	**↑8.4** ± 0.97**
Sodium [mEq/L]	161 ± 2.6	151 ± 1.6	↓149 ± 1.6	**↓146*** ± 9.1**	148 ± 1.4	↑147 ± 1.0	146 ± 1.3	↑147 ± 2.9
Potassium [mEq/L]	6.9 ± 0.79	↑7.6 ± 1.1	**↑8.2*** ± 1.2**	**↑8.0*** ± 1.4**	7.4 ± 0.73	**↑8.4*** ± 1.4**	↑8.4 ± 1.6	↑8.1 ± 0.98
Blood urea nitrogen [mg/dL]	17.2 ± 6.6	↓16.4 ± 2.0	↓14.6 ± 1.6	↑31.3 ± 53.7§	20.1 ± 2.6	↓18.1 ± 2.1	↓16.9 ± 1.9	↑20.4 ± 6.2
Alkaline phosphatase [IU/L]	204 ± 39.6	↑207 ± 27.7	↓199 ± 36.6	↑284 ± 166§	137 ± 32.0	↓112 ± 43.9	↓108 ± 23.1	↑148 ± 33.7

* Statistically significant compared to control (Dunnett's test; $p \leq 0.05$);
** Statistically significant compared to control (Dunnett's test; $p \leq 0.01$);
§ Animal M3 007 was not excluded for the statistical comparisons.

H. URINALYSIS

There were no abnormalities in urine measurements attributed to the test material. Statistically significant changes in specific gravity and pH of mid-level males were not considered toxicologically relevant.

Table 5.3.2-61: 90 Day Study of Glyphosate Administered in Feed to Sprague/Dawley Rats ([redacted] 1987): Selected urinalysis findings

Parameter	Dose group [ppm]							
	Males				Females			
	0	950	4600	19000	0	950	4600	19000
pH	6.1 ± 0.5	↑6.2 ± 0.4	**↑6.8*** ± 0.3**	↑6.4 ± 0.9	5.8 ± 0.4	↑5.7 ± 0.4	↑5.7 ± 0.5	↓5.5 ± 0.5
Specific gravity	1.058 ± 0.015	↑1.060 ± 0.010	**↓1.040*** ± 0.012**	↓1.041 ± 0.020	1.063 ± 0.019	↑1.068 ± 0.024	↑1.068 ± 0.025	↑1.068 ± 0.015

* Statistically significant compared to control (Dunnett's test; $p \leq 0.05$)

I. NECROPSY

Organ weights

There were no statistically or toxicologically significant differences in organ weights.

Gross pathology

The few macroscopic abnormalities observed at necropsy are common in rats and were not considered related to administration of the test material.

Histopathology

Microscopically, three high level male rats had chronic or active inflammation of the pancreatic islets which extended into the acinar parenchyma in two animals. This lesion is relatively common in this strain of rat and was not considered treatment-related in this study. The incidence of all other microscopic lesions observed in treatment groups was not significantly different from that of their respective controls.

Table 5.3.2-62: 90 Day Study of Glyphosate Administered in Feed to Sprague/Dawley Rats ([redacted], 1987): Selected histopathological findings

Parameter		Dose group [ppm]							
		Males				Females			
		0	950	4600	19000	0	950	4600	19000
Pancreas	Acinar atrophy/ degeneration	1	-	-	3	-	-	-	-
	Fibrosis, interstitial, islets	0	-	-	3	-	-	-	-
	Inflammation, islets	0	-	-	3	-	-	-	-
	Inflammation, acinar	0	-	-	2	-	-	-	-
	Mononuclear infiltrate	2	-	-	2	1	-	-	2

III. CONCLUSIONS

Glyphosate (Lot XLG 161) was administered to Sprague-Dawley rats at target levels of 0, 1000, 5000 or 20000 ppm in the feed for approximately three months.

The animals were examined for mortality (daily), clinical signs, body weight, food consumption (weekly), ophthalmoscopy (before treatment and termination), and haematology, clinical chemistry, urinalysis, gross pathology, organ weights and histopathology at termination.

No evidence of toxicological effects was observed in any parameter examined. Therefore, a No Observable Effect Level (NOEL) for glyphosate, as administered in this study, was apparently greater than 20000 ppm.

3. Assessment and conclusion

Assessment and conclusion by applicant:
In this study, glyphosate was administered to Sprague-Dawley rats at target levels of 0, 1000, 5000 or 20000 ppm in the feed (equivalent to actual doses of approx. 0, 63, 317 or 1267 mg/kg bw/day for males and 84, 404 or 1623 mg/kg bw/day for females) for approximately three months in general compliance to OECD 408 (1981) and GLP (no certificate of the competent authority was provided).
No evidence of toxicological effects was observed in any parameter examined. Therefore, the NOAEL for glyphosate, as administered in this study, is ≥ 19000 ppm (actual dose; equivalent to 1267 mg/kg bw/day for males and 1623 mg/kg bw/day for females).

Assessment and conclusion by RMS:
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3. Information on the study

Data point:	CA 5.3.2/015
Report author	[redacted]
Report year	1985
Report title	Subacute Oral Toxicity (90 Days) to Rats of Glyphosate (Technical) of Excel Industries Ltd.
Report No	Not reported
Document No	Not reported

Guidelines followed in study	Not reported, similar to OECD 408 (1981)
GLP	Not reported
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	Groups of ten Wistar rats per sex and dose were administered glyphosate (purity not reported) at dose levels of 0 (control group receiving the vehicle, i.e. 0.1 % Tween 80 in water), 300, 1200 or 2400 mg/kg bw/day for 90 days by oral gavage. In addition, a second group receiving the mid dose of 1200 mg/kg bw/day was sacrificed after a 30-day recovery period (reversal group). Animals were observed daily for signs of toxicity. Body weight and food consumption were determined regularly. Blood samples for haematological (red and white cell parameters) and clinical chemistry (total serum protein, alanine aminotransferase, alkaline phosphatase, blood urea nitrogen and glucose) investigations were taken prior to treatment, on day 45 and on days 91 and 121 just prior to sacrifice. Urinalysis was also performed. All animals were subjected to gross pathological examination and extended histopathology (salivary glands, however, not examined). Organ weights were determined.
Short description of results:	There were no deaths during the study and no signs of toxicity were observed. Laboratory investigations and pathological examinations did not reveal indications of adverse effects. The only findings which could be attributed to treatment were a somewhat lower body weight gain and a reduced food intake becoming more apparent towards the end of treatment period in high dose males and females. Thus, the NOAEL in this 90–day gavage study was 1200 mg/kg bw/day.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	Monograph (2000): The study was considered supportive in the Monograph (2000) due to serious reporting deficiencies, e.g. the year when the study was performed, was not indicated in the original report. Furthermore, there was no information on the guideline followed and on GLP status available from the original report. Statistical analysis of the results was not reported. RAR (2015): The study was considered unacceptable due to serious reporting deficiencies, e.g. absence of statistical analysis. Report identification and dates of experimental work not given. Purity and Batch number of the test substance not reported. Therefore and since the study report is not available, this study is considered invalid.
Reasons why the study report is not available for submission	The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL.
Category study in AIR 5 dossier (I, docs)	Category 4b

1. Information on the study

Data point:	CA 5.3.2/016
Report author	[redacted]
Report year	1981
Report title	Glyphosate subchronic toxicological study
Report No	Not reported
Document No	Not reported
Guidelines followed in study	Not reported, similar to OECD 408 (1981)
GLP	No, pre-GLP
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	Groups of 10 Wistar rats per sex and dose were administered glyphosate (purity: 96.8 %; manufacturer: Alkaloida, Tiszavasvari, Hungary) for one or for three months, respectively, at dietary levels of 0, 1000, 3000 or 10000 ppm. Observations performed were: Mortality, clinical signs, body weight, food consumption, haematology, clinical chemistry, organ weight and histopathology.
Short description of results:	There was no mortality in this study and no clinical signs of toxicity. Body weight and food consumption were similar throughout the study groups. Haematology revealed a number of changes of which a reduced red blood cell count, an increase in leucocyte count at 3000 and 10000 ppm in both sexes and a higher platelet count in all treated male groups and in high and mid dose females were probably treatment-related. In females, blood glucose levels were increased at the highest dose level. Alkaline phosphatase activity was increased in both sexes at 10000 ppm and so were alanine aminotransferase and aspartate aminotransferase activities. At 10000 ppm, the liver weights were increased in both sexes and for the males round liver edges were reported. The lowest dietary level of 1000 ppm is considered the NOAEL in this study. For male and female rats receiving the test substance for three months, a mean daily compound intake of 102 or 105.4 mg/kg bw/day, respectively, was calculated.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	Monograph (2000): The study was considered supplementary in the Monograph (2000) due to serious reporting deficiencies. When the study was performed, GLP was not compulsory. Measurement of mean daily intake of test substance for all dose levels was not performed. The study was considered unacceptable in the RAR (2015). Therefore and since the study report is not available, this study is not considered to be reliable.
Reasons why the study report is not available for submission	The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL.
Category study in AIR 5 dossier (L docs)	Category 4b

1. Information on the study

Data point	CA 5.3.2/017
Report author	[redacted]
Report year	1995
Report title	HR-001: 13-week Subchronic Oral Toxicity Study in Mice
Report No	[redacted] 94-0136
Document No	Not reported
Guidelines followed in study	Japan MAFF Guidelines 59 NohSan No.4200, 1985; U.S. EPA FIFRA Guidelines Subdivision F, 1984; OECD 408 (1981)
Deviations from current test guideline (OECD 408, 2018)	Reticulocytes not counted, clotting not evaluated, total cholesterol but not HDL and LDL measured, urea not measured, no blood hormones (T3, T4 and TSH) measured; organ weights limited to brain, liver, kidneys, testes, adrenals and caecum. Deviations from the current version of OECD 408 (2018) are basically due to the fact that the study was aligned to an older version of the OECD test guideline 408.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

In order to evaluate the sub-chronic toxicity of HR-001 in mice, the test substance was administered by incorporating it into a basal diet to each dose group of 12 males and 12 females of SPF ICR mice (Crj:CD-1) at a dose level of 0, 5000, 10000 or 50000 ppm (equivalent to 0, 600.2, 1221 or 6295 mg/kg bw/day for males and 0, 765.0, 1486 or 7435 mg/kg bw/day for females) for a period of 13 weeks.

50000 ppm group: Males showed a depressed body weight gain associated with lowered food consumption and food efficiency throughout the treatment period. Decreased food efficiency was also observed in females. In haematological examinations, females showed decreases in haematocrit (Ht), haemoglobin concentration (Hb) and erythrocyte count (RBC). Blood chemical examinations revealed increases of alkaline phosphatase (ALP) in males and females and inorganic phosphorous (P) in females. At necropsy, males and females revealed increased incidences of distention of the caecum. In organ weight analysis, males and females showed increases of absolute and relative weights of the caecum. Histopathologically, males showed an increase in incidence of cystitis of the urinary bladder.

10000 ppm group: Distention of the caecum was observed in one female at necropsy. In organ weight analysis, increasing tendencies were noted in absolute and relative weights of the caecum.

5000 ppm group: There were no treatment-related changes in either sex in any parameters.

I. MATERIALS AND METHODS

A: Materials

1. Test material: Glyphosate technical

Identification:	HR-001
Description:	White crystal
Lot/Batch #:	T-941209
Purity:	97.56 %
Stability of test compound:	26/12/1994

2. Vehicle and/or positive control: Plain diet / none

3. Test animals:

Species:	Mouse
Strain:	Crj:CD-1
Source:	[redacted]
Age:	5 weeks
Sex:	Male and female
Weight at dosing:	♂ 27.3 – 32.7 g; ♀ 22.4 – 25.8 g
Acclimation period:	9 days
Diet/Food:	MF Mash (Oriental Yeast Co., Ltd.)
Water:	Filtered and sterilized tap water, *ad libitum*
Housing:	3/cage, sexes separately in stainless steel cages 21.5 × 33.0 × 18.0 cm
Environmental conditions:	Temperature: 24 ± 2 °C Humidity: 55 ± 15 % Air changes: 15/hour 12 hours light/dark cycle

B: Study design and methods

In life dates: 1995-01-10 to 1995-04-27

Animal assignment and treatment:
The test material was offered on a continuous basis in the basal diet to groups of 24 SPF ICR mice (Crj: CD-1) (12 males + 12 females) for a minimum of 90 days. Dietary concentrations were 0, 5000, 10000 or 50000 ppm (equivalent to 0, 600.2, 1221 or 6295 mg/kg bw/day for males and 0, 765.0, 1486 or 7435 mg/kg bw/day for females).

Table 5.3.2-63: HR-001: 13-week Subchronic Oral Toxicity Study in Mice ([redacted], 1995): Study design

Test group	Dietary concentration [ppm]	Test substance intake [mg/kg bw/day]	Males	Females
Control	0	♂: 0; ♀: 0	12	12
Low	5000	♂: 600.2; ♀: 765.0	12	12
Mid	10000	♂: 1221; ♀: 1486	12	12
High	50000	♂: 6295; ♀: 7435	12	12

Chemical analysis for homogeneity and concentration of the test substance in the diet were performed on samples (about 50 g each) of each does level taken from top, middle and bottom portions of the mixer at the first diet preparation. The control diet was also sampled (50 g each) and analysed to confirm that there was no contamination with the test substance. Concentrations of the test substance in test diets at all dose levels were monitored on the same amount of samples (50 g each) every 3 weeks during the study.

Mortality
Each animal was checked for mortality or signs of morbidity at least once daily during the treatment period.

Clinical observations
Cage-side observation was performed daily on all animals to detect moribund or dead animals and abnormal clinical signs, and all findings were recorded. In addition, a detailed examination including palpation for masses was performed at least once a week.

Body weight
Body weights of all animals were recorded at initiation of treatment and weekly during the study. Group mean body weight was calculated for each dose group at each measurement. Final body weights were recorded for all animals before necropsy.

Food consumption and utilisation
Food consumption for each cage was measured weekly for a period of 3 consecutive days. Mean daily food consumption per animal in each cage was calculated by dividing the weekly food consumption by the number of animals per cage and by the number of days for measurement. Group mean food consumption (g/rat/day) was calculated at each measurement from the mean daily food consumption per animal in each cage.

Group mean chemical intake (mg/kg bw/day) was calculated from nominal dietary concentrations of the test substance, food consumption and body weight.

Group mean food efficiency for each dose group was calculated weekly from the ratio of the group body weight gain to group mean food consumption and expressed as percentage. Overall group mean efficiency throughout the treatment period was also calculated for all dose groups.

Ophthalmoscopic examination
Ophthalmological examinations including observation with a halogen ophthalmoscope were performed on all animals during acclimatisation period and on all surviving animals in the control and the highest dose groups from the main group at week 13.
The following parameters were determined: Eyeball, cornea, anterior chamber, pupil and iris.

Haematology and clinical chemistry
After 13 weeks of treatment, all surviving animals were subjected to haematological examinations. The animals were laparotomised under anaesthesia following overnight fasting, and blood samples were withdrawn from the posterior vena cava using heparinised syringes. A part of each sample was poured into a cup treated with EDTA and subjected to the examinations.

The following parameters were determined with a fully automated haematology analyser: Haematocrit (Ht), haemoglobin (Hb), erythrocyte count (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count (PLT) and total leukocyte count (WBC).

After 13 weeks of treatment, all surviving animals were subjected to blood biochemical examinations. Plasma samples obtained from the heparinised blood were used for examinations.

The following parameters were determined: Alkaline phosphatase (ALP), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), γ-glutamyl transpeptidase (GGTP), creatine phosphokinase (CPK), creatinine (Creat.), blood urea nitrogen (BUN), total protein (TP), albumin (Alb), globulin (Glob.), albumin/globulin ratio (A/G ratio), glucose (Gluc.), total cholesterol (T. Chol.), triglyceride (TG), total bilirubin (T. Bil.), calcium (Ca) and inorganic phosphorus (P).

Urinalysis
At 13 weeks of treatment, all surviving animals were subjected to urinalysis. Fresh urine samples were collected by pressing the lumbodorsal region of the animals. Specific gravity was determined with a handy refractometer. Glucose, ketones, occult blood, pH, protein, and urobilinogen were semi-quantitatively analysed by Uro-labstix. Then animals were housed individually in metabolic cages overnight, and urine samples collected were examined for volume and appearance. Urinary sediments were also examined microscopically on these samples.

Sacrifice and pathology
Clinical pathology evaluations were also conducted. Selected organs were weighed at the scheduled necropsy. Histopathological examinations were performed on selected tissues from all animals.
The following parameters were determined: Brain, spinal cord, sciatic nerve, pituitary, thyroids with parathyroids, thymus, adrenals, spleen, bone with marrow, tibio-femoral joint, lymph nodes, heart, aorta, pharynx, salivary glands, oesophagus, stomach, liver, pancreas, duodenum, jejunum, ileum, caecum, colon, rectum, head, larynx, trachea, lung, kidneys, urinary bladder, testes, prostate, seminal vesicles, epididymides, coagulating glands, ovaries, uterus, vagina, harderian glands, eyes, skeletal muscle, skin, mammary gland and all gross lesions.

Statistics
All data were evaluated using variance analysis (body weight, food consumption, urine specific gravity, urine volume, haematological parameters, blood chemical parameters and organ weights).
Data on clinical signs, mortality, ophthalmology, necropsy and histopathology were evaluated by Fisher's exact probability.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no animals found dead or killed *in extremis* in any group during the treatment period.

B. CLINICAL OBSERVATIONS
There were no treatment-related abnormalities in clinical signs in the control and treated groups during the treatment period.

C. BODY WEIGHT
In the 50000 ppm group, mean body weights of males were lower than those of the control from week 2 to the end of the treatment period. Mean body weight of males at week 13 was 91 % of that of control. Body weights of females were comparable to the control during the treatment period.
In the groups treated at 10000 ppm or less, body weights of males and females were comparable to the controls during the treatment period.

Table 5.3.2-64: HR-001: 13-week Subchronic Oral Toxicity Study in Mice ([redacted], 1995): Group mean body weights (selected weeks) and standard deviations

Dietary concentration [ppm]	Body weight [g] at week					
	0	2	6	8	12	13
Males						
0	30.1 ± 1.6	35.0 ± 2.2	39.1 ± 2.9	40.5 ± 2.9	43.0 ± 2.8	43.2 ± 3.1

Table 5.3.2-64: HR-001: 13-week Subchronic Oral Toxicity Study in Mice ([redacted] 1995): Group mean body weights (selected weeks) and standard deviations

Dietary concentration [ppm]	Body weight [g] at week					
	0	2	6	8	12	13
5000	30.1 ± 1.6	↓34.6 ± 2.3	↑39.5 ± 2.7	↑41.1 ± 3.0	↑44.1 ± 3.3	↑44.2 ± 3.3
10000	30.1 ± 1.5	↑35.2 ± 2.1	↑39.5 ± 2.9	↑41.1 ± 3.4	↑43.8 ± 3.8	↑44.4 ± 3.8
50000	↓30.0 ± 1.6	**↓32.6* ± 2.0**	↓37.3 ± 1.8	↓37.9 ± 2.2	**↓39.1* ± 3.4**	**↓39.5* ± 3.3**
Females						
0	23.9 ± 0.9	26.6 ± 1.6	30.7 ± 2.7	32.1 ± 2.2	35.2 ± 2.7	34.8 ± 2.2
5000	23.9 ± 1.0	↑26.9 ± 1.7	↑31.0 ± 2.0	32.1 ± 2.4	↓34.7 ± 3.3	↓34.5 ± 3.6
10000	23.9 ± 1.0	↑27.1 ± 1.7	↑31.5 ± 3.9	↑33.8 ± 4.4	↑36.7 ± 5.6	↑37.0 ± 5.8
50000	23.9 ± 1.0	↓26.1 ± 1.6	↓30.0 ± 1.9	↓31.3 ± 2.2	↓33.0 ± 2.1	↓33.4 ± 2.2

* Significantly different from control group ($p < 0.05$)

D. FOOD CONSUMPTION AND TEST SUBSTANCE INTAKE

In males of the 50000 ppm group, a significant depression of food consumption was recorded at week 1. Average food consumption of males during the treatment period was 94 % of the control value. Food consumption of females was comparable to the control.

In the groups treated at 10000 and 5000 ppm, food consumption of males and females was comparable to that of the controls.

The average daily test substance intake during the treatment are shown in the following table:

Table 5.3.2-65: HR-001: 13-week Subchronic Oral Toxicity Study in Mice ([redacted], 1995): Average test substance intake

Dose level [ppm]	Average test substance intake [mg/kg bw/day]	
	Males	Females
5000	600.2	765.0
10000	1221	1486
50000	6295	7435

In the 50000 ppm group, food efficiency of males and females was lower than that of the control animals at almost all measuring points during the treatment. Average food efficiency of males and females remained at 79 % and 88 % of the respective control value.

In the groups treated at 10000 and 5000 ppm, food efficiency in the treated groups of both sexes was comparable to that in the controls though some significant fluctuations were recorded sporadically.

Table 5.3.2-66: HR-001: 13-week Subchronic Oral Toxicity Study in Mice ([redacted], 1995): Average food efficiency during the treatment period [body weight gain/food consumption × 100]

Dose level [ppm]	Average food efficiency [%]	
	Males	Females
0	2.9	2.6
5000	↑3.3	↓2.5
10000	↑3.2	↑3.1
50000	↓2.3	↓2.3

E. OPHTHALMOSCOPIC EXAMINATION

There were no ophthalmological abnormalities in the animals of both sexes in the highest dose group and the control group.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

The significant changes (mean ± SD) observed after 13 weeks in the treated groups are summarised in the table below:

Table 5.3.2-67: HR-001: 13-week Subchronic Oral Toxicity Study in Mice ([redacted] 1995): Selected haematological findings

Parameter	Sex		Dose group [ppm]		
		0	5000	10000	50000
Haematocrit (Ht) [%]	female	43.6 ± 1.4	↓42.8 ± 2.4	↓42.5 ± 2.2	**↓40.0** ± 2.2**
Haemoglobin concentration (Hb) [g/dL]	female	14.1 ± 0.4	↓13.9 ± 0.7	↓13.8 ± 0.9	**↓13.0** ± 0.6**
Erythrocyte count (RBC) [10^6/mm^3]	female	8.71 ± 0.38	↓8.69 ± 0.57	↓8.57 ± 0.42	**↓8.05** ± 0.52**

** Significantly different from the control (p< 0.01, estimated by Dunnett's multiple comparison test)

In the high dose group, females showed significant decreases in haematocrit (Ht), haemoglobin concentration (Hb) and erythrocyte count (RBC), while males showed no significant differences from the control in any parameters.

There were no significant differences in any parameters between the treated groups of 10000 ppm or less and the control of either sex.

Blood clinical chemistry

The significant changes (mean ± SD) observed in the treated groups are summarised in the following table:

Table 5.3.2-68: HR-001: 13-week Subchronic Oral Toxicity Study in Mice ([redacted], 1995): Selected blood clinical chemistry findings

Parameter	Sex	Dose group [ppm]			
		0	5000	10000	50000
Alkaline phosphatase (ALP) [U/L]	Male	37 ± 8	37 ± 6	↑47 ± 9	**↑68** ± 16**
	Female	60 ± 15	↑69 ± 19	↑61 ± 11	**↑90** ± 29**
Glutamic pyrvic transaminase (GPT) [U/L]	Female	26 ± 7	↓22 ± 7	↓24 ± 7	**↓18** ± 3**
Creatine phosphokinase (CPK) [U/L]	Female	49 ± 14	**↑177** ± 239**	↑84 ± 102	**↑462** ± 894**
Blood urea nitrogen (BUN) [mg/dL]	Female	23.4 ± 3.3	↑23.8 ± 3.2	**↑27.9** ± 3.6**	↑25.4 ± 3.6
Inorganic phosphorus (P) [mg/dL]	Female	5.4 ± 0.7	↓5.3 ± 0.7	↑5.8 ± 0.8	**↑6.9** ± 0.9**

** Significantly different from control group (p< 0.01)

In the 50000 ppm group, males and females showed a small but statistically significant increase in alkaline phosphatase (ALP). In females, creatine phosphokinase (CPK) and inorganic phosphorus (P) were significantly increased, while a significant decrease in glutamic pyrvic transaminase (GPT) was noted.

In the 10000 ppm group, females exhibited a small but statistically significant increase in blood urea nitrogen (BUN). There were no significant changes in any parameters in males.

In the 5000 ppm group, females showed a significant increase in CPK, while there were no significant changes in any parameters in males.

G. URINALYSIS

In all treated groups, males showed a significant decrease in urinary pH. There were no abnormalities in females of any treated groups.

Table 5.3.2-69: HR-001: 13-week Subchronic Oral Toxicity Study in Mice ([redacted], 1995): Intergroup comparison of urinary pH (number of animals at pH value)

	Dose Group [ppm]							
	Males				Females			
pH	0	5000	10000	50000	0	5000	10000	50000
5.0	-	-	-	3	-	-	-	-
6.0	-	2	4	8	7	5	5	12
6.5	1	5	3	1**	2	4	5	-
7.0	3	3	3	-	2	2	2	-
7.5	2	2**	2**	-	-	1	-	-
8.0	6	-	-	-	1	-	-	-

** Significantly different from the control group (p< 0.01)

H. NECROPSY

Organ weights

In the 50000 ppm group, males and females showed significant increases in both absolute and relative weights of the caecum. The absolute weights of the caecum of males and females were 238 % and 187 % of that of the respective control. For relative weight, the ratio of the value to the respective control was 263 % or 195 % in males or females.

In the 10000 ppm group, absolute and relative weights of the caecum showed increasing tendencies in males and females. The absolute weight of the caecum of males and females were 115 % and 122 % of that of the respective control. For relative weight, the ratio of the value to the respective control was 111 % or 117 % in males or females.

In the 5000 ppm, there were no significant changes in any organ weights of males and females.

Table 5.3.2-70: HR-001: 13-week Subchronic Oral Toxicity Study in Mice ([redacted], 1995): Intergroup comparison of caecum weight – absolute and relative to body weight

Organ		Dose group [ppm]							
		Males				Females			
		0	5000	10000	50000	0	5000	10000	50000
Caecum	Absolute [mg]	624 ± 86	↓609 ± 116	↑718 ± 177	**↑1484* * ± 359**	497 ± 96	↓474 ± 115	↑604 ± 123	**↑928** ± 163**
	Relative [%]	1.45 ± 0.19	↓1.38 ± 0.26	↑1.61 ± 0.33	**↑3.82** ± 1.15**	1.43 ± 0.26	↓1.37 ± 0.30	↑1.67 ± 0.42	**↑2.79** ± 0.53**

** Statistically significant from controls (p< 0.01)

Gross pathology

In the 50000 ppm group, males and females showed a significant increase in incidence of distention of the caecum (12/12 in males and 10/12 in females; 0/12 in males and females of the control group).

In the 10000 ppm group, distention of the caecum was observed in one female. There were no significant changes in incidence of any macroscopic lesions in males.

In the 5000 ppm group, there were no treatment-related abnormalities in males and females.

Histopathology
In the 50000 ppm group, males showed significant increases in incidence of cystitis of the urinary bladder (4/12; 0/12 of the control group). There were no significant changes in incidence in females. Although significant increases in incidence of distention of the caecum were noted for males and females at necropsy, histopathological examinations failed to reveal any abnormalities in the caecum.
In the 10000 and 5000 ppm groups, there were no significant differences in incidence of histopathological lesions from the control in either sex.

III. CONCLUSIONS

In order to evaluate the sub-chronic toxicity of HR-001 in mice, the test substance was administered by incorporating it into a basal diet to each dose group of 12 males and 12 females of SPF ICR mice (Crj:CD-1) at a dose level of 0, 5000, 10000 or 50000 ppm for a period of 13 weeks.

50000 ppm group: Males showed a depressed body weight gain associated with lowered food consumption and food efficiency throughout the treatment period. Decreased food efficiency was also observed in females. In haematological examinations, females showed decreases in haematocrit (Ht), haemoglobin concentration (Hb) and erythrocyte count (RBC). Blood chemical examinations revealed increases of alkaline phosphatase (ALP) in males and females and inorganic phosphorous (P) in females. At necropsy, males and females revealed increased incidences of distention of the caecum. In organ weight analysis, males and females showed increases of absolute and relative weights of the caecum. Histopathologically, males showed an increase in incidence of cystitis of the urinary bladder.

10000 ppm group: Distention of the caecum was observed in one female at necropsy. In organ weight analysis, increasing tendencies were noted in absolute and relative weights of the caecum.

5000 ppm group: There were no treatment-related changes in either sex in any parameters.

Based on these results, the no observable effect level, minimal toxic level and sure toxic level of HR-001 in ICR (Crj:CD-1) mice under the conditions of the present study were determined as follow.

	Males	**Females**
No observable effect level	5000 ppm (600.2 mg/kg bw/day)	5000 ppm (765.0 mg/kg bw/day)
Minimal toxic level	10000 ppm (1221 mg/kg bw/day)	10000 ppm (1486 mg/kg bw/day)
Sure toxic level	50000 ppm (6295 mg/kg bw/day)	50000 ppm (7435 mg/kg bw/day)

3. Assessment and conclusion

Assessment and conclusion by applicant:
In this study, glyphosate technical was administered to groups of male and female SPF ICR mice (Crj:CD-1) at a dose level of 0, 5000, 10000 or 50000 ppm (equivalent to 0, 600.2, 1221 or 6295 mg/kg bw/day for males and 0, 765.0, 1486 or 7435 mg/kg bw/day for females) for a period of 13 weeks according to OECD 408 (1981) and in compliance with GLP (no certificate of the competent authority was provided).

Under the experimental conditions of the study, the NOAEL is considered to be 10000 ppm (equivalent to 1221 and 1486 mg/kg bw/day for males and females, respectively).

Assessment and conclusion by RMS:
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1. Information on the study

Data point:	CA 5.3.2/018
Report author	[redacted]
Report year	1991
Report title	Glyphosate: 13 week dietary toxicity study in mice
Report No	7024
Document No	Not reported
Guidelines followed in study	FIFRA 82-1; OECD 408 (1981)
Deviations from current test guideline (OECD 408, 2018)	Ophthalmoscopy and detailed clinical observations were not performed prior to dosing. Sensory reactivity to stimuli was not performed towards the end of exposure period. Reticulocyte count, platelet count and a measure of blood clotting time/potential was not measured during the haematological examinations. Clinical biochemistry determination did not include the following parameters: HDL, LDL and urea. Serum total T4, T3 and TSH were not measured at study termination. At necropsy, the oestrus cycle of all females was not determined. Organ weight of the thyroid gland was not determined; histopathology was performed without bone/bone marrow, cervix, coagulating glands, spinal cord and vagina. Deviations from the current version of OECD 408 (2018) are basically due to the fact that the study was aligned to an older version of the OECD test guideline 408.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

This study was designed to give toxicity information over 13 weeks on glyphosate administered to mice via the diet at concentrations calculated to achieve dose levels of 0, 200, 1000 or 4500 mg/kg bw/day. The group size was 10 animals per sex and dose group.

The animals were examined for mortality, clinical signs, body weight, food and water consumption, ophthalmoscopy, haematology, clinical chemistry, gross pathology, organ weights and histopathology.

In conclusion, dosing CD-1 mice via the diet for 13 weeks with up to and including 4500 mg glyphosate /kg bw/day produced no findings which could be directly attributed to administration of the test material.

I. MATERIALS AND METHODS

A: Materials

1. Test material: Glyphosate

Identification:	Not reported
Description:	White powder
Lot/Batch #:	161-JRJ-131-2 and 003-89-A
Purity:	99.5 % (batch 161-JRJ-131-2) and 98.0 % (batch 003-89-A)
Stability of test compound:	Not reported

2. Vehicle and/ or positive control: Diet/ none

3. Test animals:

Species:	Mouse
Strain:	CD-1
Source:	[redacted]
Age:	Ca. 4 weeks
Sex:	Male and female
Weight at dosing:	♂ ca. 21 g; ♀ ca. 18 g upon arrival. ♂ 28.4 g; ♀ 21.8 g at the beginning of dosing
Acclimation period:	13 days
Diet/Food:	SDS Expanded (Fine Ground) Maintenance Diet No. 1
Water:	Tap water, *ad libitum*
Housing:	One male or one female per cage in suspended polypropylene cages (48.0 x 15.0 x 12.0 cm) with wire grid tops. Sterilised white wood shavings were used as bedding.
Environmental conditions:	Temperature: 20 ± 2 °C Humidity: 55 ± 10 % Air changes: ca. 15 /hour 12 hours light/dark cycle

B: Study design and methods

In life dates: 1989-03-06 to 1989-06-06

Animal assignment and treatment:
The test material was offered on a continuous basis in the basal diet to groups of 10 CD-1 mice per sex for a minimum of 90 days. Male and female dietary concentrations were adjusted on a weekly basis to achieve an intake of 0, 200, 1000 or 4500 mg/kg bw/day.

Table 5.3.2-71: Glyphosate – 13 week dietary toxicity study in mice ([redacted] 1991): Study design

Test group	Test substance intake [mg/kg bw/day]	Males	Females
Control	0	10	10
Low	200	10	10
Mid	1000	10	10
High	4500	10	10

Analysis of the test diet
Fresh diets were prepared once each week in SDS Expanded (Fine Ground) Maintenance Diet No. 1. The method of preparation was by direct admixture of test material to untreated diet and blending for 20 min in a Winkworth change drum mixer. Each mixed batch was stored in a closed container at ambient temperature.

A 100 g sample of diet from each group/sex was retained immediately after each diet preparation.

In addition, usually 3 x 100 g samples were also taken for routine homogeneity and accuracy assessment from diets prepared for Weeks 1, 6 and 13.

In addition, data proving homogeneity and 21-day stability of glyphosate were generated prior to the commencement of the study.

Mortality
Viability was checked once each morning and once as late as practicable each day.

Clinical observations
All animals were examined for reaction to treatment during the day. The onset, intensity and duration of these signs were recorded.

All animals received a detailed clinical examination once each week.

Body weight
The weight of each animal was recorded once during the week before the start of treatment and once each week thereafter.

Food consumption and water consumption
The quantity of food consumed by each cage of animals was recorded once each week, commencing one week before the start of treatment and once each week thereafter.

Water consumption was monitored by visual inspection throughout the treatment period.

Ophthalmoscopy examination
The eyes of all animals in the Control and High dose groups were examined using an indirect ophthalmoscope after the application of a mydriatic agent (1 % Mydriacyl). Anterior, lenticular and fundic areas were evaluated. This ophthalmoscopic examination was undertaken during Week 12 of treatment.

Haematology and clinical chemistry
Samples were taken from all rats from each group during Week 13 of dosing.
Blood samples for haematology were collected from the orbital sinus under light ether anaesthesia and for clinical chemistry via the dorsal aorta at necropsy.

Haematology:
The following parameters were determined: Haematocrit, haemoglobin, total red blood cell count, total white blood cell count, differential white blood cell count, calculations of absolute indices and Hepato Quick (clotting time) on a sample obtained by tailsnip without anaesthesia.

Clinical chemistry
The following parameters were determined: Alkaline phosphatase (AP), aspartate aminotransferase (AST), Alanine aminotransferase (ALT), creatinine (Crea), blood urea nitrogen (BUN), total protein (TP), albumin (Alb), glucose (Glu), total cholesterol (Chol), total bilirubin (T.Bi), sodium (Na), potassium (K), chloride (Cl), calcium (Ca), phosphate (P), plasma cholinesterase (ChE), RBC cholinesterase.

Sacrifice and pathology
All animals were killed and necropsied. Method of killing was by carbon dioxide asphyxiation followed by exsanguination. The gross dissection and necropsy were performed under the supervision of a pathologist. The premature decedents were also necropsied.

Organ weights
The following organs were weighed: Adrenals, brain, heart, kidneys, liver (with gall bladder), lungs, ovaries, pituitary, prostate, spleen, testes (with epididymides), thymus and uterus.

Histopathology
The following tissues were processed and examined histopathologically from all Control and High dose animals. Only one eye per animal was processed and examined. In addition all other animals received histopathological examination of liver, kidneys and lungs. The premature decedents also underwent a full histopathological examination.

The following parameters were determined: Adrenals, aortic arch, any abnormal tissues, urinary bladder, brain, eyes, heart, intestine (duodenum, jejunum, ileum, caecum, colon), kidneys, liver (with gall bladder), lungs (perfused), mammary gland, mesenteric lymph node, muscle (thigh), oesophagus, ovaries, pancreas, pituitary, prostate, sciatic nerve, seminal vesicles, skin, spleen, stomach (glandular and non-glandular), submaxillary salivary gland, submandibular lymph node, testes (with epididymides), thymus, thyroid (with parathyroid), tongue, trachea and uterus.

Statistics
Haematology, clinical chemistry, organ weight and body weight data were statistically analysed for homogeneity of variance using the F-max test. If the group variances appeared homogeneous a parametric

ANOVA was used and pairwise comparisons made via Student's t-test using Fisher's F-protected LSD. If the variances were heterogeneous, log or square root transformations were used in an attempt to stabilise the variances. If the variances remained heterogeneous, then a non-parametric test such as a Kruskal-Wallis ANOVA was used. Organ weights were also analysed conditional on body weight (i.e. analysis of covariance).

Histopathology data were analysed using Fisher's Exact Probability test.

II. RESULTS AND DISCUSSION

A. ACHIEVED DOSAGES AND ANALYSIS OF THE FORMULATED DIETS
The group mean achieved dosages were in close agreement with the nominal values.

The majority of diets prepared for Weeks 1, 6 and 13 were seen to be within acceptable limits (± 10 %) for accuracy of concentration and homogeneity.

In Week 1, the concentration for Group 2 (male) was -10.7 % as compared to nominal concentration and the coefficient of variation was 11.6 %. A repeat analysis for this group from diets prepared for Week 2 showed an acceptable level of concentration (+1.2 %) and coefficient of variation (1.7 %). At Week 13 the concentration for Group 3 (male and female) were -14.8 % and -14.2 % respectively and the coefficients of variation were 9.8 % and 7.1 % respectively. Archive samples for these groups from diets prepared for Week 13 were analysed and showed an acceptable level of concentration (+3.0 % for female and -2.6 % for male) and coefficient of variation (3.8 % for female and 4.8 % for male).

B. MORTALITY
There were 6 unscheduled deaths; one Control dose male (killed *in extremis* because it was unable to eat), 2 control dose females (one which died at bleed and the other which was killed *in extremis* due to its general condition), one Low dose male (which died during haematology sampling) and 2 high dose females (one

of which was found dead and the other which was killed *in extremis* due to its general condition). None of these deaths could be attributed to administration of glyphosate.

C. CLINICAL OBSERVATIONS

There were no clinical signs in the control and treated groups that were considered to be due to administration of glyphosate.

D. BODY WEIGHT

There were no notable intergroup differences in either sex.

E. FOOD CONSUMPTION AND WATER CONSUMPTION

There were no notable intergroup differences in total food or water consumed in either sex at any time.

F. OPHTHALMOSCOPIC EXAMINATION

There were no notable findings in either sex.

G. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

No notable intergroup differences were found in either sex.

The statistically significant increase in Hepato Quick sampling time ($P < 0.01$), seen in the Low dose group was considered chance due to the lack of an effect in the other groups receiving glyphosate.

Table 5.3.2-72: Glyphosate – 13 week dietary toxicity study in mice [redacted] 1991): Selected haematology findings

Parameter	Dose group [mg/kg bw/day]							
	Males				Females			
	0	200	1000	4500	0	200	1000	4500
Hepato Quick (clotting time) [s]	18 ± 2	18 ± 1	18 ± 1	18 ± 1	16.6 ± 1.1	**17.9** ± 1.0**	17.3 ± 0.7	17.1 ± 0.4

** Statistically significant from controls ($p < 0.01$)

Blood clinical chemistry

In males and females, there were no statistically significant intergroup differences.

Alkaline phosphatase and plasma cholinesterase were slightly increased with no true dose response relationship and potassium was decreased in males in all dose groups. It was noted that glucose tended to increase with increasing dose level of glyphosate in males.

Glucose and alkaline phosphatase were slightly increased in females in all groups, however a dose response relationship was absent.

Limited sample volume precluded analysis of total protein, albumin and cholesterol. The number of samples available for analysis of some other parameters was also affected by the small volume of plasma obtained from many animals.

Table 5.3.2-73: Glyphosate – 13 week dietary toxicity study in mice ([redacted] 1991): Selected clinical chemistry findings

Parameter	Dose group [mg/kg bw/day]							
	Males				Females			
	0	200	1000	4500	0	200	1000	4500
Alkaline phosphatase (AP) [IU/L]	88 ± 25	111 ± 29	143 ± 79	139 ± 30	135 ± 39	173 ± 36	159 ± 46	178 ± 41
Plasma cholinesterase (ChE) [IU/L]	5843 ± 548	6821 ± 847	6919 ± 1208	6789 ± 849	11125 ± 1195	9900 ± 630	10599 ± 1302	10232 ± 1860
RBC cholinesterase (RChE) [IU/L]	1413 ± 936	1213 ± 369	1177 ± 504	1052 ± 481	1211 ± 426	1229 ± 276	1288 ± 684	2033 ± 971
Glucose [mmol/L]	14.7 ± 5.72	15.54 ± 4.05	16.12 ± 3.37	18.17 ± 5.86	14.57 ± 5.20	16.63 ± 6.02	15.01 ± 4.79	16.63 ± 4.54
Potassium [mmol/L]	13.2 ± 1.7	13.1 ± 1.0	12.8 ± 1.8	12.6 ± 2.1	12.4 ± 3.6	13.0 ± 2.0	11.7 ± 2.8	11.1 ± 2.1

G. NECROPSY

Organ weights
There were no notable intergroup differences in either sex.

The increase in testes weight in the male Low and Intermediate dose groups ($P < 0.01$), seen in absolute values and after final adjustment for body weight was considered to be chance, due to the lack of effect in the High dose group. The increases in thymus and prostate weight seen in the male Intermediate dose group only were also considered to be chance.

The increase in absolute pituitary weight in the female High dose group ($P < 0.05$) was considered to be chance, due to the lack of dose response relationship and the variability seen the other dose groups.

Table 5.3.2-74: Glyphosate – 13 week dietary toxicity study in mice ([redacted] 1991): Selected organ weights

Parameter		Dose group [mg/kg bw/day]							
		Males				Females			
		0	200	1000	4500	0	200	1000	4500
Pituitary	Absolute organ weight [g]	0.0011 ± 0.00033	0.00100 ± 0	0.00120 ± 0.00042	0.00100 ± 0	0.0013 ± 0.0005	0.0017 ± 0.0008	0.0011 ± 0.0003	**0.0019** ± 0.0006*
Prostate	Absolute organ weight [g]	0.021 ± 0.008	0.019 ± 0.011	0.032 ± 0.015	0.020 ± 0.005				
Testes	Absolute organ weight [g]	0.34 ± 0.05	**0.39** ± 0.02 **	**0.38** ± 0.03 **	0.34 ± 0.03				
Thymus	Absolute organ weight [g]	0.017 ± 0.005	0.021 ± 0.003	**0.024** ± 0.008 **	0.017 ± 0.005	0.023 ± 0.005	0.025 ± 0.008	0.024 ± 0.007	0.030 ± 0.011

* Statistically significant from controls ($p < 0.05$);
** Statistically significant from controls ($p < 0.01$)

Gross pathology
There were no findings which could be related to treatment with Glyphosate.

Histopathology
There was a decrease in diffuse vacuolation observed in the liver in the control (males: 4/10; females: 1/10), Low dose (males: 2/10) and Intermediate dose (females: 3/10) animals.

An increase in cortical tubular epithelial hypertrophy in kidney was seen in Intermediate dose females (5/10), where only 1/10 controls and 2/10 Low dose animals showed the findings, but no High dose females. There was also a marginal increase seen in High dose males (4/10), where 2/10 controls, 0/10 Low dose and 2/10 Intermediate dose animals had the finding. The significance of this finding is doubtful in view of its presence in controls and the lack of true dose response relationship.

There were also a number of histopathological changes usually seen in rats of this age and strain at IRI, many of these being of inflammatory nature, and considered to be unrelated to treatment with Glyphosate.

Table 5.3.2-75: Glyphosate – 13 week dietary toxicity study in mice ([redacted] 1991): Selected histopathological findings (incidence)

Parameter		Dose group [mg/kg bw/day]							
		Males				Females			
		0	200	1000	4500	0	200	1000	4500
Liver	No abnormalities detected	2	0	2	5	3	2	1	2
	Centrilobular vacuolation	2	2	4	1	2	3	1	3
	Diffuse vacuolation	4	2	0	0	1	0	3	0
Kidneys	No abnormalities detected	6	4	3	2	5	6	3	6
	Cortical tubular epithelial hypertrophy	2	0	2	4	1	2	5	0

III. CONCLUSIONS

This study was designed to give toxicity information over 13 weeks on glyphosate administered to mice via the diet at concentrations calculated to achieve dose levels of 0, 200, 1000 or 4500 mg/kg bw/day.
The animals were examined for mortality, clinical signs, body weight, food and water consumption, ophthalmoscopy, haematology, clinical chemistry, gross pathology, organ weights and histopathology.

In conclusion, dosing CD-1 mice via the diet for 13 weeks with up to and including 4500 mg glyphosate /kg bw/day produced no findings which could be directly attributed to administration of the test material. The highest dose of 4500 mg/kg bw/day was considered the NOEL in this study by the study authors.

3. Assessment and conclusion

Assessment and conclusion by applicant:
In this study, groups of male and female CD-1 mice were administered glyphosate in the diet at dose levels of 0, 200, 1000 or 4500 mg/kg bw/day for 13 weeks according to OECD 408 (1981) and in compliance with GLP (no certificate of the competent authority was provided).

Dosing CD-1 mice via the diet for 13 weeks with up to and including 4500 mg/kg bw/day glyphosate produced no findings which could be directly attributed to administration of the test material
.
The highest dose of 4500 mg/kg bw/day is considered the NOAEL in this study, although evaluation of clinical chemistry parameters was of limited scientific value only.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.3.2/019
Report author	[redacted]
Report year	1979
Report title	A Three Month Feeding Study of Glyphosate (Roundup® Technical) in Mice
Report No	77-2111
Document No	Not reported
Guidelines followed in study	No guideline followed, similar to OECD 408 (1981)
Deviations from current test guideline (OECD 408, 2018)	No sensory reactivity was investigated; no ophthalmoscopy was performed; no haematology or clinical chemistry was performed; organ weights of the adrenals, epididymides, prostate and seminal vesicles and coagulating glands, pituitary gland, thyroid, thymus and uterus were not determined; histopathology was performed without aorta, coagulating glands, mammary glands, seminal vesicles, skin, trachea and vagina.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No (pre-GLP).
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 3b

2. Full summary

Executive Summary

A total of 120 CD-1 mice were randomly distributed into four groups of 15 animals per sex and group. The test material, glyphosate (Roundup® Technical), was administered to the animals in Groups II (low), III (mid) and IV (high) at dose levels of 5000, 10000 or 50000 ppm (equivalent to 944.1, 1867.2 or 9707.0 mg/kg bw/day for males and 1527.7, 2734.7 or 14858.2 mg/kg bw/day for females) via dietary admixture for three months. Control animals (Group I) received untreated diet.

Animals were observed for mortality, clinical signs, body weight changes, food consumption, organ weight changes and gross pathology and histopathological evaluation of selected tissues was performed on 10 animals per sex and group from the control and high-dose group.

Body weight gain of the high-dose males and females was lower than that of the control animals after 13 weeks of test substance administration. Food consumption of the low-, mid- and high-dose males and females was generally greater than that of control animals throughout the period of test substance administration. All other parameters evaluated (mortality, physical observations, organ weights, organ/body weight ratios and organ/brain weight ratios) were considered to be either comparable between treated and control animals or of no toxicological significance.

The results of gross *post mortem* examinations as well as the histopathologic evaluations of selected tissues did not reveal any evidence of effects related to the administration of glyphosate (Roundup® Technical).

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate (Roundup® Technical)
Description:	Fine white powder
Lot/Batch #:	XHJ-64
Purity:	98.7 %
Stability of test compound:	Not reported

2. Vehicle and/or positive control: Diet / none

3. Test animals:

Species:	Mouse
Strain:	CD-1, COBS (ICR derived)
Source:	[redacted]
Age:	41 days
Sex:	Male and female
Weight at dosing:	♂ 28 g (23 – 33 g); ♀ 22 g (19 – 25 g)
Acclimation period:	13 days
Diet/Food:	Standard laboratory diet (Purina Laboratory Chow, No. 5001®) *ad libitum*; fresh food presented weekly.
Water:	Automated water system (Elizabethtown Water Company), *ad libitum*
Housing:	Individually in elevated stainless steel wire mesh cages.
Environmental conditions:	Temperature: 20.6 – 28.3 °C Humidity: Not reported Air changes: Not reported 12 hour dark/light cycle

B: Study design and methods

In life dates: 1979-05-21 to 1979-08-21

Animal assignment and treatment:
The test material was offered on a continuous basis in the basal diet to groups of 15 CD-1 mice per sex for 92 – 93 days. Dietary concentrations were 0, 5000, 10000 or 50000 ppm (equivalent to 0, 944.1, 1867.2 or 9707.0 mg/kg bw/day for males and 0, 1527.7, 2734.7 or 14858.2 mg/kg bw/day for females).

Table 5.3.2-76: A Three Month Feeding Study of Glyphosate (Roundup® Technical) in Mice ([redacted], 1979): Study design

Test group	Dietary concentration [ppm]	Compound intake [mg/kg bw/day]	Males	Females
Control	0	♂: 0; ♀: 0	15	15
Low	5000	♂: 944.1; ♀: 1527.7	15	15
Mid	10000	♂: 1867.2; ♀: 2734.7	15	15
High	50000	♂: 9707.0; ♀: 14858.2	15	15

Analysis of the test diet
Prior to initiation of the study, full sized batches of diets were prepared at dietary levels of 5000, 10000 and 50000 ppm. Duplicate samples of approximately 50 g each were taken from the right and left of the top, middle and bottom of the mixer (12 samples/batch) and sent frozen to the sponsor (1979-04-30) for analysis of homogeneity. In addition, approximate 25 g duplicate samples were taken from the feed buckets and stored frozen on Days 0, 1, 2, 5, 7 and 14. On Day 14, these samples were sent frozen to the sponsor for analytical confirmation of stability (1979-05-14).

Additional samples were taken throughout the study, frozen and half of them sent to sponsor for analysis.

Mortality
Viability was checked twice daily.

Clinical observations
All animals were examined for gross signs of toxicological or pharmacological effects twice daily.
Additionally, all animals received a detailed physical examination for signs of local or systemic toxicity and pharmacologic effects once each week.

Body weight
The weight of each animal was recorded twice prior to treatment, weekly during treatment and terminally (after fasting).

Food consumption and compound intake
The quantity of food consumed by each animal was recorded once prior to treatment and weekly during treatment. The test substance intake was calculated from food consumption data.

Please refer to the table above for information on the compound intake.

Sacrifice and pathology
All animals dying spontaneously, killed in a moribund condition and all survivors killed by exsanguination from the abdominal aorta under ether anaesthesia were subjected to necropsy.
A complete gross post-mortem examination was performed on all animals.

Organ weights
The following organs were weighed: Brain, heart, kidneys, liver, ovaries, spleen and testes.
Organ/body weight and organ/brain weight ratios were calculated.

Histopathology
Histopathological examinations of the following organs and tissues were made on sections from control and high dose animals stained with haematoxylin and eosin: Adrenals, bone and bone marrow, blood smear, brain, epididymides, eyes, gall bladder, gross lesions, heart, intestine (duodenum, jejunum, ileum, caecum, colon), kidneys, liver, lungs, lymph node, muscle, oesophagus, ovaries, pancreas, pituitary, prostate, salivary gland (mandibular), sciatic nerve, spinal cord, spleen, stomach, testes, thymus, thyroid/parathyroid, urinary bladder, uterus (with cervix), tissue masses or suspect tumours and regional lymph nodes.

Statistics
Body weight, food consumption, organ weights, organ/body weight and organ/brain weight ratios were analysed. Mean values of all dose groups were compared to control at each time interval.

II. RESULTS AND DISCUSSION

A. ACHIEVED DOSAGES AND ANALYSIS OF THE FORMULATED DIETS
The analysis of the formulated diets was in the responsibility of the sponsor.

B. MORTALITY

One control male and one mid-dose female died spontaneously during the course of the study. All other animals survived the duration of the study.

C. CLINICAL OBSERVATIONS

Daily and weekly physical observations of the males and females in all treatment groups were of the type commonly seen in the laboratory mouse and were considered comparable to those of the control animals. No pharmacological or toxicological signs or symptoms were observed which were considered related to the administration of the test substance.

D. BODY WEIGHT

Mean body weight values of the low-, mid- and high-dose males and females were generally slightly or statistically significantly lower than control values at the second pre-test interval and throughout the course of the study. However, body weight gains of the low- and mid-dose males and females were considered comparable to those of control animals. The body weight gain of the high-dose males (+8.13 g) and females (+7.2 g) was lower than that of the control animals (+10.67 g males and +8.74 g females) after 13 weeks of test substance administration.

Table 5.3.2-77: A Three Month Feeding Study of Glyphosate (Roundup® Technical) in Mice ([redacted], 1979): Summary of body weight data [g]

	Dose group [ppm]							
	Males				Females			
Week	0	5000	10000	50000	0	5000	10000	50000
-1	26.47 ± 0.70	↓25.87 ± 0.63	↓26.13 ± 0.60	↓25.73 ± 0.65	20.73 ± 0.42	↓20.67 ± 0.30	↓20.47 ± 0.31	↑20.80 ± 0.37
0	29.33 ± 0.47	↓27.67 ± 0.49	**↓27.27* ± 0.58**	**↓27.47* ± 0.54**	23.13 ± 0.35	↓22.20 ± 0.43	↓21.93 ± 0.33	↓22.00 ± 0.40
1	31.60 ± 0.55	↓31.33 ± 0.61	↓30.27 ± 0.69	**↓28.80** ± 0.48**	25.73 ± 0.37	↓24.93 ± 0.38	↓24.60 ± 0.38	**↓23.93* ± 0.43**
2	33.27 ± 0.52	↓31.47 ± 0.58	**↓31.33* ± 0.61**	**↓30.67* ± 0.49**	26.40 ± 0.42	**↓23.87** ± 0.32**	↓25.13 ± 0.43	**↓24.00** ± 0.51**
3	33.47 ± 0.62	↑33.60 ± 0.69	↓32.80 ± 0.60	**↓29.67** ± 0.49**	27.47 ± 0.42	**↓25.13** ± 0.51**	**↓25.87* ± 0.41**	**↓25.60* ± 0.46**
4	35.27 ± 0.67	↓34.60 ± 0.68	↓34.80 ± 0.63	**↓31.67** ± 0.52**	28.00 ± 0.54	↓26.40 ± 0.59	↓27.27 ± 0.42	**↓25.80* ± 0.46**
5	35.33 ± 0.64	↓33.87 ± 0.65	↓34.40 ± 0.65	**↓32.47* ± 0.50**	29.20 ± 0.54	**↓26.93** ± 0.56**	↓28.13 ± 0.41	**↓27.00** ± 0.48**
6	34.87 ± 0.66	↓33.80 ± 0.67	↓34.53 ± 0.59	**↓31.80** ± 0.59**	28.53 ± 0.55	↑28.60 ± 0.62	↓28.13 ± 0.40	**↓25.40** ± 0.43**
7	35.80 ± 0.68	↓35.60 ± 0.56	↓35.47 ± 0.62	**↓33.27* ± 0.67**	28.87 ± 0.52	↓27.00 ± 0.56	↓28.33 ± 0.39	↓27.93 ± 0.48
8	36.07 ± 1.13	↑36.13 ± 0.64	↑36.20 ± 0.56	↓33.53 ± 0.62	30.20 ± 0.56	**↓27.93** ± 0.58**	↓29.53 ± 0.38	**↓28.33* ± 0.40**
9	35.93 ± 1.25	↓35.47 ± 0.68	↑36.07 ± 0.57	**↓33.53§ ± 0.60**	30.07 ± 0.57	↓28.87 ± 0.58	↓29.67 ± 0.43	↓28.73 ± 0.36
10	35.87 ± 1.38	↑36.20 ± 0.64	↑37.33 ± 0.58	↓35.07 ± 0.63	30.13 ± 0.52	↓28.53 ± 0.65	↑30.20 ± 0.43	↓28.87 ± 0.38
11	37.50 ± 0.82	↓36.67 ± 0.66	↑37.53 ± 0.60	**↓34.87* ± 0.68**	30.00 ± 0.68	**↓27.20* ± 0.69**	↓28.53 ± 0.43	↓28.20 ± 0.48
12	37.21 ± 0.81	↓36.27 ± 0.64	↓36.60 ± 0.61	**↓34.07* ± 0.79**	30.40 ± 0.65	**↓27.80** ± 0.57**	↑30.93 ± 0.56	**↓28.47* ± 0.46**
13	40.00 ± 0.89	↓39.40 ± 0.57	↓37.93 ± 0.68	**↓35.60** ± 0.76**	31.87 ± 0.62	↓31.00 ± 0.62	**↓30.64* ± 0.44**	**↓29.20** ± 0.45**
Body weight gain week 0 to 13	10.67	11.73	10.66	8.13	8.74	8.80	8.71	7.20

* Significantly different from control (Dunnett's test; $p \leq 0.05$);

Table 5.3.2-77: A Three Month Feeding Study of Glyphosate (Roundup® Technical) in Mice ([redacted], 1979): Summary of body weight data [g]

** Significantly different from control (Dunnett's test; $p \leq 0.01$);
§ Significantly different from control (Dunn's Rank Sum test; $p \leq 0.05$)

E. FOOD CONSUMPTION AND COMPOUND INTAKE

Food consumption of the low-, mid- and high-dose males and females was generally slightly or significantly greater than that of control animals throughout the period of test substance administration.

Table 5.3.2-78: A Three Month Feeding Study of Glyphosate (Roundup® Technical) in Mice ([redacted], 1979): Summary of food consumption data [g/kg bw/day]

Week	Dose group [ppm]							
	Males				Females			
	0	5000	10000	50000	0	5000	10000	50000
0	188.98 ± 9.10	↑220.81 ± 15.65	↑224.58 ± 13.77	↑214.18 ± 19.66	280.73 ± 28.16	↑357.58 ± 26.50	↑314.42 ± 31.31	↑339.16 ± 28.38
1	186.59 ± 8.47	↑192.70 ± 7.59	↑203.20 ± 8.74	**↑240.60** ** **± 16.43**	236.17 ± 12.25	**↑287.68** * **± 12.69**	↑245.93 ± 16.75	**↑355.83** ** **± 6.99**
2	185.66 ± 4.87	↑222.49 ± 19.73	**↑247.86** §§ **± 11.80**	↑207.33 ± 17.97	269.50 ± 17.10	**↑403.54** * **± 35.71**	↑311.22 ± 24.80	↑364.73 ± 34.23
3	154.75 ± 14.57	**↑217.73** * **± 20.71**	↑206.49 ± 11.11	↑200.07 ± 16.86	261.13 ± 23.33	↑352.47 ± 32.42	↑348.84 ± 33.78	↑322.33 ± 35.80
4	171.69 ± 6.79	↑182.35 ± 5.89	**↑239.02** ** **± 11.00**	**↑207.30** * **± 10.97**	260.40 ± 16.33	↑279.24 ± 17.16	↑275.37 ± 18.53	↑269.39 ± 22.77
5	168.81 ± 8.76	↑209.64 ± 15.63	↑197.34 ± 12.57	↑186.35 ± 7.54	256.47 ± 20.22	↑314.80 ± 29.42	↑260.91 ± 26.79	↑286.65 ± 38.37
6	168.40 ± 8.29	↑215.74 ± 16.94	↑189.12 ± 10.47	↑205.03 ± 17.19	297.49 ± 26.48	↑327.30 ± 20.84	↓277.75 ± 25.63	↓272.62 ± 24.82
7	164.12 ± 5.46	↑185.65 ± 8.17	↑173.75 ± 7.47	↑204.51 ± 15.53	248.79 ± 11.55	↓248.75 ± 9.40	↑252.13 ± 13.61	↑252.93 ± 15.06
8	137.01 ± 10.32	**↑182.55** * **± 13.83**	↑173.69 ± 8.37	↑173.22 ± 10.92	286.13 ± 29.16	↑286.84 ± 25.42	↓254.55 ± 24.12	↓282.57 ± 31.90
9	140.61 ± 5.50	↑174.21 ± 16.81	↑170.99 ± 12.12	↑162.61 ± 10.70	316.03 ± 53.33	↑363.19 ± 46.71	↓289.64 ± 38.76	↑346.02 ± 46.25
10	150.61 ± 8.15	↑174.77 ± 9.40	↑155.69 ± 6.42	↑175.50 ± 13.52	226.59 ± 25.29	↑265.17 ± 26.31	↑252.99 ± 25.28	↑233.77 ± 27.80
11	136.76 ± 4.94	↑175.35 ± 15.23	↑154.51 ± 5.88	**↑183.55** § **± 12.34**	270.78 ± 26.43	↑293.56 ± 35.37	↓262.94 ± 22.68	↑288.22 ± 37.41
12	151.93 ± 6.42	↑176.09 ± 10.42	↑170.28 ± 8.13	↑188.33 ± 15.03	262.66 ± 23.70	↑293.35 ± 29.76	↑268.41 ± 22.33	↑267.07 ± 26.18
13	126.14 ± 5.71	↑155.19 ± 13.93	**↑156.22** § **± 6.82**	**↑198.71** §§ **± 15.47**	253.21 ± 23.45	↑269.36 ± 26.04	↑266.01 ± 23.29	↑333.23 ± 32.54

* Significantly different from control (Dunnett's test; $p \leq 0.05$);
** Significantly different from control (Dunnett's test; $p \leq 0.01$);
§ Significantly different from control (Dunn's Rank Sum test; $p \leq 0.05$);
§§ Significantly different from control (Dunn's Rank Sum test; $p \leq 0.01$)

CD-1 mice received dose levels of 0, 5000, 10000 or 50000 ppm which when applied to the body weight and feed consumption was equivalent to 0, 944.1, 1867.2 or 9707.0 mg/kg bw/day for males and 0, 1527.7, 2734.7 or 14858.2 mg/kg bw/day for females.

F. NECROPSY

Gross pathology
Macroscopic post mortem observations did not reveal any changes considered related to the administration of test material.

Organ weights
The mean organ/body weight ratios of the brain, heart, kidneys and liver of the high-dose males and the mean relative liver weight of the mid-dose males, were significantly greater than those of the control values. These differences were probably reflective of the slightly lower terminal body weights of the mid- and high-dose males, respectively. All other mean organ weights and organ/body weight ratios were considered comparable between the control and treated males and females.

There were no significant differences in mean absolute organ weights and organ/brain weight ratios between the controls and the low-, mid- and high-dose males and females.

Table 5.3.2-79: A Three Month Feeding Study of Glyphosate (Roundup® Technical) in Mice [redacted], 1979): Organ weight data and organ/body weight ratios

	Dose group [ppm]							
	Males				Females			
	0	5000	10000	50000	0	5000	10000	50000
Body weight [g]	40.0 ± 0.9	↓39.6 ± 0.6	↓37.9 ± 0.7	**↓35.6** ± 0.8**	26.4 ± 0.5	↑26.5 ± 0.5	↑26.8 ± 0.4	↓26.0 ± 0.3
Brain [g]	0.459 ± 0.004	↓0.449 ± 0.007	↓0.455 ± 0.007	↓0.458 ± 0.009	0.455 ± 0.007	↑0.467 ± 0.007	↑0.466 ± 0.007	0.455 ± 0.006
Brain [%]	1.154 ± 0.024	↓1.139 ± 0.027	↑1.206 ± 0.030	**↑1.291** ± 0.027**	1.728 ± 0.037	↑1.765 ± 0.037	↑1.745 ± 0.033	↑1.754 ± 0.029
Gonads [g]	0.3471 ± 0.0104	↑0.3561 ± 0.0346	↑0.3653 ± 0.0295	↓0.3219 ± 0.0083	0.0161 ± 0.0015	↑0.0262 ± 0.0096	↑0.0185 ± 0.0016	↓0.0150 ± 0.0012
Gonads [%]	0.8715 ± 0.0284	↑0.9008 ± 0.0885	↑0.9666 ± 0.0788	↑0.9096 ± 0.0297	0.0608 ± 0.0055	↑0.1008 ± 0.0386	↑0.0688 ± 0.0057	↓0.0574 ± 0.0043
Heart [g]	0.148 ± 0.003	↑0.150 ± 0.006	↑0.152 ± 0.005	↑0.153 ± 0.006	0.132 ± 0.004	↓0.128 ± 0.004	↑0.142 ± 0.008	↓0.126 ± 0.004
Heart [%]	0.3719 ± 0.0118	↑0.3796 ± 0.0143	↑0.4010 ± 0.0107	**↑0.4300* ± 0.0159**	0.5014 ± 0.0126	↓0.4856 ± 0.0151	↑0.5324 ± 0.0274	↓0.4845 ± 0.1189
Spleen [g]	0.0754 ± 0.0039	↓0.0721 ± 0.0027	↓0.0723 ± 0.0028	↓0.0644 ± 0.0036	0.0916 ± 0.0073	↑0.0933 ± 0.0035	↑0.0951 ± 0.0046	↓0.0857 ± 0.0039
Spleen [%]	0.1901 ± 0.0118	↓0.1820 ± 0.0059	↑0.1918 ± 0.0087	↓0.1818 ± 0.0109	0.3448 ± 0.0246	↑0.3526 ± 0.0143	↑0.3553 ± 0.0159	↓0.3296 ± 0.0134
Kidneys [g]	0.535 ± 0.016	0.535 ± 0.017	↑0.545 ± 0.010	↑0.566 ± 0.016	0.383 ± 0.011	↑0.387 ± 0.012	↑0.387 ± 0.014	↓0.376 ± 0.006
Kidneys [%]	1.342 ± 0.040	↑1.357 ± 0.052	↑1.441 ± 0.027	**↑1.590** ± 0.032**	1.450 ± 0.028	↑1.463 ± 0.052	↓1.448 ± 0.044	↓1.448 ± 0.024
Liver [g]	1.333 ± 0.039	↓1.331 ± 0.028	↑1.377 ± 0.026	↓1.329 ± 0.034	1.133 ± 0.028	↓1.115 ± 0.024	↓1.106 ± 0.036	↓1.068 ± 0.030
Liver [%]	3.334 ± 0.069	↑3.365 ± 0.069	**↑3.637* ± 0.066**	**↑3.737** ± 0.076**	4.293 ± 0.083	↓4.207 ± 0.077	↓4.133 ± 0.116	↓4.104 ± 0.098

* Significantly different from control (Dunnett's test; $p \leq 0.05$);
** Significantly different from control (Dunnett's test; $p \leq 0.01$)

Gross necropsy / histopathology

Results of the gross *post mortem* examinations performed on all animals as well as histopathological evaluations of selected tissues from 10 animals/sex/group in the control and high-dose groups did not reveal any evidence of effects which could be attributed to the administration of glyphosate (Roundup® Technical).

III. CONCLUSIONS

Body weight gain of the high-dose males and females was lower than that of the control animals after 13 weeks of test substance administration. Food consumption of the low-, mid- and high-dose males and females was generally greater than that of control animals throughout the period of test substance administration. All other parameters evaluated (mortality, physical observations, organ weights, organ/body weight ratios and organ/brain weight ratios) were considered to be either comparable between treated and control animals or of no toxicological significance.

The results of gross *post mortem* examinations as well as the histopathologic evaluations of selected tissues did not reveal any evidence of effects related to the administration of glyphosate (Roundup® Technical).

3. Assessment and conclusion

Assessment and conclusion by applicant:

In this study, the test material glyphosate was administered to groups of CD-1 mice at dose levels of 0, 5000, 10000 or 50000 ppm (equivalent to 0, 944.1, 1867.2 or 9707.0 mg/kg bw/day for males and 0, 1527.7, 2734.7 or 14858.2 mg/kg bw/day for females) via the diet for three months.

The study was conducted according to a testing regime similar to OECD 408 (1981) at a time when GLP was not compulsory. However, there were major deviations to current standards: no sensory reactivity tests were performed; no haematology/clinical chemistry were performed; organ weights of the adrenals, epididymides, prostate + seminal vesicles and coagulating glands, pituitary gland, thyroid, thymus and uterus were not determined; histopathology was performed without aorta, coagulating glands, mammary glands, seminal vesicles, skin, trachea and vagina.

Apart from these major deviations, the study was well conducted and can provide supplemental information for the assessment of repeated dose toxicity of glyphosate. The study is therefore considered to be only supplementary.

All in-life data (physical observations, body weight and food consumption) and gross necropsy, organ weights and histopathology observations indicated no adverse effects of glyphosate at any of the dose levels administered. Therefore, under the conditions of this study, the NOAEL for oral administration in the diet in CD mice can be set at the highest dose of 50000 ppm (equivalent to approx. 9707.0 and 14858.2 mg/kg bw/day in male and female mice, respectively).

Assessment and conclusion by RMS:

Oral 90–day toxicity (dog)

Table 5.3.2-80 summarises the 90–day studies performed in dogs previously assessed in the 2001 and 2015 EU glyphosate evaluations.

Table 5.3.2-80: Studies on oral 90–day repeated-dose toxicity with glyphosate in non-rodents

Annex Point	Study	Species Study type	Substance(s)	Reference list-related category§	Result
CA 5.3.2/020	[redacted], 2007	Beagle dog 13-week, oral capsule (0, 30, 300, 1000 mg/kg bw/day)	Glyphosate technical (Purity: 95.7 %)	Valid, Category 2a	NOAEL = 300 mg/kg bw/day
CA 5.3.2/021 CA 5.3.2/022 CA 5.3.2/023 CA 5.3.2/024	[redacted] 1999 (Study Report) [redacted] 1999 (Appendix) [redacted], 1999 (List of Tables) [redacted], 1997 (Test Compound Stability in Experimental Diet)	Beagle dog 90–day, oral diet (0, 200, 2000, 10000 ppm)	Glyphosate technical (Purity: >95 %)	Valid, Category 2a	NOAEL = 10000 ppm (equivalent to 252.6 mg/kg bw/day for males and females)
CA 5.3.2/025 CA 5.3.2/026	[redacted] 1996 (Study Report) [redacted], 1996 (Appendix)	Beagle dog 13-week, oral diet (0, 2000, 10000, 50000 ppm)	Glyphosate acid (Purity: 99.1 %)	Valid, Category 2a	NOAEL = 10000 ppm (equivalent to 323 mg/kg bw/day for males and 334 mg/kg bw/day for females)
CA 5.3.2/027	[redacted], 1996	Beagle dog 13-week, oral diet (0, 1600, 8000, 40000 ppm)	Glyphosate technical (Purity: 94.61 %)	Valid, Category 2a	NOAEL = 40000 ppm (equivalent to 1015 mg/kg bw/day for males and 1014 mg/kg bw/day for females)
CA 5.3.2/028	[redacted], 1985	Mongrel dog 90–day, oral diet (0, 100, 250, 500 mg/kg bw/day)	Glyphosate technical (Purity: unknown)	Invalid, Category 4b	NOAEL = 250 mg/kg bw/day
CA 5.3.2/029	[redacted], 1983	Beagle dog 6-month, oral capsule (0, 10, 60, 300 mg/kg bw/day)	Isopropylamine salt of glyphosate (Purity: 62.49 %)	Valid, Category 2a	NOAEL = 300 mg/kg bw/day
CA 5.3.2/030	[redacted], 1981 (Revised and English version of Hungarian report 1991)	Beagle dog 13-week, oral diet (0, 200, 600, 2000 ppm)	Glyphosate (Purity: not reported)	Supportive, Category 3a	NOAEL = 600 ppm (equivalent to approx.. 25 mg/kg bw/day for males and females)

§ The category describes the acceptability/reliability of the study within the AIR 5 submission. This category does not describe hazard classification. (for details please refer to the Doc ID: 110054-B-GRG_Jun_2020)

There are four valid 90–day repeated dose studies available in which Beagle dogs were administered glyphosate either in the diet or via capsule ([redacted], 2007; [redacted], 1999; [redacted], 1996; [redacted], 1996). All studies conform to current guidelines and were performed under GLP. NOAELs were in the range of approx. 250 to 350 mg/kg bw/day, except for [redacted] (1996) where a NOAEL of approx. 1000 mg/kg

bw/day was reported.

The study by [redacted] (1991; revised English version of 1981 hungarian report) had been submitted in the 2001 EU glyphosate evaluation, but was assessed as supportive. The revised English report dated 1991 based on the original Hungarian report (1981) is of limited reliability due to reporting deficiencies in the revised report. Numerical data for body weight, food consumption, haematology, clinical chemistry, and organ weights provided in the original study report were not included in the revised study report. But importantly, the histopathology report is available.
The 90–day feeding study in Mongrel dogs ([redacted] 1985) does not comply with modern standards and was therefore considered invalid.

In the oral sub-chronic toxicity study in the dog previously evaluated in the 2001 EU glyphosate evaluation [redacted], 1991), only unspecific signs of toxicity (decrease in body weight gain and food consumption) were observed at high dose levels. In addition, liver effects of equivocal toxicological significance were observed at the mid and high dose level (approx. 9 and 77.4 mg/kg bw/day). These changes were characterised by round shaped and enlarged hepatocytes and occasionally also by the narrowing of some of the hepatocytic trabeculae and slight dissociation of the liver structure. In addition, congestion of the liver was noted in three males and all female dogs in the highest dose group. However, the EU evaluation in 2001 found that since these findings were not confirmed in the more recent studies using much higher dose levels they were not considered to be compound-related.
The results of the four newer studies are consistent with the study previously submitted and reviewed, in that a reduction in body weight gain and food consumption was observed. In the study by [redacted] (2007) at 1000 mg/kg bw/day the test item administration induced marked clinical signs (liquid/soft faeces, dehydration, thin appearance, vomiting and pallor), caused lower body weight gain (males) and body weight loss (females) and reduced food consumption. This led to the early sacrifice of two moribund animals, and to the early termination of the entire group at week 11.

Laboratory investigations in the surviving animals of the high dose group demonstrated some abnormalities (higher alanine aminotransferase activity in both sexes and lower alkaline phosphatase activity, as well as lower protein and albumin levels in females) and urinary changes (decrease in specific gravity in both sexes and increase in urinary volume and markedly less colour of urine in females). Treatment-related histopathological changes in surviving animals consisted of an increased number of adipocytes in the sternum in both sexes, as well as prostate atrophy and uterine atrophy at 1000 mg/kg bw/day. These lesions, also noted among the moribund sacrificed animals, could be related to the low body weight of these high-dose animals caused by the test item. The relevance of these findings is uncertain given that in this study 1000 mg/kg bw/day clearly exceeded the maximum tolerated dose.

The lowest NOAEL observed was 253 mg/kg bw/d and the highest was 1000 mg/kg bw/day and the overall was NOAEL 323 mg/kg bw/day and 334 mg/kg bw/day in males and females respectively.The lowest observed adverse effect level was 1000 mg/kg bw/day ([redacted], 2007).

In addition, there is an acceptable study in which the formulation MON-0139 (62.49 % isopropylamine salt of glyphosate) had been administered for six months in gelatine capsules to Beagle dogs ([redacted], 1983). There were no effects on body weight, food consumption, haematology, urinalysis, organ weights or histopathology considered biologically adverse or related to administration of MON-0139. An apparent increase in alkaline phosphatase activity potentially associated with treatment was observed in top dose males from the second measurement onwards reaching statistical significance in month five only. No other clinical chemistry parameters were considered adversely affected. As the increase in alkaline phosphatase activity was not accompanied by any other indication of organ or tissue damage, the highest dose of 300 mg/kg bw/day can be considered the NOAEL.

1. Information on the study

Data point	CA 5.3.2/020
Report author	[redacted]
Report year	2007
Report title	Glyphosate Technical: 13-Week Toxicity Study by Oral Route (Capsule) in Beagle Dogs
Report No	29646 TCC
Document No	Not reported
Guidelines followed in study	OECD 409 (1998); JMMAF 12 NohSan No. 8147
Deviations from current test guideline (OECD 409, 1998)	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary
Groups of four Beagle dogs per sex received the test item, glyphosate technical, by daily administration (capsule) at dose-levels of 0, 30, 300 or 1000 mg/kg bw/day for 11/13 weeks. The duration of the treatment period for the high-dose group was shortened to 11 weeks for ethical reasons due to marked toxic effects. The animals were checked daily for mortality and clinical signs. Body weight was recorded weekly. Food consumption was estimated daily. Ophthalmological examinations were carried out before the beginning and at the end of the treatment period. Haematological and blood biochemical investigations, as well as urinalysis, were performed before the beginning of the treatment period, in Week 7 and at the end of the treatment period. At termination, the animals were sacrificed and subjected to a full macroscopic post-mortem examination. Designated organs were weighed and specified tissues preserved. A microscopic examination was performed on selected tissues from all the animals.

In the low- and mid-dose groups no treatment-related signs were noted. No haematological, blood biochemical, urinary or histopathological effects were observed. Only a slight increase of absolute and relative adrenal weights of males receiving 300 mg/kg bw/day was observed. However, the increase was not statistically significant
.
At 1000 mg/kg bw/day the test item administration induced marked clinical signs (liquid/soft faeces, dehydration, thin appearance, vomiting and pallor), caused lower body weight gain (males) or body weight loss (females) and reduced food consumption. This led to the early sacrifice of two moribund animals, and to the early termination of the entire group at week 11.

Laboratory investigations in the surviving animals demonstrated some abnormalities (higher alanine aminotransferase activity in both sexes and lower alkaline phosphatase activity, as well as lower protein and albumin levels in females) and urinary changes (decrease in specific gravity in both sexes and increase in urinary volume and markedly less colour of urine in females).

Treatment-related histopathological changes in surviving animals consisted of increased number of adipocytes in the sternum in both sexes, as well as prostate atrophy and uterine atrophy at 1000 mg/kg

bw/day. These lesions, also noted among the moribund sacrificed animals, could be related to the low body weight of these high-dose animals caused either directly or indirectly, by the test item. Further major microscopic changes in moribund sacrificed animals were found in the kidneys (bilateral vacuolation of cortical tubules, sometimes with pigment deposits), liver (diffuse macrovesicular vacuolation, acute inflammation and/or pigment deposits), oesophagus, lung, uterus (atrophy) and/or bone marrow (increased number of adipocytes). These findings were associated with numerous changes in laboratory parameters (haemoconcentration, increased urea and creatinine levels, decreased urea, protein, albumin and bilirubin levels and decreased liver enzyme activities).

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate Technical
Description:	White crystalline powder
Lot/Batch #:	H05H016A
Purity:	95.7 %
Stability of test compound:	Stable under storage conditions (< 30°C), light protected; Expiry date: 2008-03-25

2. Vehicle and/or positive control: Empty gelatine capsules, size 12 (Torpac, NY, US)

3. Test animals:

Species:	Dogs
Strain:	Beagle
Source:	[redacted]
Age:	Approx. 6 months
Sex:	Male and female
Weight at dosing:	♂ 6.5 – 8.0 kg; ♀ 6.6 – 7.7 kg
Acclimation period:	14 days
Diet/Food:	125 C3 pelleted diet (SAFE, Villemoisson, Epinary-sur-Orge, France), 300 g per day (Following reduced food consumption among some animals standard tinned dog food was distributed instead or in addition.)
Water:	Tap water, *ad libitum*
Housing:	Individual housing in pens containing wood shavings.
Environmental conditions:	Temperature: 20 ± 5 °C Humidity: 50 ± 20 % Air changes: 12/hour 12 hours light/dark cycle

B: Study design and methods

In life dates: 2005-06-08 to 2005-09-22

Animal assignment and treatment:
In a 13-week oral toxicity study groups of four Beagle dogs per sex received daily doses of 0, 30, 300 or 1000 mg/kg bw/day glyphosate technical by capsule application. The test item capsules were prepared weekly and delivered daily to the animal room, protected from light. As the test item was administered via

capsules, no chemical analysis was performed during the study. The purity, characteristics and identification of the test item were indicated on the certificate of analysis that accompanied the test item.

Mortality
Each animal was checked for mortality or signs of morbidity twice a day during the treatment period, including weekends and public holidays.

Clinical observations
A check for clinical signs of toxicity was made once daily on all animals. In addition, a detailed clinical examination was performed at least once before the beginning of the treatment period and then once a week until the end of the study.

Body weight
The body weight of each animal was recorded twice before group allocation, on the first day of treatment, and then once a week until the end of the study. In addition, the group 4 animals were weighed before final sacrifice on day 75 (early sacrifice of remaining top dose animals).

Food consumption
The quantity of food consumed was recorded for each animal. Food intake per animal and per day was calculated for 7 days before the beginning of the treatment period and then throughout the study.

Ophthalmoscopic examination
Ophthalmological examinations were performed on all the animals before the beginning and at the end of the treatment period.

Haematology and clinical chemistry
Haematological and blood chemical investigations were performed on all animals from each test and control group before the beginning of the treatment period, in Week 7 and at the end of the treatment period (Week 11 for Group 4 and Week 13 for Groups 1 to 3).

Prior to blood sampling the animals were deprived of food for an overnight period of at least 14 hours.
The following parameters were determined: Erythrocytes, haemoglobin, mean cell volume (MCV), mean cell haemoglobin concentration (MCHC), mean cell haemoglobin (MCH), thrombocytes, leucocytes, differential white cell count including morphology, reticulocytes, prothrombin time, activated partial thromboplastin time, sodium, potassium, chloride, calcium, inorganic phosphorous, glucose, urea, creatinine, total bilirubin, total protein, albumin, albumin/globulin ratio, total cholesterol, triglycerides, alkaline phosphatase, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT) and gamma-glutamyl transferase (GGT).

Urinalysis
Urine samples were collected from all animals of the test and control groups before the beginning of the treatment period, in Week 7 and at the end of the treatment period (Week 11 for Group 4 and Week 13 for Groups 1 to 3). During urine collection, the animals were deprived of food for an overnight period of at least 14 hours. The following parameters were assessed: Appearance, colour, volume, pH, specific gravity, proteins, glucose, ketones, bilirubin, nitrites, blood, urobilinogen and sediment.

Sacrifice and pathology
On completion of the treatment period (Week 11 or 13), after at least 14 hours fasting, all surviving animals were subjected to a gross pathological examination. The moribund animals were sacrificed in the same way. Any macroscopic findings were recorded.

The following organ weights were determined: Adrenals, brain, epididymides, heart, kidneys, liver, ovaries, pituitary gland, prostate, spleen, testes, thymus, thyroids with parathyroid and uterus.

Tissue samples were taken from the following organs and preserved in buffered formalin: Adrenals, aorta, bone & bone marrow (sternum and femur), brain (at three levels), caecum, colon, duodenum, epididymides, oesophagus, eyes, gall bladder, heart, ileum (with Peyer's patches), jejunum, kidneys, larynx, liver, lungs (with bronchi), lymph nodes (mandibular and mesenteric), mammary gland, muscle (skeletal), optic nerve, ovaries, oviducts, pancreas, pituitary gland, prostrate, rectum, salivary glands (parotid and submandibular), sciatic nerve, skin, spinal cord (cervical, thoracic and lumbar), spleen, stomach, testes, thymus, thyroid with parathyroid, tongue, trachea, ureters, urinary bladder, uterus (horns and cervix) and vagina.

Statistics
Statistical analysis of body weight, haematology, blood biochemistry, urinalysis and organ weight data was done according to the statistical decision tree shown in "*Guidance Notes for Analysis and Evaluation of Chronic Toxicity and Carcinogenicity Studies*" (OECD, 2002), summarising the most common statistical procedures used for analysis of data in toxicology studies, together with their most likely outcomes.

II. RESULTS AND DISCUSSION

A. MORTALITY
Two unscheduled sacrifices (one male and one female) were occurred in animals given 1000 mg/kg bw/day: One male was sacrificed on Day 61 on humane grounds. Vomiting was seen once in Week 7 (before dosing) and liquid faeces were noted on many occasions in Weeks 8 and 9. Prior to sacrifice, signs of poor clinical condition including thin appearance, dehydration, and pallor of lip mucosa, coldness to the touch, hypothermia (34 – 35 °C) and hypo-activity were observed. These signs were associated with a body weight loss between Weeks 7 and 9 (-34 %) and reduced food consumption from Week 7 (generally only 25 – 50 % of this animal's daily ration was consumed), followed by an absence of food intake on the day before death. Medical care (Smecta® and Lactate Ringer®) was given in order to stop the diarrhoea and rehydrate the animal.

One female was sacrificed on Day 72 for humane reasons. This animal showed liquid or soft faeces on many occasions from Week 4 and dehydration from Week 9. Vomiting was observed once in Week 10. These signs were accompanied by a body weight loss between Weeks 8 and 11 (-22 %) and decreased food consumption from Week 8 (generally only 25 – 50 % of this animal's daily ration was consumed), followed by an absence of food intake on the two days prior to sacrifice. Medical care (Smecta® and lactate Ringer®) was given in many occasions.

B. CLINICAL OBSERVATIONS
No treatment-related clinical signs were noted in control animals or those given 30 or 300 mg/kg bw/day.

The following treatment-related clinical signs were reported in animals given 1000 mg/kg bw/day (excluding those killed in extremis, which are discussed separately above):

- liquid or soft faeces on several occasions in all animals,
- vomiting in 2/3 females on one occasion within 30 minutes or 3 to 5 hours after treatment,
- thin appearance in 1/3 males and all females,
- dehydration in 1/3 males and 2/3 females,
- pallor of ears and mouth in 1/3 females.

C. BODY WEIGHT
No relevant differences in the mean body weight gain were noted between controls and animals given 30 or 300 mg/kg bw/day during the treatment period.

Due to numerous individual body weight losses recorded from Week 4 in males and from Week 1 in females, a marked lower mean body weight was noted in animals given 1000 mg/kg bw/day at termination.

At the end of the treatment period this resulted in only a slight mean body weight gain in males (+4 % vs. +31 % in controls) and a mean body weight loss in females (-7 % vs. +14 % in controls) when compared

to their body weight on Day 1. This effect on body weight was considered treatment-related (see table below).

Table 5.3.2-81: Glyphosate Technical: 13-Week Toxicity Study by Oral Route (Capsule) in Beagle Dogs ([redacted], 2007): Group mean weekly body weights

Time point	Mean body weight and body weight change [kg]						
	Day 1	Week 5	Week 9	Week 11	Change week 1-11	Week 13	Change week 1-13
Dose [mg/kg bw/day]	**Males**						
0	7.4 ± 0.24	9.0 ± 0.29	9.5 ± 0.25	9.7 ± 0.25	+2.3	10.4 ± 0.30	+3.0
30	↓7.2 ± 0.57	↓8.5 ± 0.52	↓8.9 ± 0.43	↓9.1 ± 0.62	+1.9	↓9.5 ± 0.72	+2.3
300	↓7.3 ± 0.62	↓8.5 ± 0.74	↓9.0 ± 0.75	↓9.2 ± 0.69	+1.9	↓9.7 ± 0.72	+2.4
1000	↓7.3 ± 0.48	↓8.3 ± 0.72	**↓7.7* ± 0.61**	**↓7.6* ± 0.74**	+0.3	Not reported	Not reported
	Females						
0	7.3 ± 0.18	7.8 ± 0.42	8.2 ± 0.60	8.3 ± 0.81	+1.0	8.8 ± 0.82	+1.5
30	7.3 ± 0.28	↑8.3 ± 0.47	↑8.7 ± 0.49	↑8.9 ± 0.48	+1.6	↑9.2 ± 0.55	+1.9
300	↑7.4 ± 0.32	↑8.2 ± 0.20	↑8.6 ± 0.28	↑8.7 ± 0.24	+1.3	↑9.2 ± 0.02	+1.8
1000	↓7.2 ± 0.42	↓7.0 ± 1.41	↓6.9 ± 1.21	↓6.7 ± 0.53	-0.5	Not reported	Not reported

* Statistically significant from controls ($p < 0.05$);
Not reported: not applicable, animals sacrificed at week 11.

D. FOOD CONSUMPTION

The food consumption was not affected by the test item treatment in animals given 30 or 300 mg/kg bw/day. Reduced food consumption, varying from 25 – 75 % of the amount given, was occurred on many occasions as early as Day 4 for males and Day 3 for females in animals given 1000 mg/kg bw/day. From Day 62, when tinned dog food was distributed instead of pelleted diet, all animals consumed their full daily ration.

E. OPHTHALMOSCOPIC EXAMINATION

There were no ophthalmological findings at the end of the treatment period.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

The laboratory investigations of the moribund sacrificed male showed the following changes among haematological and blood biochemical parameters when compared to pre-treatment values:

- increase in leucocyte count (WBC) mainly due to an increase in the neutrophil count (N),
- increase in haemoglobin level (Hb), erythrocyte count (RBC) and packed cell volume (PCV),
- decrease in platelet count (PLAT),
- decrease in sodium and chloride levels, as well as an increase in potassium and inorganic phosphorus levels,
- increase in glucose, protein, albumin, cholesterol, triglycerides, urea and creatinine levels.

Some of the abnormalities found in the laboratory investigations (such as the increase in red blood cell parameters and in protein and albumin levels) were indicative of haemoconcentration, which was probably secondary to the dehydration caused by the diarrhoea.

When compared to both pre-dose and control values, no biologically relevant differences were noted in surviving animals of the test item groups in Weeks 7 and 11/13.

Table 5.3.2-82: Glyphosate Technical: 13-Week Toxicity Study by Oral Route (Capsule) in Beagle Dogs ([redacted], 2007): Intergroup comparison of selected haematology parameters (mean ± SD)

Parameter	Week	Dose [mg/kg bw/day]							
		Males				Females			
		0	30	300	1000	0	30	300	1000
WBC [G/L]	Pre-Rx	12.91 ± 1.130	↓11.50 ± 1.524	↑13.28 ± 3.722	↑14.12 ± 1.640	11.78 ± 1.575	↓10.22 ± 2.187	↓11.24 ± 1.643	↓10.58 ± 1.771
	Post-Rx	9.47 ± 1.905	↓8.51 ± 0.135	↑13.11 ± 4.141	↑10.66 ± 1.505	10.91 ± 1.781	**↓8.57* ± 0.687**	↓8.91 ± 1.291	↓10.69 ± 2.195
N [G/L]	Pre-Rx	7.99 ± 1.111	↓7.77 ± 1.694	↓7.88 ± 2.395	↑8.81 ± 1.658	7.66 ± 1.003	↓5.64 ± 1.229	↓6.78 ± 1.837	↓6.51 ± 2.384
	Post-Rx	5.72 ± 1.624	↓5.37 ± 0.325	↑8.56 ± 3.403	↑6.72 ± 1.205	7.07 ± 1.587	↓5.29 ± 0.322	↓5.21 ± 0.947	↓6.51 ± 1.956
RBC [T/L]	Pre-Rx	5.59 ± 0.250	↑5.94 ± 0.344	↑6.03 ± 0.414	↑6.34 ± 0.142	6.37 ± 0.334	↑6.60 ± 0.323	↓6.36 ± 0.142	↓6.21 ± 0.396
	Post-Rx	6.94 ± 0.169	**↓6.26* ± 0.299**	↓6.57 ± 0.511	↑7.02 ± 0.278	6.62 ± 0.563	↑7.16 ± 0.224	↓6.60 ± 0.214	↓6.23 ± 0.585
Hb [g/dL]	Pre-Rx	12.7 ± 0.91	↑13.6 ± 0.75	↑13.5 ± 1.05	↑14.1 ± 0.44	14.7 ± 0.62	↑15.3 ± 0.83	14.7 ± 0.15	14.7 ± 1.0
	Post-Rx	16.5 ± 0.70	**↓14.8* ± 0.79**	**↓15.1* ± 1.14**	↓15.7 ± 0.96	15.5 ± 1.13	↑17.0 ± 0.69	15.5 ± 0.35	↓14.6 ± 1.34
PCV [L/L]	Pre-Rx	0.37 ± 0.022	↑0.39 ± 0.019	↑0.39 ± 0.025	↑0.41 ± 0.015	0.42 ± 0.017	↑0.45 ± 0.022	↑0.43 ± 0.006	0.42 ± 0.031
	Post-Rx	0.50 ± 0.021	**↓0.45* ± 0.024**	↓0.46 ± 0.031	↓0.48 ± 0.021	0.47 ± 0.037	↑0.51 ± 0.018	0.47 ± 0.013	↓0.44 ± 0.042
PLAT [G/L]	Pre-Rx	377 ± 32.8	↓341 ± 65.7	↓338 ± 74.9	↓359 ± 79.0	321 ± 97.4	↑381 ± 92.4	↑335 ± 4.1	↑337 ± 40.3
	Post-Rx	288 ± 21.2	↑316 ± 38.5	↑342 ± 90.2	↑373 ± 41.0	316 ± 44.8	↑327 ± 43.3	316 ± 5.7	↑423 ± 48.7

Pre-Rx: prior to first dose, Post-Rx: week 11 for 1000 mg/kg group (n=3 males, n=4 females), week 13 for other groups (n=4);
* Statistically significant from controls ($p < 0.05$).

The laboratory investigations performed before sacrifice of the moribund female dog showed the following changes among the blood biochemical parameters when compared to pre-treatment values:

- decrease in sodium, potassium, chloride and inorganic phosphorus levels,
- decrease in urea, protein and albumin levels and increase in total bilirubin level and alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase activities.

The abnormalities reported in blood electrolyte levels were not attributed directly to the test item treatment but were related to the poor clinical condition of the animal (diarrhoea, dehydration).

Blood chemistry

When compared to control values in Week 13, the following test item related differences were noted in animals given 1000 mg/kg bw/day in Week 11 (see table below):

- higher alanine aminotransferase (ALAT) activity in 2/3 males and 1/3 females,
- lower alkaline phosphatase (ALP) activity in 3/3 females,
- lower protein and albumin levels in 3/3 females.

Other changes were not attributed to the test item treatment.

Table 5.3.2-83: Glyphosate Technical: 13-Week Toxicity Study by Oral Route (Capsule) in Beagle Dogs ([redacted], 2007): Group mean blood chemical values and standard deviations (SD) in Week 11/13

Dose [mg/kg bw/day]	ALAT [IU/L]	ALP [IU/L]	Total protein [g/L]	Albumin [g/L]
Males				
0 (Week 13)	31 ± 4.8	303 ± 73.7	60 ± 3.0	33 ± 1.7
30 (Week 13)	↑34 ± 5.3	↑323 ± 83.0	↓58 ± 1.8	33 ± 1.0
300 (Week 13)	↓30 ± 8.9	↑305 ± 49.5	↓58 ± 2.2	33 ± 1.3
1000 (Week 11)	↑91 ± 42.5	↑304 ± 53.8	60 ± 3.2	33 ± 2.1
Females				
0 (Week 13)	29 ± 6.0	388 ± 168.0	61 ± 2.1	35 ± 1.6
30 (Week 13)	↑31 ± 10.4	↓281 ± 91.5	↑62 ± 2.1	↓34 ± 1.0
300 (Week 13)	29 ± 4.1	↓332 ± 142.6	↓59 ± 2.5	35 ± 0.6
1000 (Week 11)	↑122 ± 163.9	↓321 ± 322.0	↓55 ± 5.5	↓30 ± 2.5

G. URINALYSIS

When compared to both pre-dose and control values, the following findings were noted at 1000 mg/kg bw/day in Week 11:

- decrease in mean specific gravity in 1/3 males and 3/3 females
- increase in mean urinary volume accompanied by less marked colour of urine in 3/3 females.

As these changes were only noted at the highest dose-level, they were attributed to the test item treatment.

Table 5.3.2-84: Glyphosate Technical: 13-Week Toxicity Study by Oral Route (Capsule) in Beagle Dogs ([redacted], 2007): Intergroup comparison of urine specific gravity values (mean ± SD)

Parameter	Week	Dose [mg/kg bw/day]							
		Males				Females			
		0	30	300	1000	0	30	300	1000
Specific gravity	Pre-Rx	1043 ± 9.6	↑1045 ± 7.1	↑1044 ± 6.3	↓1038 ± 15.0	1036 ± 8.5	↑1050 ± 0.0	↑1043 ± 6.5	↑1044 ± 7.5
	Post-Rx	1045 ± 4.1	↑1048 ± 5.0	↓1043 ± 15.0	↓1028 ± 18.9	1036 ± 14.4	↑1048 ± 5.0	↑1039 ± 4.8	↓1022 ± 5.8

Pre-Rx: prior to first dose, Post-Rx: week 11 for 1000 mg/kg bw/day group (n=3), week 13 for other groups (n=4)

H. NECROPSY

Organ weights

Treatment-related, statistically significant effects were restricted to the prostate.

Table 5.3.2-85: Glyphosate Technical: 13-Week Toxicity Study by Oral Route (Capsule) in Beagle Dogs ([redacted], 2007): Intergroup comparison of prostate and uterus weight – absolute and relative to body weight

Organ		Dose [mg/kg bw/day]							
		Males				Females			
		0	30	300	1000	0	30	300	1000
Prostate	Absolute [g]	5.34 ± 1.58	↓4.86 ± 2.18	↓4.42 ± 1.56	**↓1.72* ± 0.308**	-	-	-	-
	Relative [%]	0.051 ± 0.016	↑0.052 ± 0.025	↓0.045 ± 0.012	↓0.023 ± 0.002	-	-	-	-

Table 5.3.2-85: Glyphosate Technical: 13-Week Toxicity Study by Oral Route (Capsule) in Beagle Dogs ([redacted], 2007): Intergroup comparison of prostate and uterus weight – absolute and relative to body weight

Organ		Dose [mg/kg bw/day]							
		Males				Females			
		0	30	300	1000	0	30	300	1000
Uterus	Absolute [g]	-	-	-	-	9.17 ± 6.89	↓6.03 ± 4.60	↑11.88 ± 6.38	↓5.43 ± 7.62
	Relative [%]	-	-	-	-	0.109 ± 0.085	↓0.068 ± 0.056	↑0.133 ± 0.070	↓0.078 ± 0.109

* Statistically significant from controls ($p < 0.05$);
n = 3 for 1000 mg/kg bw/day groups, n=4 for all other groups

Gross pathology
Macroscopic pathological examination of the male that was killed moribund demonstrated a reddish mucosa of the colon and rectum appeared, enlarged adrenal glands and thyroids, and reduced size of the spleen and thymus.

In the high-dose female that was killed moribund, the oesophagus, jejunum and ileum presented many greyish/white areas and the colon mucosa showed reddish/purplish foci. The gall bladder was dilated with blackish deposits and the liver was yellowish, enlarged and firm. The kidneys were pale.

All the macroscopic changes noted in surviving animals at termination were considered to be normal variations, when compared to background data, which may be seen in untreated Beagle dogs of this age, except for changes in the uterus (reduced in size) for females given 1000 mg/kg bw/day.

Histopathology
The major histopathological findings in the male dog sacrificed moribund were bilateral hyaline degeneration of the cortical tubules in the kidneys with pigment deposits, diffuse acute inflammation in the liver with pigment deposits, acute inflammation of the lamina propria of the oesophagus, bilateral hypertrophy of cortex of the adrenals, diffuse lymphoid atrophy in the spleen, acute inflammation in the lungs with alveolar spaces containing blood and increased number of adipocytes in the sternum.

The bilateral hyaline degeneration of the cortical tubules in the kidneys was considered to be test item treatment-related. However, it is not possible to determine if this lesion, which was associated with increase in urea and creatinine levels, was directly due to the test item action or the result of the dehydration caused by a severe intestinal irritation. The inflammation noted in the liver, oesophagus and lungs was considered to be test item related and was associated with change in leucocyte count. The increased number of adipocytes in the sternum seen also in the schedule killed animals was considered treatment-related. The abnormalities reported in blood electrolyte levels, glucose, triglycerides and cholesterol levels were not directly attributed to the test item treatment but were considered to be secondary to the poor clinical condition of the animal (diarrhoea, dehydration, changes in the kidneys). The modifications reported in spleen and adrenal glands were not attributed to the test item treatment, as they were non-specific changes that could be found in treated animals housed in laboratories.

At microscopic level, the major findings in the sacrificed female were bilateral vacuolation of the cortical tubules in the kidneys, macrovesicular vacuolation in the liver, diffuse hypoplasia of langerhans islet in the pancreas, severe atrophy of cortex of the thymus, increased number of adipocytes in the sternum and uterine atrophy.

The liver histopathological modification was considered to have resulted from the test item treatment and was correlated with changes in the blood biochemical parameters (i.e. urea, protein, albumin and bilirubin levels as well as liver enzyme activities). The abnormalities reported in blood electrolyte levels were not

attributed directly to the test item treatment but were related to the poor clinical condition of the animal (diarrhoea, dehydration). The uterine atrophy and increased number of adipocytes in the sternum, seen also in the schedule killed top dose animals, were considered treatment-related.

The atrophy noted in the thymus is a non-specific change that could be found in laboratory housed animals; therefore a relationship to the test treatment was excluded. The other lesions noted (i.e. in the kidneys and pancreas) can be spontaneously observed in untreated Beagle dogs of this age and sex. Therefore, a relationship to the test treatment was considered unlikely.

No test-substance related histopathological changes were observed in animals of both sexes at and below 300 mg/kg bw/day.

Treatment-related changes observed in surviving animals given 1000 mg/kg bw/day consisted of increased number of adipocytes in the sternum of 2/3 males and 3/3 females, prostate atrophy in 2/3 males and uterine atrophy in 2/3 females.

These lesions, also noted among the moribund sacrificed animals, could be related to the low body weight of these high-dose animals caused by the test item.

All the other microscopic findings observed in the organs of both male and female animals of the high-dose group were judged to be unrelated to treatment or normal background findings.

Table 5.3.2-86: Glyphosate Technical: 13-Week Toxicity Study by Oral Route (Capsule) in Beagle Dogs ([redacted] 2007): Summary incidence of selected histopathological findings – surviving animals

Finding	Dose [mg/kg bw/day]							
	Males				Females			
	0	30	300	1000	0	30	300	1000
Sternum: Adipocytes-increased number	0/4	0/4	0/4	3/3	0/4	0/4	0/4	3/3
Prostate: Atrophy-diffuse	0/4	0/4	0/4	2/3	-	-	-	-
Uterus: Atrophy	-	-	-	-	0/0	0/0	0/0	3/3

III. CONCLUSIONS

In the low- and mid-dose groups no treatment-related signs were noted. No haematological, blood biochemical, urinary or histopathological effects were observed. Only a slight increase of absolute and relative adrenal weights of males receiving 300 mg/kg bw/day was observed. However, the increase was not statistically significant.

At 1000 mg/kg bw/day the test item administration induced marked clinical signs (liquid/soft faeces, dehydration, thin appearance, vomiting and pallor), caused lower body weight gain (males) or body weight loss (females) and reduced food consumption. This led to the early sacrifice of two moribund animals, and to the early termination of the entire group at week 11.

Laboratory investigations in the surviving animals demonstrated some abnormalities (higher alanine aminotransferase activity in both sexes and lower alkaline phosphatase activity, as well as lower protein and albumin levels in females) and urinary changes (decrease in specific gravity in both sexes and increase in urinary volume and markedly less colour of urine in females).

Treatment-related histopathological changes in surviving animals consisted of increased number of adipocytes in the sternum in both sexes, as well as prostate atrophy and uterine atrophy at 1000 mg/kg bw/day. These lesions, also noted among the moribund sacrificed animals, could be related to the low body

weight of these high-dose animals caused either directly or indirectly, by the test item. Further major microscopic changes in moribund sacrificed animals were found in the kidneys (bilateral vacuolation of cortical tubules, sometimes with pigment deposits), liver (diffuse macrovesicular vacuolation, acute inflammation and/or pigment deposits), oesophagus, lung, uterus (atrophy) and/or bone marrow (increased number of adipocytes). These findings were associated with numerous changes in laboratory parameters (haemoconcentration, increased urea and creatinine levels, decreased urea, protein, albumin and bilirubin levels and decreased liver enzyme activities).

Under the experimental conditions of the study and taking into account the slight effects on organ weights at the mid-dose level, the No Observed Adverse Effect Level (NOAEL) was considered to be 300 mg/kg bw/day.

3. Assessment and conclusion

Assessment and conclusion by applicant:
In this study, groups of four Beagle dogs per sex received the test item, glyphosate technical, by daily administration (capsule) at dose-levels of 0, 30, 300 or 1000 mg/kg bw/day for 11/13 weeks according to OECD 409 (1998) and in compliance with GLP.

In the low- and mid-dose groups no treatment-related signs were noted. No haematological, blood biochemical, urinary or histopathological effects were observed. Only a slight increase of absolute and relative adrenal weights of males receiving 300 mg/kg bw/day was observed. However, the increase was not statistically significant.

Under the experimental conditions of the study and taking into account the slight effects on organ weights at the mid-dose level, the NOAEL is considered to be 300 mg/kg bw/day. The high dose of 1000 mg/kg bw/day was found to clearly exceed the MTD (Maximum Tolerated Dose).

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.3.2/021
Report author	[redacted]
Report year	1999 (study report)
Report title	Subchronic (90 Day) Oral Toxicity Study With Glyphosate Technical In Beagle Dogs AND Test compound stability in experimental diet (dog feed)
Report No	1816
Document No	Not reported
Guidelines followed in study	OECD 409 (1981)
Deviations from current test guideline (OECD 409, 1998)	Detailed clinical observations only performed monthly, not weekly. Urinalysis only performed at study termination. Several organ weights missing: epididymides, ovaries, uterus, thymus, spleen, brain, heart; several organs were not sampled: gross lesions, spinal cord, eyes with optic nerve, trachea, skin, mammary gland, prostate or other accessory sex organs. Deviations from the current version of OECD 409 (1998) are basically due to the fact that the study was aligned to an older version of the OECD test guideline 409.

Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a
Data point	CA 5.3.2/022
Report author	[redacted]
Report year	1999
Report title	Subchronic (90 Day) Oral Toxicity Study With Glyphosate Technical In Beagle Dogs AND Test compound stability in experimental diet (dog feed) - Appendix
Report No	1816
Document No	Not reported
Guidelines followed in study	OECD 409 (1981)
Deviations from current test guideline (OECD 409, 1998)	Detailed clinical observations only performed monthly, not weekly. Urinalysis only performed at study termination. Several organ weights missing: epididymides, ovaries, uterus, thymus, spleen, brain, heart; several organs were not sampled: gross lesions, spinal cord, eyes with optic nerve, trachea, skin, mammary gland, prostate or other accessory sex organs. Deviations from the current version of OECD 409 (1998) are basically due to the fact that the study was aligned to an older version of the OECD test guideline 409.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a
Data point	CA 5.3.2/023
Report author	[redacted]
Report year	1999
Report title	Subchronic (90 Day) Oral Toxicity Study With Glyphosate Technical In Beagle Dogs AND Test compound stability in experimental diet (dog feed) - List of tables
Report No	1816
Document No	Not reported
Guidelines followed in study	OECD 409 (1981)
Deviations from current test guideline (OECD 409, 1998)	Detailed clinical observations only performed monthly, not weekly. Urinalysis only performed at study termination. Several organ weights missing: epididymides, ovaries, uterus, thymus, spleen, brain, heart; several organs were not sampled: gross lesions, spinal cord, eyes with optic nerve, trachea, skin, mammary gland, prostate or other accessory sex organs. Deviations from the current version of OECD 409 (1998) are basically due to the fact that the study was aligned to an older version of the OECD test guideline 409.
Previous evaluation	Yes, accepted in RAR (2015)

GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a
Data point	CA 5.3.2/024
Report author	[redacted]
Report year	1997
Report title	Test Compound Stability in Experimental Diet (Dog feed)
Report No	1817-R.FST
Document No	Not reported
Guidelines followed in study	Not reported
Deviations from current test guideline (OECD 409, 1998)	Not reported
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

Three treated groups of four male and four female Beagle dogs received the test item, glyphosate technical, at dietary dose-levels of 0, 200, 2000 or 10000 ppm (corresponding to 0, 5.3, 53.5 or 252.6 mg/kg bw/day) for 90 days.

The animals were checked daily for mortality and clinical signs. Veterinary examination was carried out before grouping, at start of treatment, monthly throughout the study and at termination. Body weight was recorded weekly. Food consumption was determined weekly. Ophthalmological examinations were carried out before the beginning and at the end of the treatment period. Haematological and blood biochemical investigations were performed before the beginning of the treatment period, after 45 days of exposure and at termination. Urine was analysed at termination. At termination, the animals were sacrificed and subjected to a full macroscopic post-mortem examination. Designated organs were weighed and specified tissues preserved. A microscopic examination was performed on selected tissues from all the animals.

No signs of toxicity or ophthalmological findings were observed in any dose group. Food consumption was significantly reduced in the high dose group initially (week 2) while body weights remained unaffected.

Haematological parameters appeared in general unaffected (clotting time was increased after 45 days of exposure in both sexes, but no effects on this parameter were visible at termination; other parameters attaining statistical point significance fell within historical control). Slight increases on total bilirubin and gamma-glutamyl-transferase were observed in the high dose group. No effects on urine parameters, organ weights or organ histopathology were observed.

In absence of any histopathological correlate, the inconsistent effects described in haematology and clinical chemistry were considered incidental and of no toxicological significance.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate Technical
Description:	Crystalline solid
Lot/Batch #:	01.12.1997 & 01.06.1997
Purity:	>95 %
Stability of test compound:	Expiry dates: 2000-06-01 and 2000-12-01

2. Vehicle and/or positive control: Plain diet / none

3. Test animals:

Species:	Dogs
Strain:	Beagle
Source:	[redacted]
Age:	6 – 8 months
Sex:	Male and female
Weight at dosing:	♂ 10.0 – 12.2 kg; ♀ 8.8 – 11.0 kg
Acclimation period:	6 days
Diet/Food:	Nutripet Pet meal (Tetragon Chemie Pvt.Ltd., Bangalore, India), was offered daily for one hour, *ad libitum*
Water:	Deep borewell water passed through activated charcoal filter and exposed to UV rays, *ad libitum*
Housing:	Individual housing in floor pens.
Environmental conditions:	Temperature: 23 – 29 °C Humidity: 40 – 70 % Air changes: Not reported Natural daylight plus fluorescent light from 9 am to 5 pm

B: Study design and methods

In life dates: 1998-03-18 to 1998-06-26

Animal assignment and treatment:
In a 90 day feeding study groups of four Beagle dogs per sex received daily doses of 0, 200, 2000 or 10000 ppm glyphosate technical in the diet (equivalent to 5.3, 53.5 or 252.6 mg/kg bw/day).

Test diets were prepared prior to start of treatment and then twice during the three month study period by mixing a known amount of the test substance with a small amount of basal diet and blending. This pre-mix was then added to a larger amount of basal diet and blended for 20 minutes. The feed was fortified with test compound at weekly intervals.

The stability of the test compound was examined in an additional study (No. 1817-R.FST). The homogeneity of the test material in diet was determined at start of the study. Three samples from the food fortified with the test compound were taken and analysed.

Mortality
Each animal was checked for mortality or signs of morbidity daily during the treatment period.

Clinical observations
Each animal was daily checked for signs of toxicity. A more detailed veterinary investigation was performed before start of exposure, monthly throughout the study and before termination.

Body weight
The body weight of each animal was recorded before allocation and start of treatment, weekly throughout the study and before termination.

Food consumption
The quantity of food consumed was recorded for each animal on a weekly basis. Food was offered in a dedicated bowl for a period of 1 hour after which any remaining or spilled food was removed.

Ophthalmoscopic examination
Ophthalmological examinations were performed on all the animals before the beginning and at the end of the treatment period.

Haematology and clinical chemistry
Haematological, blood chemical and urinalytical investigations were performed on all animals from each test and control group before the beginning of the treatment period, after 45 days of exposure and at termination from animals fasted since the last feeding (approximately 23 hours).

The following parameters were determined: Erythrocytes (RBC), haemoglobin (HB), haematocrit (HCT), mean cell volume (MCV), mean cell haemoglobin concentration (MCHC), mean cell haemoglobin (MCH), leucocytes (WBC), differential white cell count (Neutrophils (Neut), lymphocytes (Lymp), eosinophils (Eosi), monocytes (Mono), reticulocytes (Retic)), clotting time, glucose, urea, total protein, alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), creatinine, total bilirubin, albumin, calcium, inorganic phosphorous, total cholesterol, triglycerides, chloride, sodium and potassium.

Urinalysis
Urine was collected from all animals at termination during autopsy. Urinalysis was performed for control and high-dose group animals.

The following parameters were determined: Specific gravity, pH, leucocytes, proteins, glucose, ketones, blood and urobilinogen.

Sacrifice and pathology
On completion of the treatment period, after an overnight fasting, all surviving animals were subjected to a gross pathological examination. The moribund animals were sacrificed in the same way. Any macroscopic findings were recorded.

The following organ weights were determined: Adrenals, kidneys, liver (with gall bladder), testes and thyroids with parathyroids.

Tissue samples were taken from the following organs and preserved in buffered formalin: Adrenals, aorta, bone & bone marrow (sternum), brain, caecum, colon, duodenum, gall bladder, gonads, heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes (mesenteric), oesophagus, pancreas, pituitary gland, rectum, salivary glands, sciatic nerve, spleen, stomach, thymus, thyroids with parathyroids, urinary bladder, uterus. These tissues (plus parathyroids) were microscopically investigated for all animals of the control and high dose group.

Statistics
Body weights, net body weight gain, food intake, laboratory investigations (haematology and clinical

chemistry values of days 0, 45 and 90), organ weights data and organ weight ratios were compared by Bartlett's test for homogeneity of intra group variances. When the variances proved to be heterogeneous, the data were transformed using appropriate transformation.

The data with homogeneous intra group variances were subjected to one-way analysis of variance. Following ANOVA, when F was found to be significant, Dunnett's pair wise comparison of means of treated groups with control mean was done individually. Following a significant difference of a test group with the control group, the dose-response correlation was estimated including the control and all treated groups and tested by t-test. All analyses and comparisons are evaluated at 5 % probability level.

II. RESULTS AND DISCUSSION

A. MORTALITY
All animals survived until scheduled necropsy.

B. CLINICAL OBSERVATIONS
No clinical signs of toxicity were observed.

C. BODY WEIGHT
Body weights remained statistically unaffected by treatment. No weight loss was observed.

Table 5.3.2-87: Subchronic (90 Day) Oral Toxicity Study with Glyphosate Technical in Beagle Dogs & Test compound stability in experimental diet (dog feed) ([redacted], 1999): Group mean weekly body weights

	Body weight [kg]						
Week	**Initial**	**1**	**2**	**3**	**4**	**5**	**6**
Test item [ppm]	**Males**						
0	11.1 ± 0.8	11.7 ± 0.6	12.2 ± 0.5	12.3 ± 0.6	12.5 ± 0.6	12.7 ± 0.5	12.9 ± 0.7
200	↑11.2 ± 0.7	↓11.6 ± 0.5	↓12.1 ± 0.4	12.3 ± 0.6	↑12.8 ± 0.6	12.7 ± 0.5	↑13.2 ± 0.7
2000	11.1 ± 0.7	11.7 ± 0.4	↓12.1 ± 0.6	↓12.0 ± 0.6	↑12.6 ± 0.6	12.7 ± 0.6	12.9 ± 0.6
10000	11.1 ± 0.9	↓11.4 ± 0.9	↓11.2 ± 0.8	↓11.5 ± 0.9	↓12.4 ± 0.6	↑12.4 ± 0.8	↓12.7 ± 0.5
	Females						
0	10.1 ± 1.0	10.4 ± 1.2	10.9 ± 0.9	10.7 ± 1.3	11.0 ± 1.3	11.0 ± 1.3	11.2 ± 1.5
200	↑10.2 ± 0.9	↑10.7 ± 1.2	↑11.1 ± 0.9	↑10.9 ± 1.2	↑11.3 ± 1.4	↑11.4 ± 1.3	↑11.5 ± 1.4
2000	↓9.8 ± 0.8	↓10.2 ± 0.7	↓10.3 ± 0.9	↓10.2 ± 0.4	↓10.8 ± 0.7	↓10.9 ± 0.7	↓11.0 ± 1.0
10000	↓10.0 ± 0.5	↓9.9 ± 0.6	↓10.0 ± 0.9	↓10.1 ± 0.9	↓10.6 ± 0.8	↓10.6 ± 0.8	↓10.7 ± 1.0
	Body weight [kg]						
Week	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Test item [ppm]	**Males**						
0	13.2 ± 0.7	13.3 ± 0.5	13.4 ± 0.4	13.5 ± 0.5	13.6 ± 0.4	13.7 ± 0.5	13.8 ± 0.3
200	↑13.5 ± 0.6	↑13.5 ± 0.6	↑13.5 ± 0.8	↑13.6 ± 0.8	↑13.7 ± 0.8	↓13.6 ± 0.9	↓13.5 ± 0.9
2000	13.2 ± 0.7	↑13.4 ± 0.7	13.4 ± 0.8	↓13.3 ± 0.9	↓13.3 ± 0.9	↓13.2 ± 1.0	↓13.2 ± 1.1
10000	↓12.8 ± 0.9	↓13.0 ± 0.9	↓13.3 ± 0.8	↓13.3 ± 0.8	↓13.5 ± 0.9	↓13.3 ± 0.9	↓13.6 ± 1.0
	Females						
0	11.4 ± 1.6	11.7 ± 1.4	11.9 ± 1.4	11.7 ± 1.6	12.0 ± 1.3	11.7 ± 1.3	11.8 ± 1.3
200	↑11.7 ± 1.4	↑11.8 ± 1.4	↓11.5 ± 1.6	↓11.6 ± 1.5	↓11.6 ± 1.6	↓11.6 ± 1.6	↓11.4 ± 1.7
2000	↓11.2 ± 0.9	↓11.4 ± 1.0	↓11.3 ± 1.2	↓11.4 ± 1.2	↓11.5 ± 1.2	↓11.6 ± 1.2	↓11.5 ± 1.5
10000	↓10.9 ± 1.2	↓11.2 ± 1.2	↓11.3 ± 1.1	↓11.3 ± 1.1	↓11.3 ± 1.1	↓11.3 ± 1.0	↓11.2 ± 1.3

C. FOOD CONSUMPTION

The food intake of the high dose group (10000 ppm) was significantly lower during the second week of treatment only. Except for this finding the food consumption of all the treatment groups was comparable to the control group during the study period.

Table 5.3.2-88: Subchronic (90 Day) Oral Toxicity Study with Glyphosate Technical in Beagle Dogs & Test compound stability in experimental diet (dog feed) ([redacted] 1999): Average weekly food intake

	Food consumption [g/animal/day]						
Week	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Test item [ppm]	**Males**						
0	254 ± 84.18	336 ± 34.76	342 ± 38.72	336 ± 36.74	320 ± 48.33	346 ± 13.88	345 ± 32.11
200	↑287 ± 11.12	↑354 ± 22.38	↑366 ± 42.98	↑376 ± 33.24	↑326 ± 41.72	346 ± 19.30	↑350 ± 22.46
2000	↑305 ± 34.04	↑373 ± 32.62	↑406 ± 29.60	↑347 ± 52.39	↑334 ± 27.28	↑363 ± 18.51	↑358 ± 41.16
10000	↑262 ± 14.66	**↓177* ± 21.05**	↑380 ± 69.48	↑368 ± 40.18	↑342 ± 44.68	↓332 ± 17.02	↓330 ± 23.85
Test item [ppm]	**Females**						
0	247 ± 48.56	263 ± 64.46	278 ± 64.82	302 ± 52.58	295 ± 48.42	298 ± 41.60	283 ± 43.19
200	↑285 ± 41.19	↑332 ± 44.57	↑324 ± 11.87	↑352 ± 37.92	↑323 ± 47.21	↑321 ± 46.27	↑292 ± 41.66
2000	↓212 ± 56.44	↑306 ± 35.34	↑338 ± 39.11	↑309 ± 40.42	↑298 ± 35.71	↓286 ± 40.52	↑290 ± 25.84
10000	↓212 ± 56.85	**↓166* ± 49.84**	↑348 ± 38.54	↑327 ± 12.23	↑303 ± 32.63	↓261 ± 73.55	↑288 ± 51.41

	Food consumption [g/animal/day]					
Week	**8**	**9**	**10**	**11**	**12**	**13**
Test item [ppm]	**Males**					
0	325 ± 32.85	312 ± 36.42	332 ± 30.75	356 ± 34.71	368 ± 39.65	369 ± 39.22
200	↑342 ± 9.63	↑325 ± 27.23	↑343 ± 23.34	↓318 ± 31.48	↓347 ± 37.37	↓312 ± 38.62
2000	↑366 ± 27.74	↑339 ± 35.81	↓321 ± 59.31	↓330 ± 43.36	↓329 ± 50.93	↓339 ± 41.15
10000	↓324 ± 1.50	↑328 ± 14.35	↓303 ± 20.89	↓341 ± 8.66	↓333 ± 29.42	↓330 ± 20.32
Test item [ppm]	**Females**					
0	297 ± 42.76	275 ± 40.08	290 ± 32.70	295 ± 29.64	292 ± 24.20	285 ± 30.58
200	↓294 ± 68.51	↑294 ± 40.30	↑325 ± 19.41	↑317 ± 54.56	↓290 ± 83.86	↓271 ± 68.36
2000	↑298 ± 26.68	↑284 ± 39.81	↓278 ± 22.77	↓285 ± 20.64	↑303 ± 33.72	↓283 ± 36.84
10000	↓294 ± 40.57	↑298 ± 42.13	↓262 ± 31.61	↓268 ± 35.84	↓262 ± 40.44	↓256 ± 27.40

* Statistically significant from controls ($p \leq 0.05$)

The calculated mean daily test substance intake is summarised in the table below (Table 5.3.2-89).

Table 5.3.2-89: Subchronic (90 Day) Oral Toxicity Study with Glyphosate Technical in Beagle Dogs & Test compound stability in experimental diet (dog feed) ([redacted], 1999): Group mean compound intake levels

Dose group	Dietary concentration [ppm]	Mean daily test substance intake [mg/kg bw/day]*		
		Males	Females	Combined
1 (control)	0	0.0	0.0	0.0
2 (low)	200	5.2	5.4	5.3
3 (mid)	2000	54.2	52.8	53.5

Table 5.3.2-89: Subchronic (90 Day) Oral Toxicity Study with Glyphosate Technical in Beagle Dogs & Test compound stability in experimental diet (dog feed) ([redacted], 1999): Group mean compound intake levels

Dose group	Dietary concentration [ppm]	Mean daily test substance intake [mg/kg bw/day]*		
		Males	Females	Combined
4 (high)	10000	252.4	252.7	252.6

* Based on actual food intake and body weight data

D. OPHTHALMOSCOPIC EXAMINATION

There were no ophthalmological findings at the beginning and at the end of the treatment period.

E. HAEMATOLOGY AND CLINICAL CHEMISTRY

A significant increase in clotting time and GGT-activity was observed in both sexes at the 45-day interim bleed; however, in absence of any corresponding changes at terminal bleed or any histopathological correlate in the liver, this observation is considered to rather reflect a systemic error during determination than a real effect of the test item.

Table 5.3.2-90: Subchronic (90 Day) Oral Toxicity Study with Glyphosate Technical in Beagle Dogs & Test compound stability in experimental diet (dog feed) ([redacted], 1999): Summary of results for clotting time and GGT-activity

	Males				Females			
[ppm]	0	200	2000	10000	0	200	2000	10000
	Clotting time [s]							
Pre-exposure bleed	145 ± 32.47	↑150 ± 22.37	↑147 ± 12.69	↓144 ± 37.47	154 ± 28.62	↑162 ± 17.31	↓149 ± 17.17	↓131 ± 24.21
45 day interim bleed	131 ± 3.77	**↑153* ± 4.76**	**↑172* ± 6.61**	**↑183*$ ± 2.94**	141 ± 6.68	**↑161* ± 1.50**	**↑173* ± 8.66**	**↑182*$ ± 2.36**
90 d final bleed	134 ± 10.85	134 ± 18.84	↑136 ± 12.40	↑139 ± 4.79	142 ± 7.89	142 ± 10.08	↓134 ± 11.90	↓138 ± 21.92
	GGT [U/L]							
Pre-exposure bleed	9 ± 2.5	↑10 ± 2.16	↓8 ± 2.31	↓7 ± 0.82	9 ± 3.51	↓7 ± 2.58	↓7 ± 1.89	↑11 ± 5.56
45 day interim bleed	13 ± 3.56	13 ± 1.91	↑16 ± 1.73	**↑19*$ ± 2.22**	14 ± 1.71	14 ± 3.74	14 ± 2.94	**↑21* ± 2.87**
90 d final bleed	11 ± 7.27	↑12 ± 2.00	↑16 ± 4.50	↑18 ± 1.50	17 ± 6.06	↓16 ± 5.56	↓16 ± 6.99	↑29 ± 7.89

* Statistically significant from controls ($p \leq 0.05$);
$ Significant dose correlation

Total bilirubin seemed affected; however, in absence of a histopathological correlate on the liver, the effect was not considered adverse.

Table 5.3.2-91: Subchronic (90 Day) Oral Toxicity Study with Glyphosate Technical in Beagle Dogs & Test compound stability in experimental diet (dog feed) ([redacted], 1999): Summary of results for total bilirubin

	Males				Females			
[ppm]	0	200	2000	10000	0	200	2000	10000
	Total bilirubin [µmol/L]							
Pre-exposure bleed	3.71 ± 0.43	↑3.99 ± 0.39	3.71 ± 0.57	↓3.14 ± 0.24	3.67 ± 0.35	↓3.51 ± 0.62	↑3.96 ± 0.80	↑4.02 ± 0.36
45 day interim	5.25 ±	↓5.10 ±	↑5.93 ±	↑5.97 ±	5.22 ±	↑5.23 ±	**↑6.49* ±**	**↑6.54*$ ±**

Table 5.3.2-91: Subchronic (90 Day) Oral Toxicity Study with Glyphosate Technical in Beagle Dogs & Test compound stability in experimental diet (dog feed) ([redacted], 1999): Summary of results for total bilirubin

	Males				Females			
[ppm]	**0**	**200**	**2000**	**10000**	**0**	**200**	**2000**	**10000**
	Total bilirubin [µmol/L]							
bleed	0.75	0.70	0.45	0.26	0.30	0.38	**0.47**	**0.34**
90 d final bleed	4.21 ± 0.90	**↑5.65* ± 0.54**	**↑5.95* ± 0.73**	**↑6.21*$ ± 0.54**	4.00 ±0.52	**↑6.57* ± 0.29**	**↑7.08* ± 0.35**	**↑7.18*$ ± 0.75**

* Statistically significant from controls ($p \leq 0.05$);
$ Significant dose correlation

F. URINALYSIS

All parameters were in the normal range and comparable between control and treated animals.

G. NECROPSY

Organ weights
No treatment-related effects were observed.

Necropsy
No treatment-related effects were observed.

Histopathology
There were a few incidental findings with equal distribution across control and treated groups – no relation to treatment was observed.

III. CONCLUSIONS

No signs of toxicity or ophthalmological findings were observed in any dose group. Food consumption was significantly reduced in the high dose group initially (week 2) while body weights remained unaffected. Haematological parameters appeared in general unaffected (clotting time was increased after 45 days of exposure in both sexes, but no effects on this parameter were visible at termination; other parameters attaining statistical point significance fell within historical control). Slight increases on total bilirubin and gamma-glutamyl-transferase were observed in the high dose group. No effects on urine parameters, organ weights or organ histopathology were observed.

In absence of any histopathological correlate, the inconsistent effects described in haematology and clinical chemistry were considered incidental and of no toxicological significance. The No Observed Adverse Effect Level (NOAEL) was considered to be 10000 ppm.

3. Assessment and conclusion

Assessment and conclusion by applicant:
In this study, three treated groups of four male and four female Beagle dogs received the test item, glyphosate technical, at dietary dose levels of 0, 200, 2000 or 10000 ppm (corresponding to 0, 5.3, 53.5 or 252.6 mg/kg bw/day) for 90 days according to OECD 409 (1981) and in compliance with GLP (no certificate of the competent authority was provided).

Oral administration of glyphosate technical via feed to Beagle dogs at concentrations of 200, 2000 or 10000 ppm had no adverse effect on general health, growth, haematological, clinical chemistry or urinalysis parameters, organ weights, gross or histopathological changes. The NOAEL is considered to be 10000 ppm, corresponding to 252.6 mg/kg bw/day.

Assessment and conclusion by RMS:
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1. Information on the study

Data point	CA 5.3.2/025
Report author	[redacted]
Report year	1996 (Study report)
Report title	First Revision to Glyphosate Acid: 90-Day Oral Toxicity Study in Dogs
Report No	[redacted]/P/1802
Document No	Not reported
Guidelines followed in study	OECD 409 (1981); US EPA Subdivision F 82-1(b)
Deviations from current test guideline (OECD 409, 1998)	Heart, thymus, spleen and uterus were not weighed; microscopic examination of spinal cord was performed only at lumbar level. Deviations from the current version of OECD 409 (1998) are basically due to the fact that the study was aligned to an older version of the OECD test guideline 409.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a
Data point	CA 5.3.2/026
Report author	[redacted]
Report year	1996
Report title	First Revision to Glyphosate Acid: 90 Day Oral Toxicity Study in Dogs - Appendix
Report No	[redacted]/P/1802
Document No	Not reported
Guidelines followed in study	OECD 409 (1981); US EPA Subdivision F 82-1(b)
Deviations from current test guideline (OECD 409, 1998)	Heart, thymus, spleen and uterus were not weighed; microscopic examination of spinal cord was performed only at lumbar level. Deviations from the current version of OECD 409 (1998) are basically due to the fact that the study was aligned to an older version of the OECD test guideline 409.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

In a repeated-dose toxicity study glyphosate acid was administered to groups of four male and four female Beagle dogs at dose levels of 0 (control), 2000, 10000 or 50000 ppm (equivalent to 0, 68, 323 or 1680 mg/kg bw/day for males and 0, 68, 334 or 1750 mg/kg bw/day for females) glyphosate acid in the diet for a period of at least 90 days. The clinical condition and body weights of the dogs were monitored during the study, as was their biochemical and haematological status. At the end of the study the dogs were subjected to an examination *post mortem*. The major organs were fixed, processed and examined microscopically.

Glyphosate acid was palatable to dogs in dietary concentrations up to and including the limit dose of 50000 ppm in the diet.

Toxic effects were confined to dogs given 50000 ppm glyphosate acid, these being small reductions of body weight gain. Males also had slightly reduced plasma protein and calcium concentrations.

Liver and kidney weights of males given 10000 and 50000 ppm glyphosate acid were increased. Plasma alkaline phosphatase activity of females given 50000 ppm glyphosate acid was slightly increased. These effects were not accompanied by a histopathological lesion and are considered to be of no toxicological significance.

There were no haematological, clinical or pathological changes associated with glyphosate acid treatment. The toxicological no observed adverse effect level of glyphosate acid given in the diet to dogs for 90 days was 10000 ppm, with only minimal effects at 50000 ppm.

An absolute no effect level was 2000 ppm glyphosate acid.

Minimal toxicity was seen when glyphosate acid was administered in the diet for 90 days at the limit dose of 50000 ppm.

I. MATERIALS AND METHODS

A: Materials

1. Test material:	Glyphosate acid
Description:	Technical, white solid (passed through a 75 µm mesh)
Lot/Batch number:	D4490/1, P18
Purity:	99.1 % w/w a.s.
CAS#:	Not reported
Stability of test compound:	Not reported
2. Vehicle and/ or positive control:	Glyphosate acid was administered in diet
3. Test animals:	
Species	Dog
Strain	Beagle
Age/weight at dosing	22 – 26 weeks
Source	[redacted]
Housing	Individually in indoor pens, with a floor area of 345 × 115 cm. Each pen consisted of an exercise area and separate sleeping quarters with a heated floor
Acclimatisation period	4 – 5 weeks
Diet	Laboratory Diet A (Special Diet Services Ltd., Witham, Essex, UK), *ad libitum*

Water	Mains water, *ad libitum*
Environmental conditions	Temperature: 19 – 22 °C Humidity: Not reported Air changes: Approximately 12 changes / hour Photoperiod: 11 hours light / 13 hours dark

B: Study design and methods

In-life dates: 12 August 1986 19 November 1986

Animal assignment
The study consisted of one control and three treatment groups each containing 4 male and 4 female dogs. The randomisation procedure employed ensured the even distribution of animals across replicates (randomised blocks) and treatment groups, by body weight, placing litter mates in different treatment groups. The sexes were randomised separately.

Male dogs received 400 g and females 350 g of the appropriate diet, in the morning between 9 am and 12 am each day. During the pre-study period, the food was removed 2 – 5 hours after presentation in an attempt to ensure that the dogs ate the diet rapidly. Several batches of test diets were prepared so that no one batch was fed for longer than 5 weeks.

The clinical condition and body weights of the dogs were monitored during the study, as was their biochemical and haematological status. At the end of the study the dogs were subjected to an examination *post mortem*. The major organs were fixed, processed and examined.

Diet preparation and analysis
All experimental diets were based on expanded, ground Laboratory Diet A.

The glyphosate acid concentration was determined for each occasion diet was mixed. The homogeneity of diets containing glyphosate acid was established by analysis of aliquots of diet taken from each mix of the low and high dose diet on the first occasion on which diets were prepared. The stability of the low and high dose diets was determined over a 39 day period on one mix from the first occasion on which diets were prepared.

Concentration analysis results: The achieved dietary concentrations of glyphosate acid were all within ± 9 % of the target concentrations.

Homogeneity results: The homogeneity was considered to be satisfactory with all the mean values from the analysis at the different sampling points being within 6 % of the overall mean.

Stability results: Over a period of 39 days, no significant change was seen in the chemical stability at 2000 and 50000 ppm glyphosate acid.

Observations:
A detailed clinical examination, which included cardiac and pulmonary auscultation, was made on all dogs pre-experimentally and in week 13. In the treatment period, the dogs were observed at least twice during the working day for gross clinical and behavioural abnormalities.

A daily record of faecal consistency was made during the pre-experimental and dosing periods.

Body weight
All dogs were weighed weekly, before feeding, throughout the pre-study period, on day 1 and thereafter at weekly intervals, until termination.

Food consumption
Food residues were recorded daily and were then discarded. These measurements were made usually 4 hours (between 2 – 5 hours) after presentation of the diet during the pre-experimental period and approximately 24 hours after presentation of the diet during the dosing period.

Ophthalmoscopic examination
The eyes of all dogs were examined by indirect ophthalmoscopy pre-experimentally and in week 13.

Haematology and clinical chemistry
Jugular vein blood samples were taken before feeding from all dogs in weeks -1, 4, 8 and 13 and the following parameters measured: Haemoglobin, haematocrit, red blood cell count, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration, platelet count, total white cell count, differential white cell count, blood cell morphology, kaolin-cephalin time and prothrombin time.

Bone marrow smears were taken from a femur of all dogs at necropsy, air dried, fixed in absolute methanol and stored but not examined.

Clinical chemistry
Jugular vein blood samples were taken before feeding from all dogs in weeks -1, 4, 8 and 13 and the following parameters assessed in plasma: Urea, glucose, albumin, total protein, cholesterol, triglycerides, creatine kinase activity, alkaline phosphatase activity, aspartate aminotransferase activity, alanine aminotransferase activity, gamma-glutamyl transferase activity, calcium, sodium and potassium

Urinalysis
Urine was collected by catheterisation from all dogs, once pre-experimentally and in week 13. Microscopic examination of the centrifuged deposits, from all dogs, was made pre-experimentally and in week 13 on the samples taken for biochemical analysis. The following parameters were determined: Urobilinogen, specific gravity, pH, bilirubin, protein, ketones, glucose and blood.

Investigations *post mortem*

Macroscopic examination
At the end of the 90 day dosing period, all animals were killed and examined *post mortem*. This involved an external observation and an internal examination of all organs and structures.

Organ weights
From all animals surviving to scheduled termination, the following organs were removed, trimmed free of extraneous tissue and weighed: Adrenal glands, brain, epididymides, kidneys, ovaries, liver, testes and thyroid glands (with parathyroids).

The left and right components of paired organs were weighed separately.

Tissue submission
The following tissues were examined *in situ*, removed and examined and fixed in an appropriate fixative: Gross lesions including masses, adrenal gland, aorta, brain, bone and bone marrow (rib), caecum, colon, duodenum, gall bladder, epididymis, eyes, femur (including stifle joint), heart, ileum, jejunum, kidney, liver, lung, lymph node – prescapular, lymph node – mesenteric, mammary gland (females only), oesophagus, ovary, pancreas, pituitary gland, prostate gland, rectum, salivary gland, spinal cord (lumbar), skin, spleen, sternum, stomach, testis, thymus, thyroid/parathyroid gland, trachea, urinary bladder, uterus, voluntary muscle, cervix and nerve – sciatic.

Microscopic examination
All processed tissues were examined by light microscopy.

Statistics
Body weight gains from the start of the study to each week and final body weights were considered by analysis of variance, separately for males and females.

Haematology, blood and urine biochemistry data were considered, at each sampling time after the start of the study, by analysis of co-variance on pre-experimental values. Male and female data were analysed together and the results examined to determine whether differences between control and treated groups were consistent between sexes.

Organ weights at termination were considered by analysis of variance and analysis of co-variance on the last measured body weight, separately for males and females. Left and right components of paired organs were considered separately and combined to investigate for any differential effects.

All analyses allowed for the replicate design of the study and were carried out using SAS (1982). Unbiased estimates of the treatment group means were provided by least square means (LSMEANS option in SAS). Each treatment group was compared to the control group mean using a two-sided Student's t-test, based on the error mean square from the appropriate analysis. Where male and female data were analysed together, these comparisons were made separately.

All data were checked for atypical values and where such values were detected the analyses were repeated omitting these values to determine their influence on the conclusions.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities.

B. CLINICAL OBSERVATIONS
The clinical observations noted were of a minor nature, often seen in studies of this duration using this strain of dog and are considered to be unrelated to treatment with glyphosate acid.

C. BODY WEIGHT AND BODY WEIGHT GAIN
Body weight gain of males given 50000 ppm glyphosate acid showed a slight depression throughout the study, but the differences were not statistically significant.

Females given 50000 ppm glyphosate acid showed slightly reduced body weight gains throughout the study and these were occasionally statistically significantly different from the controls.

There was no effect on growth in dogs given 2000 or 10000 ppm glyphosate acid.

Table 5.3.2-92: First Revision to Glyphosate Acid: 90 Day Oral Toxicity Study in Dogs ([redacted], 1996): Intergroup comparison of body weight gain [g] (selected time points)

Week	Dietary Concentration of glyphosate acid [ppm]							
	Males				Females			
	0	2000	10000	50000	0	2000	10000	50000
Initial weight	10.97	10.60	11.00	10.90	9.70	9.40	9.47	9.47
4	1.00	↑1.13	↑1.07	↓0.65	0.64	↑0.75	↑0.85	↓**0.38***
9	2.07	↓1.92	2.07	↓1.65	1.31	↑1.42	↑1.52	↓**0.97***
Final weight	13.03	↓13.00	↑13.37	↓12.50	11.31	↓11.13	↑11.40	↓10.95

* Statistically significant difference from control group mean ($p < 0.05$; Student's t-test, 2-sided)

D. FOOD CONSUMPTION AND UTILISATION

All dogs ate all the diet presented during the dosing period. The dose received (in mg glyphosate acid/kg bw/day) was similar for both males and females. During the study, there was the expected decrease in the dose received, due to the increasing weight of the dogs.

One dog fed 10000 ppm glyphosate acid was given cubed diet for two days in week 5 to prevent it scooping up powdered diet and thereby allowing healing to a wound in its front paw. No glyphosate acid was received by this dog on these two days.

Dose rates (based on nominal dietary levels of glyphosate acid) were calculated in terms of mg/kg bw/day. Mean values are shown below:

Table 5.3.2-93: First Revision to Glyphosate Acid: 90 Day Oral Toxicity Study in Dogs ([redacted], 1996): Mean Dose Received [mg/kg bw/day]

Glyphosate acid [ppm]	2000	10000	50000
Males	68	323	1680
Females	68	334	1750

E. OPHTHALMOSCOPIC EXAMINATION

There were no treatment-related ophthalmological findings.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

There were no differences in haematological parameters which were considered to be related to treatment.

Blood clinical chemistry

Male dogs fed 50000 ppm glyphosate acid showed slightly reduced plasma albumin and total protein concentrations, possibly representing the start of the expected effect of feeding an inert substance at a sufficiently high level to reduce the intake of nutrients. Plasma calcium levels were also minimally reduced in these animals, possibly a result of calcium sequestration which occurs with compounds structurally-related to glyphosate acid.

Female dogs given 50000 ppm glyphosate acid had slightly elevated plasma alkaline phosphatase activities throughout the study.

There were no treatment-related changes in dogs fed 2000 or 10000 ppm glyphosate acid. There were other isolated instances where results were statistically significantly different from control, but these were considered to be unrelated to treatment.

Table 5.3.2-94: First Revision to Glyphosate Acid: 90 Day Oral Toxicity Study in Dogs ([redacted], 1996): Intergroup comparison of clinical chemistry – selected parameters and time points

Parameter	Week	Dose Level of glyphosate acid [ppm]							
		Males				Females			
		0	2000	10000	50000	0	2000	10000	50000
Albumin [g/100 mL]	4	3.70	3.70	↑3.73	↓**3.43***	3.76	↓3.65	↑3.89	↓**3.51***
	8	3.77	↓3.74	↓3.69	↓**3.53***	3.72	↓3.71	↑3.92	↓3.63
	13	3.92	↑3.97	↓3.77	↓**3.66***	3.84	↓3.70	↑3.94	↓3.78
Total protein [g/100 mL]	4	5.57	↓5.42	↓5.34	↓**5.14****	5.36	↑5.40	↑5.42	↓5.22
	8	5.44	↑5.49	↓5.32	↓**5.22***	5.32	↓5.30	↑**5.52***	↓5.19
	13	5.60	↑5.70	↓5.45	↓5.38	5.39	↓5.34	↑**5.65***	↓5.30

Table 5.3.2-94: First Revision to Glyphosate Acid: 90 Day Oral Toxicity Study in Dogs ([redacted], 1996): Intergroup comparison of clinical chemistry – selected parameters and time points

Parameter	Week	Dose Level of glyphosate acid [ppm]							
		Males				Females			
		0	2000	10000	50000	0	2000	10000	50000
Calcium [mg/100 mL]	4	11.2	11.2	↓11.1	↓**10.5****	10.9	↑11.1	↑11.2	↓10.7
	8	11.2	↓11.1	↓**10.9***	↓**10.8****	10.9	↑11.0	↑**11.2***	10.9
	13	10.7	↓10.5	↑10.8	↓**10.0****	10.4	↓10.3	↑10.6	10.4
Plasma alkaline phosphatase [mU/mL]	4	182	↑190	↑188	↑193	176	↑181	↑182	↑**220****
	8	155	↑168	↑164	↑177	152	↑155	↑155	↑**181***
	13	149	↑165	↑160	↑161	140	↑143	↑145	↑**166***

* Statistically significant difference from control group mean ($p < 0.05$; Student's t-test, 2-sided);
** Statistically significant difference from control group mean ($p < 0.01$; Student's t-test, 2-sided)

G. URINALYSIS

There were no differences in urine clinical chemistry parameters, nor in urinary sediment examinations, which were considered to be related to treatment.

F. SACRIFICE AND PATHOLOGY

Organ weights

Kidney weights of males given 10000 or 50000 ppm glyphosate acid were slightly increased above control values. There was also a small increase in liver weight at these dose levels, but in male dogs only.

Thyroid weights, adjusted for body weight, of females given 2000 or 10000 ppm glyphosate acid were statistically significantly reduced from control values. In the absence of any dose response relationship across all groups, this is considered not to be of toxicological significance.

Table 5.3.2-95: First Revision to Glyphosate Acid: 90 Day Oral Toxicity Study in Dogs ([redacted], 1996): Intergroup comparison of selected organ weights [g] in dogs (adjusted for body weight)

Organ	Dose Level of glyphosate acid [ppm]							
	Males				Females			
	0	2000	10000	50000	0	2000	10000	50000
Kidneys	55.7	58.2	**61.6***	**62.7***	53.6	50.4	53.0	56.2
Liver	385	409	**427***	**436****	362	357	367	362
Thyroid	0.893	0.966	0.989	0.890	0.949	0.811*	**0.735****	0.860

* Statistically significant difference from control group mean ($p < 0.05$; Student's t-test, 2-sided);
** Statistically significant difference from control group mean ($p < 0.01$; Student's t-test, 2-sided)

Macroscopic findings

No macroscopic findings were observed attributable to the administration of glyphosate acid.

Microscopic findings

There was no microscopic pathology attributable to the administration of glyphosate acid.

Incidental findings included minor granulomatous/inflammatory lesions in lung, alimentary tract and lymph node associated with ascarid migration. Imperfect spermatogenesis and minimal secretory activity of the prostate were observed in several sexually immature males. Minimal cystitis manifest as infiltration of the mucosa by inflammatory cells and small haemorrhages were found in several animals and were consistent with a subclinical bacterial infection of the lower urinary tract.

III. CONCLUSIONS

Glyphosate acid was palatable to dogs in dietary concentrations up to and including the limit dose of 50000 ppm in the diet.

Toxic effects were confined to dogs given 50000 ppm glyphosate acid, these being small reductions of body weight gain. Males also had slightly reduced plasma protein and calcium concentrations.

Liver and kidney weights of males given 10000 and 50000 ppm glyphosate acid were increased. Plasma alkaline phosphatase activity of females given 50000 ppm glyphosate acid was slightly increased. These effects were not accompanied by a histopathological lesion and are considered to be of no toxicological significance.

There were no haematological, clinical or pathological changes associated with glyphosate acid treatment. The toxicological no observed adverse effect level of glyphosate acid given in the diet to dogs for 90 days was 10000 ppm, with only minimal effects at 50000 ppm.

An absolute no effect level was 2000 ppm glyphosate acid.

Minimal toxicity was seen when glyphosate acid was administered in the diet for 90 days at the limit dose of 50000 ppm. The toxicological no adverse effect level for glyphosate acid from this study was 10000 ppm in the diet (equivalent to a dose of more than 300 mg glyphosate acid/kg bw/day).

3. Assessment and conclusion

Assessment and conclusion by applicant:

In this sub-chronic toxicity study glyphosate acid was administered to groups of four male and four female Beagle dogs at dose levels of 0 (control), 2000, 10000 or 50000 ppm (equivalent to 0, 68, 323 or 1680 mg/kg bw/day for males and 0, 68, 334 or 1750 mg/kg bw/day for females) glyphosate acid in the diet for a period of at least 90 days according to OECD 409 (1981) and in compliance with GLP (no certificate of the competent authority was provided).

Minimal toxicity including minimally reduced body weight gain was seen when glyphosate acid was administered in the diet for 90 days at the limit dose of 50000 ppm. Reduced plasma albumin and total protein concentrations in male dogs fed 50000 ppm glyphosate acid may represent the start of the expected effect of feeding an inert substance at a sufficiently high level to reduce the intake of nutrients. Minimal reduction of plasma calcium may be a result of calcium sequestration which occurs with compounds structurally-related to glyphosate acid. The NOAEL for glyphosate acid from this study was 10000 ppm (equivalent to 323 mg/kg bw/day for males and 334 mg/kg bw/day for females).

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.3.2/027
Report author	[redacted]
Report year	1996
Report title	HR-001: 13-week Oral Subchronic Toxicity Study in Dogs
Report No	[redacted] 94-0158
Document No	Not reported
Guidelines followed in study	Japan MAFF Guidelines 59 NohSan No.4200, 1985; U.S. EPA FIFRA Guidelines Subdivision F, 1984; OECD 409 (1981)
Deviations from current test guideline (OECD 409, 1998)	Reticulocytes not counted, clotting not evaluated; blood chloride, sodium and potassium not measured; uterus and thymus not weighed. Deviations from the current version of OECD 409 (1998) are basically due to the fact that the study was aligned to an older version of the OECD test guideline 409.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

An oral 90–day toxicity study of HR-001 (Glyphosate technical) was conducted in Beagle dogs of both sexes. Groups of 4 males and 4 females were given the test item by incorporating it into a basal diet at a level of 0, 1600, 8000 or 40000 ppm (equivalent to 0, 39.7, 198 or 1015 mg/kg bw/day for males and 0, 39.8, 201 or 1014 mg/kg bw/day for females) for a period of 13 weeks. Animals were checked daily for general conditions, mortality and individual food consumption was also measured daily. Body weights were recorded weekly. All animals were subjected to ophthalmology, urinalysis, haematology and blood biochemistry periodically. At termination of treatment, the animals were euthanised and subjected to necropsy and organ weight analysis. Histopathological examination was performed on all animals.

40000 ppm group: Three of the four females showed decrease in urine pH value. However, since the test item is degraded into free acid inducing acidified urine, the decrease in urine pH value without concomitant signs of renal toxicity was not considered of toxicological significance.

8000 and 1600 ppm groups: There were no treatment-related abnormalities in any parameters in either sex.

No significantly adverse effects were observed in Beagle dogs of both sexes following the dietary treatment with HR-001 at a concentration as high as 40000 ppm for 13 weeks

I. MATERIALS AND METHODS

A: Materials

1. Test material: Glyphosate technical

Identification:	HR-001
Description:	White crystal
Lot/Batch #:	T-940308
Purity:	94.61 %
Stability of test compound:	Not mentioned in the report

2. Vehicle and/ or positive control: Plain diet / none

3. Test animals:

Species:	Dog
Strain:	Beagle
Source:	[redacted]
Age:	♂ 5 months; ♀ 6 months
Sex:	Male and female
Weight at dosing:	♂ 27.3 – 32.7 g; ♀ 22.4 – 25.8 g
Acclimation period:	♂ 21 days; ♀50 days
Diet/Food:	Solid diet DS (Oriental Yeast, Co.) restricted at 250 g/dog/day
Water:	Filtered and sterilised tap water, *ad libitum*
Housing:	Individually in stainless steel cages 83.5 × 90.0 × 80.0 cm
Environmental conditions:	Temperature: 24 ± 2 °C Humidity: 55 ± 10 % Air changes: 15/hour 12 hours light/dark cycle

B: Study design and methods

In life dates: 1995-09-20 to 1996-02-08

Animal assignment and treatment:
The test material was offered on a continuous basis in the basal diet to groups of 4 males and 4 females Beagle dogs for a minimum of 90 days. Dietary concentrations were 0, 1600, 8000 and 40000 ppm (equivalent to an intake of 0. 39.7, 198 or 1015 mg/kg bw/day for males and 0, 39.8, 201 or 1014 mg/kg bw/day for females).

Table 5.3.2-96: HR-001: 13-week Subchronic Oral Toxicity Study in Dogs ([redacted] 1996): Study design

Test group	Dietary concentration [ppm]	Achieved test concentration [mg/kg bw/day]	Males	Females
Control	0	♂: 0; ♀: 0	4	4
Low	1600	♂: 39.7; ♀: 39.8	4	4
Mid	8000	♂: 198; ♀: 201	4	4
High	40000	♂: 1015; ♀: 1014	4	4

Homogeneity of the test substance in diet was ascertained for all dose levels using the samples taken from the top, middle and bottom portions of the mixer at the first diet preparation (before initiation of the study). The coefficient of variation of the concentrations of technical glyphosate was 2.3 % or less for all test diets and confirmed that the test substance was mixed in the basal diet at good homogeneity.

Concentrations of technical glyphosate in test diets were monitored for all batches of test diets of all dose levels during the study. The overall mean concentrations found in test diets were within a range of 94 – 101 % to the nominal levels and confirmed that the test substance was mixed in the test diets at acceptable concentrations.

Mortality
Mortality was expressed weekly as a ratio of the cumulative number of animals found dead or killed in extremis to the effective number of animals per dose group.

Clinical observations
Cage-side observation was performed daily on all animals to detect moribund or dead animals and abnormal clinical signs, and all findings were recorded. In addition, a detailed examination including palpation for masses was performed at least once a week.

Body weight
Body weights of all animals were recorded at initiation of treatment and weekly during the study. In addition, final body weight of each animal was measured before necropsy.

Food consumption and utilisation
Food residues, if any, were collected and weighted every morning. Daily food consumption by each animal was calculated as follows:

$$Food\ consumption = \frac{[Feeding\ amount\ (250g\ diet + 250g\ water) - \ food\ residue]}{2}$$

Chemical intake [mg/kg bw/day] was calculated weekly from food consumption and body weight data and the nominal dose level.

Ophthalmoscopic examination
Prior to initiation of treatment and at week 13, all animals were subjected to ophthalmological examinations with a direct ophthalmoscope.

The following parameters were determined: Eyeball, eyelid, conjunctiva, cornea, anterior chamber, pupil, iris, lens, vitreous body and fundus.

Haematology and clinical chemistry
Prior to initiation of treatment and at weeks 7 and 13, all animals were subjected to haematological examinations. Blood samples were withdrawn with heparinised syringes from the cephalic vein of the animals following overnight starvation. A part of each sample was transferred to a cup treated with EDTA and subjected to the haematological examination.

The following parameters were determined with a fully automated haematology analyser: Haematocrit (Ht), haemoglobin (Hb), erythrocyte count (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count (PLT) and total leukocyte count (WBC).

Prior to initiation of treatment and at weeks 7 and 13, all animals were subjected to biochemical

examinations. Plasma from heparinised blood samples from haematological tests was used.

The following parameters were determined: Alkaline phosphatase (ALP), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), γ-glutamyl transpeptidase (GGTP), creatine phosphokinase (CPK), creatinine (Creat.), blood urea nitrogen (BUN), total protein (TP), albumin (Alb), globulin (Glob.), albumin/globulin ratio (A/G ratio), glucose (Gluc.), total cholesterol (T. Chol.), triglyceride (TG), total bilirubin (T. Bil.), calcium (Ca) and inorganic phosphorus (P).

Urinalysis
Prior to initiation of treatment and at week 13 of treatment, all animals were subjected to urinalysis.
Volume and sediments were determined on urine samples collected for 24 hours using trays. The other parameters were determined on fresh urine samples.

The following parameters were determined: specific gravity, pH, protein, glucose, ketones, occult blood, urobilinogen, bilirubin, appearance urine volume and urinary sediments.

Sacrifice and pathology
All animals were subjected to a complete necropsy and all gross findings were recorded. After 13 weeks of treatment, all animals were anesthetised and euthanised by exsanguinations from the carotid artery before necropsy. At necropsy the organs and tissues except eyes were removed and preserved in neutral-buffered 10 % formalin. The eyes were fixed in a phosphate-buffered mixed solution of formalin and glutaraldehyde for about 3 days and transferred to neutral-buffered 10 % formalin.

Weights of the following organs were recorded for all animals and the ratios to the final body weight were calculated: Brain, heart, adrenals, thyroids with parathyroids, liver, ovaries, kidneys, prostate and spleen.

The following organs and tissues from all animals were histopathologically examined: Brain, spinal cord, peripheral nerve, pituitary, thyroids with parathyroids, thymus, adrenals, tonsil, spleen, bone with marrow, lymph nodes, heart, aorta, tongue, pharynx, buccal mucosa of oral cavity, salivary glands, oesophagus, stomach, liver, gall bladder, pancreas, duodenum, jejunum, ileum, caecum, colon, rectum, nasal cavity, larynx, trachea, lung, kidneys, urinary bladder, testes, prostate, epididymides, penis, ovaries, oviducts, uterus, vagina, diaphragm, eyes, femoral muscle, skin, mammary gland and all gross lesions.

Statistics
All data were evaluated using variance analysis (body weight, food consumption, urine specific gravity, urine volume, haematological parameters, blood chemistry parameters and organ weights).
Data on clinical signs, mortality, ophthalmology, necropsy and histopathology were evaluated by Fisher's exact probability.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no animals found dead or killed *in extremis* in any groups during the treatment period.

B. CLINICAL OBSERVATIONS
Statistically significant differences in incidence of clinical signs were not observed between the control and treated groups in either sex. There were sporadic incidences of loose stools in one male in the 8000 ppm group during week 10 and two males in the 40000 ppm group during weeks 5 and 9 for one dog and weeks 5, 6 and 8 for the other dog.

C. BODY WEIGHT
Statistically significant differences in body weights were not observed between the control and treated groups in either sex throughout the treatment.

D. FOOD CONSUMPTION AND TEST SUBSTANCE INTAKE

There were no significant changes in food consumption and chemical intake in either sex of the treated groups.

The overall group mean chemical intakes [mg/kg bw/day] over the whole treatment period were calculated from food consumption, body weights and the nominal dose levels. The results are shown in the table below:

Table 5.3.2-97: HR-001: 13-week Subchronic Oral Toxicity Study in Dogs ([redacted], 1996): Summary of compound intake

Dose level [ppm]	Overall group mean chemical intake [mg/kg bw/day]	
	Males	Females
1600	39.7	39.8
8000	198	201
40000	1015	1014

E. OPHTHALMOSCOPIC EXAMINATION

No ocular changes were detected in any dose groups of both sexes.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

Statistically significant changes in haematology parameters were observed in the treated groups as shown in the following table:

Table 5.3.2-98: HR-001: 13-week Subchronic Oral Toxicity Study in Dogs ([redacted], 1996): Results of haematological examinations week 13 (mean ± SD)

Parameter	Dose group [ppm]							
	Males				Females			
	0	1600	8000	40000	0	1600	8000	40000
Erythrocyte count (RBC) [$10^6/mm^3$]	6.73 ± 0.35	**↑7.57* ± 0.44**	**↑7.77** ± 0.23**	↑6.84 ± 0.41	7.20 ± 0.64	↑7.55 ± 0.34	↑7.64 ± 0.46	↑8.02 ± 1.08
Haemoglobin concentration (Hb) [g/dL]	15.2 ± 1.1	↑15.9 ± 0.7	↑15.8 ± 1.0	↓14.8 ± 1.0	15.9 ± 1.0	↑16.8 ± 0.2	↑17.2 ± 0.7	↓14.9 ± 1.3
Haematocrit (Ht) [%]	45.4 ± 3.3	↑47.0 ± 2.1	↑47.6 ± 2.1	↓44.5 ± 3.2	47.3 ± 3.0	↑50.1 ± 0.5	↑51.3 ± 1.8	↓45.8 ± 3.6
Mean corpuscular volume (MCV) [fL]	67.5 ± 1.7	↓62.1 ± 3.0	↓61.3 ± 3.9	↓65.2 ± 4.6	65.9 ± 2.8	↑66.5 ± 2.7	↑67.3 ± 2.8	↓58.1 ± 10.9
Mean corpuscular haemoglobin concentration (MCHC) [g/dL]	33.5 ± 0.2	↑33.8 ± 0.3	↓33.3 ± 0.7	↓33.2 ± 0.9	33.5 ± 0.6	33.5 ± 0.2	33.5 ± 0.3	**↓32.6* ± 0.5**
Lymphocytes (Lym) [$10^6/mm^3$]	3.6 ± 0.6	↑4.7 ± 1.7	↑3.9 ± 0.5	↓3.5 ± 0.5	3.0 ± 0.9	↑4.1 ± 0.7	↑4.1 ± 1.4	↑3.6 ± 1.0

* Statistically significant from controls ($p < 0.05$);
** Statistically significant from controls ($p < 0.01$)

Although there were statistically significant differences in some parameters in the treated groups of both sexes, no dose-response relationship was observed. A significant decrease in mean corpuscular haemoglobin concentration (MCHC) observed in females of the 40000 ppm group was considered to be incidental, because the change was also noted for the pre-treatment measurement and was not accompanied with significant abnormalities of erythrocyte count (RBC), haematocrit (Ht) and haemoglobin (Hb).

Blood clinical chemistry

Occasional statistically significant changes in blood biochemistry parameters were observed in the treated groups and are shown in the following table:

Table 5.3.2-99: HR-001: 13-week Subchronic Oral Toxicity Study in Dogs ([redacted], 1996): Results of clinical chemistry examinations week 13 (mean ± SD)

Parameter	Dose group [ppm]							
	Males				Females			
	0	1600	8000	40000	0	1600	8000	40000
Albumin (Alb) [g/dL]	3.03 ± 0.21	↓2.95 ± 0.24	↑3.07 ± 0.10	↓2.89 ± 0.06	3.02 ± 0.10	**↑3.24* ± 0.06**	↑3.18 ± 0.11	↓2.98 ± 0.10
Chloride (Cl) [mEq/L]	112.4 ± 1.0	**↑114.1* ± 1.0**	↓112.2 ± 0.9	**↑114.5* ± 0.3**	109.8 ± 0.6	↑109.9 ± 1.0	↓108.8 ± 0.9	↓109.0 ± 1.1
Glucose (Gluc) [mg/dL]	104 ± 4	↓98 ± 7	↓100 ± 2	↓101 ± 3	89 ± 8	↑94 ± 4	↑96 ± 4	89 ± 4

* Statistically significant from controls ($p < 0.05$)

Although there were statistically significant differences in some parameters in the treated groups of both sexes, no dose response relationship was evident. Although significant increases in chloride (Cl) were observed in males of the 1600 and 40000 ppm groups at week 13, the changes were considered to be incidental because of no dose dependency and their small degrees of alteration.

G. URINALYSIS

In the 40000 ppm group, 3 of 4 females showed decrease in urine pH at week 13, although there were no statistically significant differences between the control and treated groups of both sexes in any parameters of urinalysis.

There were no significant changes in urinalysis in males and females treated at 8000 ppm or less.

Table 5.3.2-100: HR-001: 13-week Subchronic Oral Toxicity Study in Dogs ([redacted], 1996): Intergroup comparison of urinary pH week 13 (number of animals at pH value)

pH	Dose Group [ppm]							
	Males				Females			
	0	1600	8000	40000	0	1600	8000	40000
6.0	-	-	-	-	-	-	-	1
6.5	-	-	-	-	-	-	-	-
7.0	-	-	-	-	-	-	-	1
7.5	-	1	-	1	-	-	-	1
8.0	-	-	-	1	-	-	-	-
8.5	4	3	4	2	4	4	4	1

H. NECROPSY

Organ weights

There were no gross findings with statistically significant differences in incidence and relationship to the treatment in the treated groups of either sex. Although a statistically significant increase was noted for the relative weight of the adrenals in females of the 1600 ppm group, the change was considered to be incidental due to the lack of dose-dependency.

Table 5.3.2-101: HR-001: 13-week Subchronic Oral Toxicity Study in Dogs ([redacted] 1996): Intergroup comparison of adrenal weight relative to body weight (mean ± SD)

Organ		Dose Group [ppm]							
		Males				Females			
		0	1600	8000	40000	0	1600	8000	40000
Adrenal	Relative [%]	0.008 ± 0.001	↑0.009 ± 0.001	↑0.010 ± 0.001	0.008 ± 0.002	0.009 ± 0.001	↑**0.011*** ± **0.001**	↑0.010 ± 0.001	↑0.010 ± 0.001

* Statistically significant from controls ($p < 0.05$)

Gross pathology

Histopathology
There were no histopathological changes related to the treatment in the treated groups of either sex. A female in the 40000 ppm group showed cutaneous histiocytoma which is a non-specific lesion in young dogs.

III. CONCLUSIONS

40000 ppm group: Three of the four females showed decrease in urine pH value. However, since the test item is degraded into free acid inducing acidified urine, the decrease in urine pH value without concomitant signs of renal toxicity was not considered of toxicological significance.

8000 and 1600 ppm groups: There were no-treatment related abnormalities in any parameters in either sex.

No significantly adverse effects were observed in Beagle dogs of both sexes following the dietary treatment with HR-001 at a concentration as high as 40000 ppm for 13 weeks. It was determined that the no-observable-effect level of HR-001 was 40000 ppm (equivalent to 1015 and 1014 mg/kg bw/day for males and females, respectively).

3. Assessment and conclusion

Assessment and conclusion by applicant:
In this study, groups of 4 male and 4 female Beagle dogs were given the test item (glyphosate technical) via the diet at dose levels of 0, 1600, 8000 or 40000 ppm (equivalent to 0, 39.7, 198 or 1015 mg/kg bw/day for males and 0, 39.8, 201 or 1014 mg/kg bw/day for females) for a period of 13 weeks according to OECD 409 (1981) and in compliance with GLP (no certificate of the competent authority was provided).

No toxicologically relevant adverse effects were observed in Beagle dogs of both sexes following the dietary treatment with HR-001 (glyphosate technical) at a concentration as high as 40000 ppm for 13 weeks.

Under the experimental conditions of the study, the NOAEL is considered to be 40000 ppm (equivalent to 1015 and 1014 mg/kg bw/day for males and females, respectively).

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.3.2/028
Report author	[redacted]
Report year	1985
Report title	Subacute oral toxicity in dogs (for 90 days) of glyphosate (technical) of Excel Industries Limited Bombay
Report No	Not known
Document No	Not reported
Guidelines followed in study	Not known
GLP	Not known
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	Groups of three Mongrel dogs per sex and dose were administered glyphosate (purity not reported); orally via their food at target dose levels of 0 (control group receiving 0.2 % agar solution mixed in mutton soup), 100, 250 or 500 mg/kg bw/day for 90 days. In addition, a second group (reversal group) receiving the mid dose of 250 mg/kg bw/day was sacrificed after a 30-day recovery period following treatment. Animals were observed daily for signs of toxicity. Body weight and food consumption were determined regularly. Blood samples for haematological (red and white cell parameters) and clinical chemistry (total serum protein, alanine aminotransferase, alkaline phosphatase, blood urea nitrogen, glucose) evaluations were performed at pretest, on study day 46 and on days 91 and 121 just prior to sacrifice. Urinalysis was also performed. All animals were subjected to gross pathological examination and histopathology. Organ weights were determined.
Short description of results:	There were no deaths during the study and no signs of toxicity were observed. Laboratory investigations and pathological examinations did not reveal indications of adverse effects. According to the study scientists, alanine aminotransferase was increased in high dose animals, however, the respective mean values were exceptionally high at pretest already. The only findings that could be attributed to treatment were a reduction in body weight gain and, during the second part of the study, a decrease in food consumption. These effects were noted in both sexes but were confined to the highest dose level. Thus, the mid dose of 250 mg/kg bw/day was considered the NOEL in this study.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	Monograph (2000): The study was considered supportive only due to reporting deficiencies. For examples, the year when the study was performed was not indicated in the original report, test substance purity not reported, statistical analysis of the results was not reported. RAR (2015): The study was considered invalid due to serious reporting deficiencies, e.g., absence of information on batch and purity of the test material. Therefore and since the study report is not available, this study is not considered to be reliable.
Reasons why the study report is not available for submission	The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL.
Category study in AIR 5 dossier (L docs)	Category 4b

1. Information on the study

Data point	CA 5.3.2/029
Report author	[redacted]
Report year	1983
Report title	Six Month Study of Mon 0139 by Gelatin Capsule to Beagle Dogs
Report No	810166
Document No	Not reported
Guidelines followed in study	No guideline statement, but in general accordance with OECD 409 (1981)
Deviations from current test guideline (OECD 409, 1998; OECD 452, 2018)	Blood chloride and urine volume were not measured, unclear if a middle section of the spinal cord was observed microscopically. Deviations from the current version of OECD 409 (1998) are basically due to the fact that the study was aligned to an older version of the OECD test guideline 409.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

MON-0139 (aqueous solution of the isopropylamine salt of glyphosate) was administered orally by gelatin capsule to groups of six male and six female Beagle dogs at daily doses of 0, 10, 60 or 300 mg/kg bw/day for approximately six months.

Clinical observations were done twice daily. Weekly determinations of individual body weight and daily food consumption were performed. Ophthalmologic examinations were performed at pretest and prior to final necropsy on all animals. Haematological and blood biochemistry parameters were evaluated prior to start of treatment and monthly during treatment. Urinalysis was performed pre-test and during months 2, 4 and 6 of treatment. At the end of the scheduled dosing period, the animals were sacrificed and subjected to a full examination post mortem. Selected organs were weighed and specified tissues were taken for subsequent histopathology examination.

There were no mortalities in any of the dose groups. No unusual changes occurred in body weight, food consumption or clinical signs as a result of MON-0139 exposure. The only change which potentially related to MON-0139 administration was an elevation of serum alkaline phosphatase levels in males and females at all sampling intervals. There was no indication of the source of the increased levels of this enzyme as no microscopic evidence of lesions in the organs usually responsible for elevations in serum alkaline phosphatase levels were found. This, together with the generally small magnitude of these elevations and the lack of dose response in females makes the interpretation of this change equivocal as regards biological significance and correlation with MON-0139 administration. Changes in total LDH and LDH isoenzymes were identified but the occurrences were erratic. Total LDH levels were depressed in males especially from higher dosage levels at 4, 5 and 6 months of treatment primarily from decreases in LDH 5. Other changes in LDH isoenzymes included mild elevations of LDH 2 and LDH 3 in males and decreases of these isoenzymes in females. This lack of consistency in the direction of change between males and females and a lack of consistent response from one sampling period to another, along with the lack of correlation of

these changes with microscopic lesions or other serum chemistry changes, suggested an unlikely relationship to MON-0139 exposure. Other changes in serum chemical and changes in haematological and urinalysis parameters were isolated events and/or were within the range of normal values and were not considered related to administration of the test substance. *Post mortem*, there were no gross lesions, organ weight changes or microscopic findings that were considered associated with administration of MON-0139.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	MON-0139 (aqueous solution of the isopropylamine salt of glyphosate)
Description:	Amber liquid
Lot/Batch #:	LURT-12011
Purity:	62.49 % for isopropylamine salt of glyphosate
Stability of test compound:	Shown stable during the dosing period

2. Vehicle and/or positive control: Empty gelatin capsule

3. Test animals:

Species:	Dog
Strain:	Beagle
Source:	[redacted]
Age:	Approx. 6 months
Sex:	Male and female
Weight at dosing:	♂ 6.3 – 10.6 kg; ♀ 5.4 – 8.1 kg
Acclimation period:	Approx. 6 weeks
Diet/Food:	Purina Certified Dog Chow® 5007 limited to a 2 – 3 hour period
Water:	Tap water, *ad libitum*
Housing:	Individually in stainless steel dog cages
Environmental conditions:	Temperature: 20 – 22 °C (68 – 72 °F) Humidity: Not reported Air changes: Not reported, assume standard 12 hours light/dark cycle

B: Study design and methods

In life dates: 1982-01-26 to 1982-08-13

Animal assignment and treatment:
Six male and six female dogs per dose level received MON-0139 orally, by gelatin capsule administration, once daily for approximately 6 consecutive months. The liquid MON-0139 was placed into empty gelatin capsules (size 1/8 oz.). Doses were adjusted weekly to correspond with each animal's body weight. Capsules were prepared daily. Each dog received one capsule approximately one to four hours after removing its food each day. Control animals were each administered one empty gelatin capsule daily. Animals were acclimated to the laboratory for approximately 6 weeks and passed a veterinary health check prior to

assignment to the study. Animals were individually housed in stainless steel dog cages.
Analyses after the completion of the study indicated 63.2 % MON-0139 compared to an assay (conducted prior to the study) provided by the sponsor of 62.49 % MON-0139. This +0.7 % variation of analyses was within normal limits and no decomposition of MON-0139 were demonstrated.

Table 5.3.2-102: Six Month Study of Mon 0139 by Gelatin Capsule to Beagle Dogs ([redacted] 1983): Study design

Test group	Dose Level [mg/kg bw/day]	Males	Females
Control	0	6	6
Low	10	6	6
Mid	60	6	6
High	300	6	6

Mortality
Each animal was checked for mortality or signs of morbidity at least twice daily during the treatment period.

Clinical observations
A check for clinical signs of toxicity was made at least twice daily (morning and afternoon) on all animals.

Body weight
Weekly determinations of individual body weight were performed.

Food consumption and utilisation
Daily determinations of food consumption were performed.

Ophthalmoscopic examination
Ophthalmologic examinations were performed at pretest and prior to final necropsy on all animals.

Haematology and clinical chemistry
Laboratory investigations of haematology and clinical chemistry were performed after overnight fasting on all the dogs before dosing started and approximately monthly during the treatment period. The blood samples were taken from the jugular vein.

EDTA was used as an anti-coagulant for evaluation of all haematology parameters with the exception of prothrombin time for which citrate was used. The following haematological parameters were measured: Haemoglobin, haematocrit, red blood cell count, mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), reticulocyte count, total white blood cell and differential counts, platelet count and prothrombin time.

For clinical chemistry evaluations, serum was harvested from whole blood and analysed for the following parameters: Blood urea nitrogen, glucose, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, lactic dehydrogenase (total and isozyme determination), sodium, potassium, calcium, creatinine, total protein, albumin, globulin (calculated), cholesterol, phosphorus, direct and total bilirubin.

Urinalysis
Urinalysis was performed on all the dogs before dosing started and again during months 2, 4, and 6 of treatment. Urine was collected using metabolism cages. The following parameters were measured: Appearance, pH, specific gravity, proteins, glucose, ketones, blood pigments, bilirubin and urobilinogen. Microscopic examination of the spun urine deposit was performed.

Sacrifice and pathology
After 6 months of consecutive treatment, all surviving animals were fasted overnight, sacrificed by

intravenous sodium pentobarbital followed by exsanguination and subjected to a gross pathological examination. Terminal body weights were recorded immediately prior to sacrifice.

The following organ weights were determined: Adrenals, thyroids with parathyroids, pituitary, brain, heart, liver, kidneys, testes with epididymides and ovaries.

Tissue samples were taken from the following organs and preserved in buffered formalin: All gross lesions, adrenals, aorta, brain (cerebrum, cerebellum, medulla), caecum, colon, duodenum, epididymides, eyes, heart, ileum, jejunum, kidneys, liver, gallbladder, lungs, lymph nodes (mesenteric), mammary gland, oesophagus, ovaries, pancreas, pituitary gland, prostrate, rib with marrow, salivary gland (mandibular), spinal cord (cervical, lumbar), sciatic nerve, skeletal muscle, skin, spleen, stomach, testes, thymus, thyroid/parathyroids, trachea, urinary bladder, and uterus. All tissues above from the control and high dose groups were examined histopathologically. Only tissues with gross lesions were microscopically observed from the mid and low dose groups as no target organs were identified in the high dose animals.

Statistics
Non-categorical data from haematological, serum chemical and urinalysis were statistically examined by Dunnett's test for the comparison of multiple treatments with a control, and/or by inspection. Categorical data were examined to determine any remarkable group differences. Statistical evaluation of differences in body weights, food consumptions, terminal body weights and absolute organ weights between treated and control groups was accomplished by the use of Dunnett's test. The Mann-Whitney test with Bonferroni's Inequality was used to assess the organ/body weight ratios. A comparison of the frequency of microscopic lesions between treated groups and controls was evaluated by the use of Fisher's Exact Test with Bonferroni's Inequality.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities.

B. CLINICAL OBSERVATIONS
No unusual clinical signs were noted that could be attributed to MON-0139 exposure.

C. BODY WEIGHT
No significant body weight changes occurred between any of the dose groups.

D. FOOD CONSUMPTION AND TEST SUBSTANCE INTAKE
There were no treatment-related effects. Food consumption in treatment groups was comparable to controls. Test compound was administered by gelatine capsule with dosages adjusted according to individual animal weight. Determination of the degree of absorption of the test item following dosing was not performed.

E. OPHTHALMOSCOPIC EXAMINATION
There were no test substance-related ophthalmological findings at the end of the treatment period.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY
Haematology
Statistically significant changes were observed in several haematological parameters. However, the only parameter in which changes occurred in the same sex at several different sampling periods was mean cell haemoglobin concentration (MCHC).

Table 5.3.2-103: Six Month Study of Mon 0139 by Gelatin Capsule to Beagle Dogs ([redacted] 1983): Intergroup comparison of MCHC at selected time periods (group means)

Parameter	Dose Level [mg/kg bw/day]							
	Males				Females			
	0	10	60	300	0	10	60	300
MCHC [g/dL]								
Pre-test[a]	34.3	-	-	-	34.3	-	-	-
1 month	33.6	↑**34.2***	↑**34.2***	↑**34.2***	33.8	↑**34.2***	↑**34.3****	33.8
3 months	33.3	↑33.8	↓33.2	↑33.6	33.4	↑33.7	↓33.1	33.4
6 months	31.9	↑**32.5****	↑**32.6****	↑**32.8****	32.1	↑32.4	↑**32.6***	↑**32.7****

[a] Mean for all animals on study by sex;
* Statistically significant from controls ($p < 0.05$);
** Statistically significant from controls ($p < 0.01$)

Blood clinical chemistry

Statistically significant changes were observed in several serum chemistry parameters. The only parameters in which changes occurred in the same sex at several different sampling periods were total LDH and LDH isoenzymes.

Alkaline phosphatase (ALP) levels were generally slightly elevated in both males and females at all sampling periods, although it was statistically significant only in males from Group 3 at 5 months of treatment. This change in males was most evident at the highest dosage level while in females it was usually present among all treated groups often with no dose response. This effect was considered as possibly related to administration of the test substance. Changes in other clinical chemistry parameters had no biological significance and were apparently unrelated to compound administration.

Table 5.3.2-104: Six Month Study of Mon 0139 by Gelatin Capsule to Beagle Dogs ([redacted], 1983): Intergroup comparison of specific clinical chemistry parameters at selected time periods (group means)

Parameter	Dose Level [mg/kg bw/day]							
	Males				Females			
	0	10	60	300	0	10	60	300
LDH (total) [IU/L]								
Pre-test[a]	107	-	-	-	147	-	-	-
1 month	168	↑183	↓134	↓125	103	↑**183****	↑121	↓80.8
3 months	70.3	↓**42.8****	↓61.3	↓62.5	83.6	↓80.1	↑89.5	↓53.7
6 months	76.8	↓57.6	↓**45.0****	↓**44.5****	32.9	↑40.4	↑43.3	↑40.3
LDH 1 [%]								
Pre-test[a]	24	-	-	-	17	-	-	-
1 month	15	↓**7****	15	15	22	↓17	22	↑23
3 months	29.3	↓26.3	↓20.3	↑31.0	24.8	↑30.7	↑26.7	↑31.5
6 months	42.3	↓38.2	↓33.5	↓41.2	36.0	↑41.0	↑39.5	↓32.8
LDH 2 [%]								
Pre-test[a]	24	-	-	-	23	-	-	-
1 month	18	↓15	18	↓17	22	↓**15****	↓18	↑23
3 months	18.0	↑**22.3***	↑**22.7***	↓15.8	24.0	↓**17.2****	↓**17.2****	↑24.2
6 months	16.0	↑19.8	↑**22.3***	↑**21.7***	23.0	↓20.5	↑23.8	↓23.3
LDH 3 [%]								
Pre-test[a]	26	-	-	-	30	-	-	-
1 month	23	↓20	↑**30***	↑26	27	↓**23***	↓**23***	↓26
3 months	25.2	↑**30.7***	↑27.7	↓20.5	30.2	↓**21.3****	↓**23.0***	↓**21.3****
6 months	22.7	↑22.8	↑27.8	↑24.0	30.0	↓27.0	↓26.8	↑31.7

Table 5.3.2-104: Six Month Study of Mon 0139 by Gelatin Capsule to Beagle Dogs ([redacted] 1983): Intergroup comparison of specific clinical chemistry parameters at selected time periods (group means)

Parameter	Dose Level [mg/kg bw/day]							
	Males				Females			
	0	10	60	300	0	10	60	300
LDH 4 [%]								
Pre-test[a]	15	-	-	-	17	-	-	-
1 month	21	21	↓20	↑22	17	↑20	↑19	↓**14***
3 months	13.8	↓12.8	↓13.2	↓13.7	13.7	↓12.8	↑15.2	↓11.2
6 months	12.2	↑13.0	↑12.7	↓9.3	9.7	↓7.5	↓8.7	↑10.1
LDH 5 [%]								
Pre-test[a]	11	-	-	-	13	-	-	-
1 month	23	↑**38****	↓17	↓20	12	↑**26****	↑19	↑**14***
3 months	13.7	↓9.2	↑16.2	↑19.0	7.3	↑**18.0****	↑**18.0****	↑11.8
6 months	7.5	↓6.2	↓3.7	↓3.8	1.7	↑4.0	↓1.3	↓1.0
ALP [IU/L]								
Pre-test[a]	383	-	-	-	351	-	-	-
1 month	345	↓343	↑347	↑382	273	↑295	↑330	↑302
3 months	238	↑245	↑251	↑285	246	↑289	↑300	↑263
5 months	146	↑151	↑156	↑**205****	152	↑235	↑233	↑207
6 months	137	↑169	↑157	↑177	152	↑205	↑191	↑210

[a] Mean for all animals on study by sex;
* Statistically significant from controls ($p < 0.05$);
** Statistically significant from controls ($p < 0.01$)

G. URINALYSIS

Statistically significant changes were observed in several urinalysis parameters. However, there were no parameters in which changes occurred in the same sex at several different sampling periods.

H. NECROPSY

Organ weights

The only mean absolute organ weight value that was statistically different from the control value was a decreased mean thyroid gland weight in males from the intermediate dosage group. This change was not present in the highest dosage group, therefore, it was not considered to have resulted from MON-0139 administration. The mean right testicular weight of the highest dosage group was increased (not statistically significant) due to an increase in the recorded testicular weight of one animal. There were, however, no gross or microscopic lesions recorded for the right testis of that animal. The only changes in organ to body weight ratios were increases in heart and thyroid weights relative to terminal body weights. The increased relative heart weights occurred in females from the intermediate dosage level and the increased relative thyroid weights occurred in females from the lowest dosage level, both of which resulted from non-significant increases in the absolute organ weights that were apparently unrelated to chemical administration.

Gross pathology

There were no gross lesions that were associated with administration of MON-0139.

Histopathology

There were no microscopic lesions that were associated with administration of MON-0139.

III. CONCLUSIONS

There were no mortalities in any of the dose groups. No unusual changes occurred in body weight, food

consumption or clinical signs as a result of MON-0139 exposure. The only change which potentially related to MON-0139 administration was an elevation of serum alkaline phosphatase levels in males and females at all sampling intervals. There was no indication of the source of the increased levels of this enzyme as no microscopic evidence of lesions in the organs usually responsible for elevations in serum alkaline phosphatase levels were found. This, together with the generally small magnitude of these elevations and the lack of dose response in females makes the interpretation of this change equivocal as regards biological significance and correlation with MON-0139 administration. Other changes in serum chemical and changes in haematological and urinalysis parameters were isolated events and/or were within the range of normal values and were not considered related to administration of the test substance. *Post mortem*, there were no gross lesions, organ weight changes or microscopic findings that were considered associated with administration of MON-0139.

3. Assessment and conclusion

Assessment and conclusion by applicant:
In this study, the test item MON-0139 (aqueous solution of the isopropylamine salt of glyphosate) was administered orally by gelatin capsule to groups of six male and six female Beagle dogs at daily doses of 0, 10, 60 or 300 mg/kg bw/day for approximately six months according to a testing regime similar to OECD 409 (1981) and in compliance with GLP (no certificate of the competent authority was provided).

All animals survived until termination of the study. There were no clinical signs of toxicity. There were no effects on body weight, food consumption, ophthalmoscopy, haematology, urinalysis, gross postmortem findings, organ weights or microscopic pathology considered biologically adverse or related to administration of MON-0139. An apparent increase in alkaline phosphatase activity potentially associated with treatment was observed in top dose males from the second measurement onwards reaching statistical significance in month five only. Changes in total LDH and LDH isoenzymes were identified but the occurrences were erratic. No other clinical chemistry parameters were considered adversely affected. As the increase in alkaline phosphatase activity was not accompanied by any other indication of organ or tissue damage, the highest dose of 300 mg/kg bw/day can be considered the NOAEL.

Assessment and conclusion by RMS:
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1. Information on the study

Data point	CA 5.3.2/030
Report author	[redacted]
Report year	1981 (Hungarian report, Revised and English version 1991)
Report title	3 Month Oral Dietary Toxicity Study with Glyphosate in Dogs
Report No	8011
Document No	Not reported
Guidelines followed in study	None indicated, study preceded OECD 409 guideline, but the study design was similar to OECD 409 (1981)
Deviations from current test guideline (OECD 409, 1998)	Not all required haematological, clinical chemistry, urinalysis parameters were evaluated; some organs were not weighed or microscopically examined. Formulated diets were not analysed for concentration, homogeneity or stability. Purity of the test substance not stated in the revised report since the respective supplement to the original report was missing to the author of the revised report.

	Deviations from the current version of OECD 409 (1998) are basically due to the fact that the study was aligned to an older version of the OECD test guideline 409.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory when study was performed
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

Executive Summary

This is a revised and English report version of the 1981 Hungarian report. The revision was regarded as necessary by the study sponsor in order to eliminate slight deficiencies and to analyse more deeply the obtained experimental data. For this revision, none of the original data of the study were modified or omitted according to the best of the revision author's knowledge.

Glyphosate (purity and manufacturer not stated since the respective supplement as mentioned in the original study report was not included in the revised report) was administered to groups of four male and four female Beagle dogs for three months via food at target dietary concentrations of 0, 200, 600 or 2000 ppm (equivalent to 0, 9.08, 24.92 or 77.43 mg/kg bw/day).

All animals survived the dosing period and there were no clinical signs of toxicity. Body weight and food consumption were overall not affected. Haematology, clinical chemistry, urinalysis and gross pathological examination did not reveal adverse effects. Liver weight (values not reported in the revised report) was marginally lower at the highest dose level (2000 ppm). In two high dose males and in all high dose females, a histopathological feature called "indistinct structure" was described. This was also seen at the mid dose (600 ppm) level in a smaller number of dogs (2 males and one female). This change was characterised by round shaped and enlarged hepatocytes and occasionally also by the narrowing of some of the hepatocytic trabeculae and slight dissociation of the liver structure. In addition, congestion of the liver was noted in three males and all female dogs in the highest dose group.

II. MATERIALS AND METHODS

A: Materials

1. **Test material:** Glyphosate
   - Identification: 14/980
   - Description: Not reported
   - Lot/Batch #: 03090380
   - Purity: Purity not stated since the respective supplement mentioned in the original study report was not provided in the revised report
   - Stability of test compound: Not reported
2. **Vehicle and/or positive control:** Plain diet / none
3. **Test animals:**
   - Species: Dog
   - Strain: Beagle

Source:	[redacted]
Age:	9 – 11 months
Sex:	Male and female
Weight at dosing:	Body weight data at test start not included in revised report
Acclimation period:	3 weeks
Diet/Food:	Composition not provided in the revised report.
Water:	Tap water, *ad libitum*
Housing:	Individually in kennels (1 square meter)
Environmental conditions:	Temperature: 18 ± 2 °C Humidity: 50 ± 5 % Air changes: 5-7 /hour Light/dark cycle: Not reported

B: Study design and methods

In life dates: 1980-11-24 to 1981-02-26

Animal assignment and treatment:
The test material was offered daily in diet to groups of 4 males and 4 females Beagle dogs for 13 weeks. Target dietary concentrations were 0, 200, 600 or 2000 ppm (equivalent to 0, 9.08, 24.92 or 77.43 mg/kg bw/day). Food was mixed with appropriate amounts of test substance one week before the start of the study and monthly thereafter. Food was provided to the animals for 3 hours each day between 0900 and 1200 hours and unconsumed food was weighed and food consumption calculated weekly. The report does not indicate that formulated diet was analysed for concentration, homogeneity or stability. The study design is summarised in the table below:

Table 5.3.2-105: 3 Month Oral Dietary Toxicity Study with Glyphosate in Dogs ([redacted], 1981): Study design

Test group	Dietary concentration [ppm]	Males	Females
Control	0	4	4
Low	200	4	4
Mid	600	4	4
High	2000	4	4

Mortality
Animals were observed daily.

Clinical observations
Animals were observed daily.

Body weight
Body weight measurement was done weekly.

Food consumption and utilisation
Each day, unconsumed food was weighed and food consumption calculated weekly. Chemical intake [mg/kg bw/day] was calculated weekly from food consumption and body weight data and the nominal dose level.

Ophthalmoscopic examination
Not performed.

Haematology and clinical chemistry
Blood samples were collected from the antebrachial vein prior to initiation of treatment and on study days 42 and 85±2 days. The following haematology parameters were measured: haematocrit, haemoglobin, erythrocyte count, and total and differential leukocyte counts.

The following clinical chemistry parameters were measured: Alkaline phosphatase, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, creatinine, blood urea nitrogen, total protein, albumin, globulin, albumin/globulin ratio, glucose, bilirubin, potassium and sodium.

Urinalysis
Urine was collected using metabolic cages at termination of the dosing period. The following urine parameters were evaluated: specific gravity, pH, protein, glucose and microscopic examination of sediment was performed.

Sacrifice and pathology
After 13 weeks of treatment, all animals were subjected to a complete necropsy and all gross findings were recorded. Animals were anaesthetised with phenobarbital and euthanised by exsanguinations from the carotid artery before necropsy. At necropsy, the organs and tissues were removed and preserved in 8 % formalin.

Weights of the following organs were recorded for all animals and the ratios to the final body weight were calculated: Brain, pituitary, heart, adrenals, thyroids, liver, gonads, and kidneys.

The following organs and tissues from all animals were examined microscopically: Brain (cerebrum, cerebellum pons), spinal cord, peripheral nerve, pituitary, thyroids, adrenals, spleen, lymph nodes (cervical, mesenteric), heart, aorta (thoracic, abdominal), salivary gland (submandibular), oesophagus, stomach (cardiac, fundus, pylorus), liver, gall bladder, pancreas, duodenum, jejunum, ileum, large intestine, trachea, lungs, kidneys, urinary bladder, testes, prostate, ovaries, uterus, eyes, femoral muscle, and all gross lesions.

Statistics
Differences between the control and treated groups were analysed using Student's two samples t-test.

II. RESULTS AND DISCUSSION

A. MORTALITY
All animals survived the study.

B. CLINICAL OBSERVATIONS
No signs of toxicity were observed during the study.

C. BODY WEIGHT
There were no statistically or biologically significant differences in weight variations occurring during the study between control and glyphosate treated animals. The supplement containing the data was not included in the revised study report.

D. FOOD CONSUMPTION AND TEST SUBSTANCE INTAKE
There was an isolated reduction in food consumption in some of the high dose (2000 ppm) group animals at or around study week 9 primarily associated with 3 dogs. Reduced food intake occurred at other times during the study similarly in controls and treated dogs but these reductions were no longer observed by the end of the study period. However, food intake was reduced in all study groups at the end of the dosing period causing a moderately lower substance intake. The supplement containing the food consumption data

was not included in the revised study report.

The overall group mean chemical intakes [mg/kg bw/day] over the whole treatment period were calculated from food consumption, body weights and the nominal dose levels. The results are shown in the table below:

Table 5.3.2-106: 3 Month Oral Dietary Toxicity Study with Glyphosate in Dogs ([redacted], 1981): Summary of mean group compound uptake, selected weeks [mg/kg bw/day]

Study Week	Dose levels for both sexes combined [mg/kg bw/day]			
	Dose group [ppm]			
	0	200	600	2000
1	-	9.07	25.9	83.85
3	-	10.54	25.86	91.88
6	-	10.95	24.73	88.90
9	-	9.86	28.31	68.41
10	-	8.14	25.78	64.54
11	-	6.26	21.98	71.49
12	-	7.28	19.66	50.93
13	-	4.58	15.18	44.73
Weeks 1 – 13	-	9.08	24.92	77.43

E. OPHTHALMOSCOPIC EXAMINATION

Not performed.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

There were no significant differences in haematology values in measured parameters between control and treated dogs during the study. The supplement containing the data was not included in the revised study report.

Blood clinical chemistry

There were no significant differences in clinical chemistry values in measured parameters between control and treated dogs during the study. The supplement containing the data was not included in the revised study report.

G. URINALYSIS

There were no significant differences in clinical chemistry values in measured parameters between control and treated dogs during the study. The supplement containing the data was not included in the revised study report.

H. NECROPSY

Organ weights

Marginally lower absolute liver weights resulted in lower relative liver weights for high dose animals. There were no differences in weights of the other organs between controls and treated animals. The supplement containing the data was not included in the revised study report.

Gross pathology

The revised report indicates that the findings at necropsy were rare, slight and nonspecific to treatment with glyphosate with no differences observed between control and treated groups. The report indicates a "weaker general condition of the animals at necropsy" that did not correlate with the clinical laboratory and pathology findings. One 600 ppm group female and one 2000 ppm group female were graded as in poor general condition at necropsy.

Histopathology
No findings considered indicative of severe toxicity were observed in any of the organs examined. Alterations considered mild were found only in the livers of some higher dose group dogs that were described as "indistinct structure" that did "not transfer to degeneration and was regarded as fully reversible". The revised report presents a histopathology report indicated to be prepared from the primary histopathology findings from 1981. The changes observed in the livers of the high dose group dogs (2000 ppm) were described in the revised report as round shaped, enlarged hepatocytes and occasionally narrowing of some of the hepatocytic trabeculae and slight dissociation of the structure. Non-pathologic congestions seen the liver of one lower dose animal was more pronounced in the animals with "indistinct liver structure". The abnormal presence or localisation of connective tissue or fibrosis were not observed. No other changes observed were considered attributable to treatment with glyphosate. It was concluded that treatment-related findings occurred only in the liver of the high dose group animals in the form of increased congestion and "indistinct structure". These liver lesions were considered functional and reversible. No other findings were considered of toxicological or pathological significance.

Table 5.3.2-107: 3 Month Oral Dietary Toxicity Study with Glyphosate in Dogs ([redacted], 1981): Summary incidence of liver histopathological findings

Finding	Dietary Concentration [ppm]							
	Males				Females			
	0	200	600	2000	0	200	600	2000
Focal subacute inflammation	4/4	1/4	2/4	3/4	4/4	2/4	1/4	3/4
Congestion	0/4	1/4	0/4	3/4	0/4	0/4	0/4	4/4
Indistinct structure	0/4	0/4	2/4	2/4	0/4	0/4	1/4	4/4

III. CONCLUSIONS

All animals survived the dosing period and there were no clinical signs of toxicity. Body weight and food consumption were overall not affected. Haematology, clinical chemistry, urinalysis and gross pathological examination did not reveal adverse effects. Liver weight (values not reported in the revised report) was marginally lower at the highest dose level (2000 ppm). In two high dose males and in all high dose females, a histopathological feature called "indistinct structure" was described. This was also seen at the mid dose (600 ppm) level in a smaller number of dogs (2 males and one female). This change was characterised by round shaped and enlarged hepatocytes and occasionally also by the narrowing of some of the hepatocytic trabeculae and slight dissociation of the liver structure. In addition, congestion of the liver was noted in three males and all female dogs in the highest dose group.
None of the parameters recorded or measured revealed treatment related changes, except histopathology, where more or less functional type morphological changes were described in the livers of some dogs of the highest dose group.

3. Assessment and conclusion

Assessment and conclusion by applicant:
Groups of four male and four female Beagle dogs were treated for three months with glyphosate via their food at target dietary concentrations of 0, 200, 600 or 2000 ppm (equivalent to 0, 9.08, 24.92 or 77.43 mg/kg bw/day). All animals survived the dosing period and there were no clinical signs of toxicity. Body weight and food consumption were not affected. Haematology, clinical chemistry, urinalysis and gross pathological examination did not reveal adverse effects. Liver weight (value not reported in the revised

report) was marginally lower at the highest dose level (2000 ppm). In two high dose males and in all high dose females, a histopathological feature called "indistinct structure" was described. This was also seen at the mid dose (600 ppm) level in a smaller number of dogs (2 males and one female). This change was characterised by round shaped and enlarged hepatocytes and occasionally also by the narrowing of some of the hepatocytic trabeculae and slight dissociation of the liver structure. In addition, congestion of the liver was noted in three male and all female dogs in the highest dose group. The toxicological significance of these findings is equivocal. Based on these results, an average daily glyphosate intake of approximately 25 mg/kg bw/day calculated from the 600 ppm mid-dose target concentration can be considered a NOAEL for dogs under the conditions of this study.

Assessment and conclusion by RMS:
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Oral 1 year toxicity (dog)

Table 5.3.2-108 summarises the oral 1-year studies performed in dogs previously assessed in the 2001 and 2015 EU glyphosate evaluations.

Table 5.3.2-108: Studies on oral 1-year repeated-dose toxicity with glyphosate in non-rodents

Annex Point	Study	Species Study type	Substance(s)	Reference list-related category§	Result
CA 5.3.2/031	[redacted], 2007	Beagle dog, 12-month, oral capsule (0, 30, 125, 500 mg/kg bw/day)	Glyphosate technical (Purity: 95.7 %)	Valid, Category 2a	NO(A)EL = 500 mg/kg bw/day
CA 5.3.2/032	[redacted], 1997	Beagle dog 12-month, oral diet (0, 1600, 8000, 50000 ppm)	Glyphosate technical (Purity: 94.61 %)	Valid, Category 2a	NOAEL = 8000 ppm (equivalent to 182 mg/kg bw/day for males and 184 mg/kg bw/day for females)
CA 5.3.2/033 CA 5.3.2/034	[redacted], 1996 (Study Report) [redacted], 1996 (Appendix)	Beagle dog 12-month, oral diet (0, 3000, 15000, 30000 ppm)	Glyphosate acid (Purity: 95.6 %)	Valid, Category 2a	NOAEL = 15000 ppm (equivalent to 447 mg/kg bw/day for females) and 30000 ppm (equivalent to 906 mg/kg bw/day for males)
CA 5.3.2/035	[redacted], 1990	Beagle dog 12-month, oral capsule (0, 30, 300, 1000 mg/kg bw/day)	Glyphosate technical (Purity: 98.6 - 99.5 %)	Valid, Category 2a	NOAEL = 300 mg/kg bw/day
CA 5.3.2/036	[redacted], 1985	Beagle dog 12-month, oral capsule (0, 20, 100, 500 mg/kg bw/day)	Glyphosate (Purity: 96.17 %)	Valid, Category 2a	NOAEL = 500 mg/kg bw/day

Table 5.3.2-108: Studies on oral 1-year repeated-dose toxicity with glyphosate in non-rodents

Annex Point	Study	Species Study type	Substance(s)	Reference list-related category§	Result
CA 5.3.2/037	[redacted], 1982 (Revised and English version of Hungarian report 1992)	Beagle dog 12-month, oral diet (0, 30, 100, 300 ppm)	Glyphosate (Purity: not reported)	Invalid, Category 4b	NOAEL = 300 ppm (equivalent to approx. 8 mg/kg bw/day for the sexes combined)

§: The category describes the acceptability/reliability of the study within the AIR 5 submission. This category does not describe hazard classification. (for details please refer to the Doc ID: 110054-B-GRG_Jun_2020)

There are five valid 1-year studies available in which Beagle dogs were administered glyphosate in the diet or via capsule ([redacted], 2007; [redacted], 1997; [redacted], 1996; [redacted], 1990; [redacted], 1985). These studies were previously assessed in the 2001 and 2015 EU glyphosate evaluations.

In all of these studies only non-specific signs of toxicity (slight effect body weight and an increase in clinical signs of soft, liquid stools) were observed at or close to limit dose (1000 mg/kg bw/day). These effects were characterised as reduction in body weight gain, reduction in urinary pH and minor effects on clinical pathology parameters. An increase in focal pneumonia and focal granulomatous pneumonia in the lung in all females dosed at 1259 mg/kg bw/day was observed in the study by [redacted] (1997). In the other female groups, this lesion was observed in only one out of four dogs each. However, the extent of this lesion was very focal and the degree of intensity was slight in all cases. Statistically, no significant differences between control and dose groups were found in the incidence of this lesion. In this study no treatment-related effects were observed.

Overall, the lowest dose level where treatment-related effects were observed was 926 mg/kg bw/day in female dogs in the study by [redacted] (1996). The lowest one-year oral dog NOAEL for glyphosate technical is 182 mg/kg bw/day for males and 184 mg/kg bw/day for females ([redacted] 1997). But since in this study the dose levels were set at wide intervals, this rather low NOAEL seems to be an artefact of dose selection. The most relevant one-year oral dog NOAEL for glyphosate technical is 500 mg/kg bw/day ([redacted], 2007; [redacted], 1985).

The additional study by [redacted] (1992; revised and English version of 1982 hungarian report) was considered invalid due to serious reporting deficiencies.

1. Information on the study

Data point	CA 5.3.2/031
Report author	[redacted]
Report year	2007
Report title	Glyphosate technical: 52-week Toxicity Study by Oral Route (Capsule) in Beagle Dogs
Report No	29647 TCC
Document No	Not reported
Guidelines followed in study	OECD 452 (1981); JMAFF 2-1-14 (2001)
Deviations from current test guideline (OECD 452, 2018)	None
Previous evaluation	Yes, accepted in RAR (2015)

GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary
The toxicity potential of glyphosate technical was assessed in a 1-year oral toxicity study in male and female Beagle dogs. Groups of four dogs per sex received daily doses (capsules) of 0, 30, 125, or 500 mg/kg bw/day for 52 consecutive weeks (dose level selection was based on the results of a 13-week study run in the same laboratory). Observations covered mortality, clinical signs, body weight, food consumption, ophthalmological examinations, haematology, clinical chemistry, urine analysis, organ weights, necropsy and histopathological examination.

No unscheduled deaths or premature sacrifices occurred during the study. There were no treatment-related effects on clinical signs, eyes, body weight, body weight gain, food consumption, haematology, clinical chemistry or urine analysis parameters in both sexes. Gross pathology, organ weight data and histopathological examination demonstrated no treatment-related effects.

I. MATERIALS AND METHODS

A: Materials

1. Test material: Glyphosate technical

Identification:	Glyphosate tech
Description:	White crystalline powder
Lot/Batch #:	H05H016A
Purity:	95.7 %
Stability of test compound:	Expiry date: 2008-03-25

2. Vehicle and/ or positive control: Gelatine capsules size 12 (Torpac, New York, USA) / none

3. Test animals:

Species:	Dog
Strain:	Beagle
Source:	[redacted]
Age:	Approx. 6 months
Sex:	Males and females
Weight at dosing:	7.8 – 8.9 kg (males); 7.2 – 7.9 kg (females)
Acclimation period:	13 days + 20 days pre-treatment period
Diet/Food:	125 C3 pelleted diet (SAFE, Villemoisson, Epinay-sur-Orge, France), approx. 300 g per day. Due to weight loss in three animals the amount for these dogs was increased to 350 g/day from day 149, 180, and 185, respectively. From day 191 onwards all animals received 350 g/day. One male received 400 g from day 221 onwards
Water:	Tap water, *ad libitum*

Housing:	Individually in pens containing wood shavings for bedding, except when a urine sample was required. The dogs were group-housed once a week, by sex and dose group, after the last recording of clinical signs in the afternoon, until the next morning
Environmental conditions:	Temperature: 20 ± 5 °C Humidity: 50 ± 20 % Air changes: approx. 12 / hour 12 hours light/dark cycle

B: Study design and methods

In life dates: 2005-09-27 to 2006-10-17

Animal assignment and treatment:
In a chronic oral toxicity study groups of four Beagle dogs per sex received daily doses of 0, 30, 125 or 500 mg/kg bw/day glyphosate technical in gelatine capsules for 52 consecutive weeks. The dose levels were selected based on results of a 13-week oral (capsule) toxicity study in dogs. Dose formulations were prepared weekly by adding the required amount to the capsules. The dosages were calculated based on minimum nominal active substance content of 950 g/kg glyphosate in the test item. Analyses of the test item showed a glyphosate content consistently above 95 %. Thus, no adjustment was considered necessary. Since the test item was added under GLP conditions, no additional analyses of dose formulations were deemed necessary.

Administrations of dose capsules were done approximately the same daily time each day. The low and mid-dose animals received one capsule per day, the high-dose and control dogs received three capsules per day. The quantity of dosage form applied to each animal was adjusted weekly based on the most recently recorded body weight.

Clinical observations
Observations for morbidity, and mortality were made twice daily. A check for clinical signs of toxicity was made at least once daily on all animals. In addition, a detailed clinical examination was performed once before start of treatment and weekly thereafter until termination.

Body weight
Individual body weights were recorded three times before group allocation, on day 1 (prior to treatment) and weekly thereafter during the conduct of study and at termination.

Food consumption and compound intake
Food consumption of each animal was estimated daily by noting the difference between the amount provided and the remaining amount on the next morning. Food consumption was expressed as percentage of quantity provided. Whenever fasting was required, food was removed at the end of the day and estimation of food consumption was made at that time.

Ophthalmological examination
Ophthalmological examinations were performed on all dogs prior to start and at the end of the treatment period. Pupillary light and blink reflexes were evaluated first. Mydriasis was then induced by adding Tropicamide solution into the eyes and the appendages, optic media and fundus were examined by indirect ophthalmoscopy.

Haematology and clinical chemistry
Blood samples were collected from all dogs prior to treatment, in week 25 and at the end of the treatment period in week 51. For sampling dogs were fasted overnight for at least 14 hours. The following haematological parameters were examined: Haemoglobin concentration (HB), erythrocyte count (RBC),

mean cell volume (MCV), packed cell volume (PCV), mean cell haemoglobin concentration (MCHC), mean cell haemoglobin (MCH), thrombocytes (PLAT), leukocytes (WBC), differential white cell count with cell morphology, neutrophils (N), eosinophils (E), basophils (B), lymphocytes (L), monocytes ♂, reticulocytes (RETIC), prothrombin time (PT) and activated partial thromboplastin time (APTT). The following clinical chemistry parameters were examined: Alkaline phosphatase (ALP), alanine aminotransferase activity (ALAT), aspartate amino transferase (ASAT), albumin, albumin/globulin ratio, total bilirubin, glucose, urea, calcium, chloride, total cholesterol, creatinine, γ-glutamyl-transferase (GGT), inorganic phosphorus, total protein, sodium, potassium and triglycerides.

Urinanalysis
Individual urine samples were collected from all dogs prior to treatment, in week 25 and at the end of the treatment period in week 51. For sampling dogs were fasted overnight for at least 14 hours. Urine was collected in the presence of thymol crystals. The following examinations were made: Appearance, colour, specific gravity, pH, volume, proteins, glucose, ketones, bilirubin, nitrites, blood and urobilinogen. The sediment was examined microscopically for leukocytes, erythrocytes, cylinders, magnesium ammonium phosphate crystals, calcium phosphate crystals, calcium oxalate crystals and cells.

Sacrifice and pathology
All surviving dogs were killed after completion of 52 weeks treatment and were subjected to a gross pathological examination. The following organs were weighed: Adrenals, brain, epididymides, heart, kidneys, liver, spleen, thymus, uterus, pituitary, prostate, ovaries, testes and thyroids with parathyroid. Organ to body weight ratios were calculated.

Tissue samples were taken from the following organs of all dogs and preserved in 10 % buffered formalin (except for the eyes with the optic nerve which were fixed in Davidson´s fixative, and testes and epididymides which were preserved in Bouin´s fluid): Adrenals, aorta, brain, caecum, colon, duodenum, oesophagus, eyes and optic nerve, epididymides, femur with articulation, gall bladder, heart, ileum, jejunum, kidneys, larynx, liver, lungs with bronchi, mammary gland, mandibular lymph node, mesenteric lymph node, skeletal muscle, ovaries, oviducts, parathyroid, pancreas, pituitary, prostate, rectum, salivary glands (parotid and submandibular), skin, spinal cord (cervical, thoracic and lumbar), spleen, sternum with bone marrow, stomach, sciatic nerve, testes, thymus, thyroids with parathyroid, tongue, trachea, urinary bladder, ureters and uterus (horns and cervix).

A detailed histopathological examination was performed on all sampled tissues of all dogs, except for femur, larynx, oviducts, tongue, ureter and vagina.

Statistics
Statistical analysis of body weight, haematology, blood biochemistry, urinalysis and organ weight data was done according to the statistical decision tree shown in "*Guidance Notes for Analysis and Evaluation of Chronic Toxicity and Carcinogenicity Studies*" (OECD, 2002), summarising the most common statistical procedures used for analysis of data in toxicology studies, together with their most likely outcomes.

II. RESULTS AND DISCUSSION

A. MORTALITY
No mortalities or premature sacrifices occurred during the treatment period.

B. CLINICAL OBSERVATIONS
There were no treatment-related clinical signs observed during the study period.

Observed clinical signs consisted of vomiting or soft faeces, thin appearance, hyperactivity, ptyalism, skin findings (scabs and erythema, generally localized on the ear(s)) and nodules on the ears. These clinical observations were seen transiently, and were encountered with a similar incidence in both control and treated animals and/or were independent to the administered dose-level and/or were already present before

the beginning of the treatment period.

C. BODY WEIGHT

There was no treatment-related effect on body weight development. The lower mean body weight recorded in high dose males at the end of the treatment period was due to the lower mean body weight gain during the first month of the study (see Table 5.3.2-109**Error! Reference source not found.** below). Individual body weight changes were within the range of physiological variations. In addition, such body weight changes were observed in both control and treated dogs.

Table 5.3.2-109: Glyphosate technical: 52-week Toxicity Study by Oral Route (Capsule) in Beagle Dogs ([redacted] 2007): Mean body weight and body weight changes [kg]

Dose level [mg/kg bw/day]	Males				Females			
	0	30	125	500	0	30	125	500
Mean bw prior to start (day -1)	8.2	8.3	8.3	8.3	7.4	7.4	7.6	7.4
Weeks 1 – 4	+0.6	+0.3	+0.5	**+0.2***	+0.3	+0.3	+0.3	+0.3
Weeks 4 – 26	+1.4	+0.9	+1.4	+1.1	+1.2	+1.1	+1.5	+1.6
Weeks 26 – 52	+0.9	+1.4	+1.1	+0.8	+0.6	+0.2	+0.5	+1.1
Weeks 1 – 52/53	+2.8	+2.6	+2.9	+2.0	+2.1	+1.6	+2.3	+3.0
Mean bw in week 52/53	11.2	11.0	11.2	10.5	9.6	9.2	10.0	10.6

* Statistically significant from control ($p < 0.05$)

The weight loss of some dogs observed in the control, and low-dose group during some periods of the study were resolved when the daily food quantity was increased. Therefore, these changes were considered not test substance related.

D. FOOD CONSUMPTION

There was no treatment-related effect on food consumption noted during the study.

The reduced food consumptions noted during the study were not considered test substance related, since they occurred only on some occasions and in control and treated dogs.

Due to weight loss one male each of the low and mid dose group, and one control female received 350 g/day from day 149, 180 and 185, respectively. From day 191 onwards all animals received 350 g/day. One male received 400 g from day 221 onwards.

E. OPHTHALMOLOGY

There were no ophthalmological findings observed at the end of the study period.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

There were no treatment-related effects noted in the haematological parameters.

The differences observed for the activated partial thromboplastin time, MCHC and eosinophil counts in the treated animals when compared to control dogs were only slight and/or not dose-related.

Table 5.3.2-110: Glyphosate technical: 52-week Toxicity Study by Oral Route (Capsule) in Beagle Dogs ([redacted], 2007): Results of haematological examinations week 51 (mean ± SD)

Parameter	Dose level [mg/kg bw/day]							
	Males				Females			
	0	30	125	500	0	30	125	500
Mean corpuscular haemoglobin	35.4 ± 0.42	↓35.2 ± 0.84	↑35.5 ± 0.24	↓35.3 ± 0.19	36.0 ± 0.32	↓35.4 ± 0.56	↓35.5 ± 0.29	**↓35.0 ± 0.48***

Table 5.3.2-110: Glyphosate technical: 52-week Toxicity Study by Oral Route (Capsule) in Beagle Dogs ([redacted] 2007): Results of haematological examinations week 51 (mean ± SD)

Parameter	Dose level [mg/kg bw/day]							
	Males				Females			
	0	30	125	500	0	30	125	500
concentration (MCHC) [g/dL]								
Eosinophils [G/L]	0.27 ± 0.141	↑0.29 ± 0.087	↑0.33 ± 0.324	↑0.49 ± 0.275	0.37 ± 0.139	↓0.26 ± 0.127	↓0.30 ± 0.090	**↓0.19 ± 0.042***
Partial thromboplastin time [sec]	6.1 ± 0.05	6.1 ± 0.10	6.1 ± 0.14	6.1 ± 0.13	6.1 ± 0.10	6.1 ± 0.06	↓6.0 ± 0.05	↑6.2 ± 0.06

* Statistically significant from controls ($p < 0.05$);
** Statistically significant from controls ($p < 0.01$)

Clinical chemistry

There were no treatment-related effects noted in the clinical chemistry parameters.
The differences observed for the inorganic phosphorous, calcium, protein, glucose, albumin/globulin ratio and AP values in the treated animals when compared to control dogs were only slight and/or not dose-related.

Table 5.3.2-111: Glyphosate technical: 52-week Toxicity Study by Oral Route (Capsule) in Beagle Dogs ([redacted], 2007): Results of clinical chemistry examinations week 51 (mean ± SD)

Parameter	Dose level [mg/kg bw/day]							
	Males				Females			
	0	30	125	500	0	30	125	500
Inorganic phosphorus [mmol/L]	1.18 ± 0.174	↓1.06 ± 0.333	↑1.24 ± 0.196	↓1.04 ± 0.148	1.19 ± 0.105	↓1.06 ± 0.134	↓0.89 ± 0.146	↓1.01 ± 0.177
Calcium [mmol/L]	2.80 ± 0.128	↓2.68 ± 0.125	↓2.75 ± 0.055	**↓2.63 ± 0.022***	2.75 ± 0.033	↓2.66 ± 0.085	↑2.77 ± 0.108	↓2.67 ± 0.092
Protein [g/L]	67 ± 4.3	↓64 ± 3.0	↓63 ± 2.2	**↓60 ± 1.9****	62 ± 2.2	↓60 ± 2.1	↓61 ± 2.1	↑64 ± 2.5
Glucose [mmol/L]	5.16 ± 0.332	↑5.61 ± 0.377	↓4.55 ± 0.386	↑5.69 ± 0.465	5.09 ± 0.314	↑5.63 ± 0.660	↑5.74 ± 0.416	↑5.23 ± 0.409
Albumin/globulin ratio	1.21 ± 0.106	↑1.31 ± 0.090	↑1.30 ± 0.042	**↑1.39 ± 0.064***	1.40 ± 0.070	↑1.43 ± 0.048	↓1.38 ± 0.129	↓1.29 ± 0.088
Alkaline phosphatase [IU/L]	169 ± 79.5	↓167 ± 51.9	↓166 ± 47.9	↓119 ± 25.1	114 ± 29.7	↑212 ± 39.8	**↑230 ± 38.4***	↑177 ± 85.0

* Statistically significant from controls ($p < 0.05$);
** Statistically significant from controls ($p < 0.01$)

G. URINANALYSIS

There were no findings among the quantitative or semi-quantitative and qualitative parameters during the treatment period.

H. NECROPSY

Organ weights

The statistically significant lower brain weight (see table below Table 5.3.2-112) observed in males at 125 mg/kg bw/day was dose-independent. In addition, there were no macroscopic or histopathological findings noted in this organ. Thus, this finding is considered incidental.
There were no other statistically significant differences in organ weights and organ to body weight ratios between control and treated dogs.

Table 5.3.2-112: Glyphosate technical: 52-week Toxicity Study by Oral Route (Capsule) in Beagle Dogs ([redacted], 2007): Body/Brain weights of male dogs

Dose group [mg/kg bw/day]	0	30	125	500
No of animals	4	4	4	4
Mean final body weight [g]	11165.0	10830.0	11090.0	10255.0
Mean brain weight [g]	87.41	80.06	**73.96****	84.09
Mean % of body weight	0.78978	0.74484	0.67578	0.82550

** DUNNETT'S TEST based on pooled variances at 1 % level; assigned control group(s): 1.

Gross pathology
There were no test substance related macroscopic findings observed in any animal of all dose groups.

Histopathology
There were no test substance related microscopic findings observed in any tissue sample of any dose group.

III. CONCLUSIONS

No unscheduled deaths or premature sacrifices occurred during the study. There were no treatment-related effects on clinical signs, eyes, body weight, body weight gain, food consumption, haematology, clinical chemistry or urine analysis parameters in both sexes. Gross pathology, organ weight data and histopathological examination demonstrated no treatment-related effects.

The test item was clinically well tolerated and did not result in any laboratory or histological changes.
In conclusion under the experimental conditions of this study, the NOEL for oral toxicity of glyphosate technical was established at 500 mg/kg bw/day by the study authors.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The toxicity potential of glyphosate technical was assessed in a 1-year oral toxicity study in male and female Beagle dogs. Groups of four dogs per sex received daily doses (capsules) of 0, 30, 125, or 500 mg/kg bw/day for 52 consecutive weeks. The study was conducted according to OECD 452 (1981) and in compliance with GLP regulations

Glyphosate technical was well tolerated and did not produce any laboratory or histological effects in Beagle dogs when administered daily for 52 weeks by the oral route at all dose levels. Based on these results, the NO(A)EL in Beagle dogs after 1 year of oral exposure was 500 mg/kg bw/day.

Assessment and conclusion by RMS:
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1. Information on the study

Data point	CA 5.3.2/032
Report author	[redacted]
Report year	1997
Report title	HR-001: 12-Month Oral Chronic Toxicity Study in Dogs
Report No	[redacted] 94-0157
Document No	Not reported
Guidelines followed in study	Japan MAFF Guidelines 59 NohSan No.4200, 1985; U.S. EPA FIFRA Guidelines Subdivision F, 1984; OECD 409 (1981; in general compliance to OECD 452, 1981)
Deviations from current test guideline (OECD 409, 1998; OECD 452, 2018)	Blood clotting time parameters not evaluated; epididymis and uterus weights not reported. Deviations from the current versions of OECD 409 (1998) and OECD 452 (2018) are basically due to the fact that the study was aligned to older versions of the OECD test guidelines.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

An oral 12-month toxicity study of HR-001 (Glyphosate technical) was conducted in Beagle dogs of both sexes. Groups of 4 males and 4 females each were given the test substance by incorporating it into basal diet at a level of 0, 1600, 8000 or 50000 ppm (equivalent to 0, 34.1, 182 or 1203 mg/kg bw/day for males and 0, 37.1, 184 or 1259 mg/kg bw/day for females) for a period of 12 months. Animals were checked daily for general conditions. Body weights and food consumption were measured periodically. All animals were subjected to urinalysis at weeks 25 and 51 and to haematology and blood chemistry at weeks 26 and 52. Ophthalmological examinations were performed at week 52. At termination of treatment, animals were euthanised and subjected to organ weight analysis and necropsy. Histopathological examinations were performed on representative organs/tissues from all animal used.

Findings related to the treatment were demonstrated in clinical observation, body weight, urinalysis, haematology and blood chemistry.

50000 ppm group: Loose stool was observed in 3 of 4 males and 4 of 4 females. These animals frequently showed this clinical sign through the treatment period, whereas in the control group, only one animal in each sex showed the sign over a limited period during treatment. Body weight gain was retarded gradually with progression of the treatment in both sexes, when compared to the controls. Consequently, the difference in mean body weight between the 50000 ppm and control groups became great with time, although statistical significance was not observed. Haematologically, slight anaemic changes were noted for females at weeks 26 and 52. Females also showed significantly increased plasma level of chloride at week 26 and significantly decreased plasma levels of albumin and inorganic phosphorous at week 52. Significantly lowered urine pH values were continuously observed in males and females. However, this finding was not recognised as a toxic change since it is known that the test substance is secreted with little metabolism into urine, is degraded to a free acid in urine, and consequently, makes the urine acidic.

8000 and 1600 ppm groups: There were no treatment-related abnormalities in either sex.

I. MATERIALS AND METHODS

A: Materials

1. **Test material:** Glyphosate technical

Identification:	HR-001
Description:	White crystals
Lot/Batch #:	T-950308
Purity:	94.61 %
Stability of test compound:	Not mentioned in the report

2. **Vehicle and/or positive control:** None

3. **Test animals:**

Species:	Dog
Strain:	Beagle
Source:	[redacted]
Age:	5 months
Sex:	Males and females
Weight at dosing:	7.8 – 8.9 kg (males); 7.2 – 7.9 kg (females)
Acclimation period:	23 and 31 days for males and females, respectively
Diet/Food:	Solid diet DS (Oriental Yeast, Co.) restricted at 250 g/dog/day
Water:	Tap water, *ad libitum*
Housing:	Individually in stainless steel cages 83.5 × 90.0 × 80.0 cm
Environmental conditions:	Temperature: 24 ± 2 °C Humidity: 55 ± 10 % Air changes: 15 / hour 12 hours light/dark cycle

B: Study design and methods

In life dates: 1996-03-05 to 1997-04-03

Animal assignment and treatment:
Groups of 4 males and 4 females Beagle dogs received the test material by incorporating it into the basal diet at a level of 0, 1600, 8000 or 50000 ppm for a period of 12 months.

Clinical observations
All animals were observed daily for clinical signs. Detailed clinical observations were performed at least once per week.

Body weight
Individual body weights were recorded at initiation of treatment, weekly from weeks 1 to 13, and every 4 weeks from weeks 16 to 52. In addition, final body weight was measured before necropsy.

Food consumption and compound intake
Food consumption of each animal was recorded weekly from week 1 to 13 and every 4 weeks from week

16 to 52. Food residues, if any, were collected and weighed every morning. Daily food consumption by each animal was calculated as follows:

$$Food\ consumption = \frac{[Feeding\ amount\ (250\ g\ diet + 250\ g\ water) - food\ residue]}{2}$$

Chemical intake (mg/kg bw/day) was calculated weekly from food consumption and body weight data and the nominal level.

Ophthalmological examination
Ophthalmological examinations were performed on all dogs prior to start of the treatment period and at week 52. The following items were examined: eyeball, eyelid, conjunctiva, cornea, anterior chamber, pupil, iris, lens, vitreous body and fundus.

Haematology and clinical chemistry
Blood samples were collected from all dogs prior to treatment, in weeks 25 and 52. The following haematological parameters were examined: Haematocrit, haemoglobin concentration, erythrocyte count, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, platelet count and total leukocyte count.

All animals were subjected to blood biochemical examinations at weeks 26 and 52.

The following clinical chemistry parameters were examined: Alkaline phosphatase (ALP), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), γ-glutamyl transpeptidase (GGTP), creatine phosphokinase (CPK), creatinine (Creat.), blood urea nitrogen (BUN), total protein (TP), albumin (Alb), globulin (Glob.), albumin/globulin ratio (A/G ratio), glucose (Gluc.), total cholesterol (T. Chol.), triglyceride (TG), total bilirubin (T. Bil.), calcium (Ca), inorganic phosphorus (P), sodium (Na), Potassium (K) and chloride (Cl).

Urinalysis
Prior to initiation of treatment and at weeks 25 and 51, all animals were subjected to urinalysis on the following parameters: Appearance, colour, specific gravity, pH, volume, proteins, glucose, ketones, bilirubin, nitrites, blood and urobilinogen.

Sacrifice and pathology
All surviving dogs were killed after completion of 52 weeks treatment and were subjected to a gross pathological examination. The following organs were weight: Adrenals, brain, epididymides, heart, kidneys, liver, spleen, thymus, uterus, pituitary, prostate, ovaries, testes, thyroids with parathyroid. Organ to body weight ratios were calculated.

Tissue samples were taken from the following organs of all dogs and preserved in 10 % buffered formalin (except for the eyes with the optic nerve which were fixed in Davidson´s fixative, and testes and epididymides which were preserved in Bouin´s fluid): Brain, spinal cord, peripheral nerve, pituitary, thymus, thyroids with parathyroids, adrenals, tonsil, spleen, bone with marrow, lymph nodes, heart, aorta, tongue, buccal mucosa of oral cavity, pharynx, salivary glands, oesophagus, stomach, liver with gallbladder , pancreas, duodenum, jejunum, ileum, caecum, colon, rectum, nasal cavity, larynx, trachea, lung, kidneys, urinary bladder, testes, prostate, penis, epididymides, ovaries, oviducts, uterus, vagina, diaphragm, eyes, femoral muscle, skin, mammary gland and all gross lesions.

A detailed histopathological examination was performed on all sampled tissues of all dogs, except for femur, larynx, oviducts, tongue, ureter and vagina.

Statistics
Statistical analysis of body weight, haematology, blood biochemistry, urinalysis and organ weight data was done according to the statistical decision tree shown in "*Guidance Notes for Analysis and Evaluation of Chronic Toxicity and Carcinogenicity Studies*" (OECD, 2002), summarising the most common statistical procedures used for analysis of data in toxicology studies, together with their most likely outcomes.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no deaths in any dose groups of either sex.

B. CLINICAL OBSERVATIONS
In the 50000 ppm group, loose stool was observed in 3 of 4 males and 4 of 4 females. The animals in the 8000 and 1600 ppm groups did not show the clinical sign at all. In the control group, only one animal in each sex showed it. Most of the animals in the 50000 ppm group frequently showed the sign throughout the treatment period, whereas the occurrence in the suffering animals of the control group was restricted to a limited period.

For other clinical signs observed, the occurrence was sporadic in all dose groups, or the incidence was almost comparable among the dose groups.

C. BODY WEIGHT
In the 50000 ppm group of both sexes, retarded body weight gain became evident gradually as the study progressed. Consequently, the mean body weights in this group at termination of treatment were 6 % in males and 11 % in females lower than those in the controls. However, statistically significant differences in mean body weights were not observed throughout the treatment between the control and treated groups including the 50000 ppm.

Table 5.3.2-113: HR-001: 12-Month Oral Chronic Toxicity Study in Dogs ([redacted], 1997): Mean body weight and body weight changes [kg]

Dose level [ppm]	Males				Females			
	0	1600	8000	50000	0	1600	8000	50000
Mean bw prior to start (day -1)	8.4	8.3	8.3	8.3	8.2	8.2	8.2	8.2
Weeks 0 – 4	+1.1	+1.2	+1.1	+0.9	+1.0	+0.9	+0.8	+0.6
Weeks 4 – 24	+2	+2.7	+1.8	+1.5	+1.9	+1.7	+2	+1.3
Weeks 24 – 52	0	+0.6	+0.5	+0.1	+0.8	+0.8	+1.4	+0.5
Weeks 0 – 52	+3.1	+4.5	+3.4	+2.5	+3.7	+3.4	+4.2	+2.4
Mean bw in week 52/53	11.5	12.8	11.7	10.8	11.9	11.6	12.4	10.6

* Statistically significant from control ($p < 0.05$)

D. FOOD CONSUMPTION
Decreased food consumption was noted for one female in the 1600 ppm group at weeks 24, 28 and 52 and for another female in the same group at week 32. Consequently, group mean food consumption in this group was decreased at those weeks. However, food consumption in this group recorded at other weeks was comparable to that of the controls. Moreover, the averaged group mean food consumption through the treatment period was almost comparable between the 1600 ppm and control groups of females.

All males in all dose groups and females except the above 2 animals in the 1600 ppm group consumed whole amount of diet offered every day.

Group mean chemical intakes were calculated from group mean values of food consumption and body weight, and the nominal dose levels. The overall group mean chemical intakes [mg/kg bw/day] through the whole treatment period are presented in the table below:

Table 5.3.2-114: HR-001: 12-Month Oral Chronic Toxicity Study in Dogs ([redacted], 1997): Mean test substance intake

Dose level [ppm]	Test substance intake [mg/kg bw/day]	
	Males	Females
1600	34.1	37.1
8000	182	184
50000	1203	1259

E. OPHTHALMOLOGY

No remarkable ocular changes were detected in animals in any dose group at week 52.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

Statistically significant changes in haematology that were observed in treated groups are presented in the following table:

Table 5.3.2-115: HR-001: 12-Month Oral Chronic Toxicity Study in Dogs ([redacted], 1997): Results of haematological examination – selected parameters (mean ± SD)

Parameter	Week	Dose level [ppm]							
		Males				Females			
		0	1600	8000	50000	0	1600	8000	50000
Haematocrit (Ht) [%]	0	41.5 ± 1.7	↓40.9 ± 2.9	↓38.2 ± 1.4	↓39.5 ± 3.0	45.6 ± 0.6	↓42.1 ± 3.8	45.6 ± 1.8	**↓41.8* ± 1.2**
	26	48.2 ± 1.8	↑50.6 ± 2.1	↑48.4 ± 3.6	↑45.3 ± 2.8	49.1 ± 4.0	↓48.7 ± 1.6	↓45.8 ± 2.3	↓44.3 ± 1.9
	52	50.5 ± 2.0	↑53.8 ± 2.4	↓49.8 ± 3.3	↓47.6 ± 3.5	54.2 ± 3.7	↓53.1 ± 3.5	↓49.6 ± 4.3	**↓46.5* ± 3.1**
Haemoglobin (Hb) [g/dL]	0	14.2 ± 0.5	↓13.8 ± 1.1	↓13.0 ± 0.4	↓13.6 ± 1.0	15.1 ± 0.4	↓14.2 ± 1.2	↑15.5 ± 0.5	↓14.1 ± 0.4
	26	17.1 ± 0.7	↑17.5 ± 0.6	↓17.0 ± 1.0	↓16.0 ± 1.0	17.2 ± 1.2	↓17.1 ± 0.7	↓16.1 ± 0.6	**↓15.4* ± 0.5**
	52	17.3 ± 0.5	↑18.1 ± 0.7	↓16.8 ± 1.0	↓16.2 ± 1.1	18.2 ± 1.2	↓17.9 ± 1.0	↓16.8 ± 1.4	**↓15.7* ± 0.7**
Erythrocyte count (RBC) [10^6/mm^3]	0	6.31 ± 0.38	↓6.22 ± 0.70	↓5.68 ± 0.35	↓6.13 ± 0.69	7.08 ± 0.41	↓6.41 ± 0.50	↓6.94 ± 0.40	**↓6.26* ± 0.29**
	26	7.39 ± 0.41	↑7.83 ± 0.47	↓7.27 ± 0.62	↓7.09 ± 0.62	7.69 ± 0.47	↓7.37 ± 0.19	↓7.04 ± 0.53	↓6.94 ± 0.47
	52	7.68 ± 0.35	↑8.05 ± 0.63	↓7.31 ± 0.52	↓7.13 ± 0.71	8.40 ± 0.42	↓7.86 ± 0.62	↓7.46 ± 1.03	**↓6.87* ± 0.42**

* Statistically significant from controls ($p < 0.05$)

Male groups showed no significant changes in any parameters.

Female in the 50000 ppm group showed significantly decreased values of haematocrit (Ht), haemoglobin concentration (Hb), and erythrocyte count (RBC) at week 52. Haemoglobin concentration in this group was also significantly lower at week 26. This group had already showed lower values for these 3 parameters than the controls before initiation of treatment (at week 0). In particular, the differences from the control values in haematocrit and erythrocyte count at week 0 were statistically significant. However, the rates of deviation from the control values were, though slightly, augmented in the treatment period when compared to those at week 0.

Females in the 8000 and 1600 ppm groups showed no significant changes in haematological examinations.

Clinical chemistry
Statistically significant changes in blood biochemistry that were observed in treated groups are presented in the table hereafter:

Table 5.3.2-116: HR-001: 12-Month Oral Chronic Toxicity Study in Dogs ([redacted] 1997): Results of week 52 clinical chemistry examination- selected parameters (mean ± SD)

Parameter	Dose level [ppm]							
	Males				Females			
	0	1600	8000	50000	0	1600	8000	50000
Creatine phosphokinase (CPK) [U/L]	111 ± 21	↓93 ± 7	**↓82* ± 8**	↑121 ± 16	78 ± 14	↑96 ± 9	↑103 ± 22	↑100 ± 20
Albumin (Alb) [g/dL]	3.07 ± 0.19	↑3.22 ± 0.18	↓2.99 ±0.26	↓2.88 ± 0.09	3.19 ± 0.14	↓3.04 ± 0.20	↓3.13 ± 0.12	**↓2.84* ± 0.17**
Calcium (Ca) [mg/dL]	10.2 ± 0.2	↑10.4 ± 0.3	↓10.0 ± 0.3	↓9.8 ± 0.2	10.2 ± 0.1	**↓10.0* ± 0.1**	↑10.3 ± 0.2	**↓9.7** ± 0.1**
Inorganic phosphorus (P) [mg/dL]	3.8 ± 0.9	↑3.9 ± 0.6	↓3.3 ± 0.3	↓2.8 ± 0.2	3.9 ± 0.3	3.9 ± 0.3	↑4.2 ± 0.5	**↓2.8* ± 0.5**
Chloride (Cl) [mEq/L]	112.3 ± 1.3	↓110.2 ± 1.8	↑112.5 ± 2.2	↑114.0 ± 1.0	112.0 ± 1.3	↑112.4 ± 1.5	↓109.3 ± 2.0	↑114.0 ± 1.7

* Statistically significant from controls ($p < 0.05$);
** Statistically significant from controls ($p < 0.01$)

Females in the 50000 ppm group showed a significant increase in chloride (Cl) at week 26 and significant decreases in albumin (Alb), calcium (Ca) and inorganic phosphorous (P) at week 52. A significant decrease in calcium was also noted for females in the 1600 ppm group at 52 weeks.

For male groups, the 8000 ppm group showed a significant decrease in creatine phosphokinase (CPK) at week 52. But this change was not observed in the 50000 ppm group.

G. URINALYSIS
There were no findings among the quantitative or semi-quantitative and qualitative parameters during the treatment period.

H. NECROPSY
Organ weights
Males in the 1600 ppm group showed statistically significant increases in both absolute and relative weights of the pituitary. However, these changes were not observed in the 50000 or 8000 ppm groups of males.

In the 50000 or 8000 ppm groups, neither males nor females showed statistically significant changes in any organ weights.

Gross pathology
The macroscopic lesions observed in the present study were all sporadic in nature and there were no statistically significant differences in the incidence between the control and treated groups.

Histopathology
In the 50000 ppm group, focal pneumonia / focal granulomatous pneumonia in the lung was observed in all females. In the other female groups including the control group, the lesion was observed in only one of 4 animals each. However, the extent of the lesions was very focal and the degree of intensity was slight in all cases including those of the 50000 ppm group. Statistically, no significant differences between the control and dose groups were found in incidence of any histological lesions, including the pulmonary lesion.

III. CONCLUSIONS

Findings related to the treatment were demonstrated in clinical observation, body weight, urinalysis, haematology and blood chemistry.

50000 ppm group: Loose stool was observed in 3 of 4 males and 4 of 4 females. These animals frequently showed this clinical sign through the treatment period, whereas in the control group, only one animal in each sex showed the sign over a limited period during treatment. Body weight gain was retarded gradually with progression of the treatment in both sexes, when compared to the controls. Consequently, the difference in mean body weight between the 50000 ppm and control groups became great with time, although statistical significance was not observed. Haematologically, slight anaemic changes were noted for females at weeks 26 and 52. Females also showed significantly increased plasma level of chloride at week 26 and significantly decreased plasma levels of albumin and inorganic phosphorous at week 52. Significantly lowered urine pH values were continuously observed in males and females. However, this finding was not recognised as a toxic change since it is known that the test substance is secreted with little metabolism into urine, is degraded to a free acid in urine, and consequently, makes the urine acidic.

8000 and 1600 ppm groups: There were no treatment-related abnormalities in either sex.

Based on the results, the no-observable-effect level, minimum toxic level and sure toxic level of HR-001 to Beagle dogs under the conditions of the present study were determined as follow.

	Males	Females
No observable effect level	8000 ppm (182 mg/kg bw/day)	8000 ppm (184 mg/kg bw/day)
Minimum toxic level	50000 ppm (1203 mg/kg bw/day)	50000 ppm (1259 mg/kg bw/day)
Sure toxic level	≥50000 ppm (≥1203 mg/kg bw/day)	≥50000 ppm (≥1259 mg/kg bw/day)

3. Assessment and conclusion

Assessment and conclusion by applicant:
This oral 12-month toxicity study with glyphosate technical (HR-001) was conducted in Beagle dogs of both sexes. Groups of 4 males and 4 females each were given the test material via the diet at dose levels of 0, 1600, 8000 or 50000 ppm (equivalent to 0, 34.1, 182 or 1203 mg/kg bw/day for males and 0, 37.1, 184 or 1259 mg/kg bw/day for females) for a period of 12 months. The study was conducted according to OECD 409 (1981) and in general accordance with OECD 452 (1981).
Based on the study results the NOAEL in Beagle dogs after 1-year oral exposure to HR-001 is 8000 ppm (equivalent to 182 and 184 mg/kg bw/day for males and females, respectively).

Assessment and conclusion by RMS:
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1. Information on the study

Data point	CA 5.3.2/033
Report author	[redacted]
Report year	1996 (Study report)
Report title	Glyphosate Acid: 1 Year Dietary Toxicity Study in Dogs
Report No	[redacted]/P/5079
Document No	Not reported
Guidelines followed in study	OECD 452 (1981): OPPTS 870.4100 (1998): 87/302/EEC B.30 (1988)
Deviations from current test guideline (OECD 452, 2018)	Organ weight of heart, spleen, ovaries and uterus was not determined. Deviations from the current version of OECD 452 (2018) are basically due to the fact that the study was aligned to an older version of the OECD test guideline 452.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a
Data point	CA 5.3.2/034
Report author	[redacted]
Report year	1996
Report title	Glyphosate Acid: 1 Year Dietary Toxicity Study in Dogs - Appendix
Report No	[redacted]/P/5079
Document No	Not reported
Guidelines followed in study	OECD 452 (1981): OPPTS 870.4100 (1998): 87/302/EEC B.30 (1988)
Deviations from current test guideline (OECD 452, 2018)	Organ weight of heart, spleen, ovaries and uterus was not determined. Deviations from the current version of OECD 452 (2018) are basically due to the fact that the study was aligned to an older version of the OECD test guideline 452.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

In a toxicity study, groups of four male and four female Beagle dogs were fed diets containing 0 (control), 3000, 15000 or 30000 ppm glyphosate acid (equivalent to 0, 90.9, 440.3 or 906.5 mg/kg bw/day for males and 0, 92.1, 447.8 or 926.2 mg/kg bw/day for females) for a period of at least 1 year.

Clinical observations and veterinary examinations (including ophthalmoscopy) were made and body weights, food consumption and clinical pathology parameters were measured and at the end of the

scheduled period, the animals were killed and subjected to a full examination *post mortem*. Selected organs were weighed and specified tissues were taken for subsequent histopathology examination.

Mild toxicity was evident at 30000 ppm glyphosate acid, as a slight reduction in body weight in females throughout the latter half of the study. This reduction was generally independent of any reduction in food consumption and does not, therefore, reflect a palatability effect. There were no other toxicologically significant effects and the pathological no-effect level was 30000 ppm glyphosate acid.

Oral administration of 0, 3000, 15000 or 30000 ppm glyphosate acid in the diet for 52 weeks caused minimal toxicity at 30000 ppm, evident as a slight reduction in body weight in females. This dose level was equivalent to an overall mean dose of 906 mg/kg bw/day for males and 926 mg/kg bw/day for females.

I. MATERIALS AND METHODS

A: Materials

1. Test material:	Glyphosate acid
Description:	Technical, white solid
Lot/Batch number:	P24
Purity:	As given in report 95.6 % a.s.
CAS#:	Not reported
Stability of test compound:	Confirmed by the Sponsor
2. Vehicle and/ or positive control:	Diet / none
3. Test animals:	
Species	Dog
Strain	Beagle
Age/weight at dosing	20 – 29 weeks
Source	[redacted]
Housing	Housed by treatment group (sexes separately) in indoor pens. The pens had a sleeping platform with heated floor underneath and interlinking gates which enable the dogs to be separated for feeding and dosing
Acclimatisation period	4 – 5 weeks
Diet	Laboratory Diet A (Special Diet Services Ltd., Stepfield, Witham, Essex, UK), *ad libitum*
Water	Mains water, *ad libitum*
Environmental conditions	Temperature: 19 ± 2 °C Humidity: 40 – 70 % Air changes: Approximately 15 changes / hour Photoperiod: 12 hours light / 12 hours dark

B: Study design and methods

In-life dates: 1995-04-11 to 1996-04-12

Animal assignment

In a chronic toxicity study, groups of four male and four female Beagle dogs were fed diets containing 0 (control), 3000, 15000 or 30000 ppm glyphosate acid (equivalent to 0, 90.9, 440.3 and 906.5 mg/kg bw/day for males and 0, 92.1, 447.8 and 926.2 mg/kg bw/day for females) for a period of at least 1 year. A randomisation procedure was used which resulted in the even distribution of dogs (16 males and 16 females) to treatment groups according to body weight ensuring that litter mates were in different groups. Each morning, male dogs received 400 g and female dogs received 350 g of their appropriate experimental diet.

Table 5.3.2-117: Glyphosate Acid: 1 Year Dietary Toxicity Study in Dogs ([redacted], 1996): Study design

Test group	Dietary concentration [ppm]	Dose to animal (Males / Females) [mg/kg bw/day]	Males	Females
Control	0	0 / 0	1 – 4	5 – 8
Low	3000	90.9 / 92.1	9 – 12	13 – 16
Mid	15000	440.3 / 447.8	17 – 20	21 – 24
High	30000	906.5 / 926.2	25 – 28	29 – 32

Diet preparation and analysis

The experimental diets were made in 60 kg batches, by direct addition of glyphosate acid (allowing for purity) to ground Laboratory A diet, and mixed thoroughly. Water was then added to each batch and mixed prior to pelleting. The pellets were dried in the residual heat of an autoclave, allowed to cool and were then stored in bins at room temperature.

Samples from all dietary levels (including controls) were taken at approximately two-monthly intervals throughout the study and analysed quantitatively for glyphosate acid. The homogeneity of glyphosate acid in Lab diet A was determined by analysing samples from the low and high dose levels. The chemical stability of glyphosate acid in diet was determined over a period of up to 10 weeks (69 days) for these same diets.

Samples were extracted with water. Portions of the supernatant were diluted with water to give sample solution concentrations within the range of the calibration standards. These were derivatised using 9-fluorenylmethylchloroformate (FMOCCL) and analysed by High Performance Liquid Chromatography (HPLC).

Concentration analysis results: The mean achieved concentrations of glyphosate acid in analysed dietary preparation were typically within 12 % of nominal concentration.
The overall mean concentrations were within 9 % of target.

Homogeneity results: The homogeneity of glyphosate acid in diet at concentrations of 3000 ppm and 30000 ppm for a batch size of 60 kg was determined and considered satisfactory; percentage deviations from the overall mean where within 11 %.

Stability results: The chemical stability of glyphosate acid in experimental diets (determined at concentrations of 3000 ppm and 30000 ppm) when stored at room temperature, was shown to be satisfactory for 69 days. This covered the period of usage on the present study.

Observations

All dogs were observed at least three times daily for clinical behavioural abnormalities (at dosing, after dosing and at the end of the working day) and, on a weekly basis, they were given a thorough examination. Individual, daily assessments of gastro-intestinal findings were made for up to 5 hours post dosing: any subsequent assessments were made on a group basis. All dogs were also given a full clinical examination by a veterinarian pre-study, during weeks 13, 26, 39 and prior to termination. The examination included cardiac and pulmonary auscultation.

Body weight

All dogs were weighed weekly, before feeding, throughout the pre-study period, on day 1 and thereafter at weekly intervals until termination.

Food consumption and test substance intake
Food residues were recorded daily, approximately 4 hours after feeding and any residual food was discarded. These measurements were made for at least 2 weeks pre-study and throughout the treatment period.

Ophthalmoscopic examination
The eyes of all dogs were examined pre-study, during weeks 13, 26, 39 and prior to termination.

Haematology and clinical chemistry
Blood was collected from all dogs in weeks -1, 4, 13, 26 and prior to termination into tubes containing EDTA or trisodium citrate and the following parameters measured: Haemoglobin, haematocrit, red blood cell count, mean cell volume, mean cell haemoglobin, prothrombin time, blood cell morphology, mean cell haemoglobin concentration, platelet count, total white cell count, differential white cell count, red cell distribution width activated partial thromboplastin time and bone marrow smears (taken but not examined).

Clinical chemistry
Blood was collected from all dogs in weeks -1, 4, 13, 26 and prior to termination into tubes containing lithium heparin and the following parameters measured: Urea, creatinine, glucose, albumin, total protein, cholesterol, triglycerides, total bilirubin, creatine kinase activity, alkaline phosphatase activity, aspartate aminotransferase activity, alanine aminotransferase activity, gamma-glutamyl transferase activity, calcium, phosphorus (as phosphate), sodium, potassium and chloride.

Urinalysis
Urine was collected by catheterisation, pre-experimentally, in week 26 and during the week prior to termination. The following parameters were measured and recorded on each urine sample: Volume, colour (if abnormal), specific gravity, pH, glucose, ketones, protein, bilirubin and blood.
In addition, each urine sample was centrifuged and the sediment stained and examined microscopically to identify the components.

Investigations *post mortem*

Macroscopic examination
All animals were killed by exsanguination under terminal anaesthesia induced by intravenous administration of sodium pentobarbitone and examined *post mortem*.

Organ weights
From all animals surviving to scheduled termination, the following organs were removed, trimmed free of extraneous tissue and weighed: Adrenal glands, brain, epididymides, thyroid glands, kidneys, liver and testes.

The left and right components of paired organs were weighed separately.

Tissue submission
The following tissues were examined *in situ*, removed and examined and fixed in an appropriate fixative: Gross lesions including masses, adrenal gland, aorta, brain (cerebrum, cerebellum and brainstem), bone marrow (sternum), caecum, colon, duodenum, epididymides, eyes (retina, optic nerve), femur (including stifle joint, stored not examined), gall bladder, heart, ileum, jejunum, kidney, larynx, liver, lung, lymph node – prescapular, lymph node – mesenteric, mammary gland (females only), peripheral nerve (sciatic), oesophagus, ovary, pancreas, parathyroid gland, pituitary gland, prostate gland, rectum, salivary gland, spinal cord (cervical, thoracic, lumbar), skin, spleen, sternum, stomach, testis, thymus, thyroid gland, trachea, urinary bladder, uterus (with cervix) and voluntary muscle.

Microscopic examination
All processed tissues were examined by light microscopy.

Statistics
All data were evaluated using analysis of variance and / or covariance for each specified parameter using the GLM procedure in SAS (1989).

II. RESULTS AND DISCUSSION

A. MORTALITY
None of the dogs died.

B. CLINICAL OBSERVATIONS
There were no toxicologically significant findings. Salivation at dosing was observed in individual dogs in all treatment groups throughout the study. The apparent increased incidence in two top dose males and one female was considered to be related to anticipation of feeding and not to treatment with glyphosate acid. There was also a low incidence of scrotal skin reddening seen in one male in each treatment group; this was considered to be incidental to treatment with glyphosate acid.

There was no increased incidence of faecal abnormalities in dogs treated with glyphosate acid.

C. BODY WEIGHT AND BODY WEIGHT GAIN
There was a slight body weight effect evident in females fed 30000 ppm glyphosate acid with a maximum reduction of 11 % (compared to controls) in week 51. These dogs showed a gradual reduction in growth rate, compared to the controls, which was consistently statistically significant from week 23 onwards. One female lost 0.6 kg during week 32 but this was related to a loss of appetite during this time. There were no effects in males at any dose level or in females at 15000 ppm but females fed 3000 ppm glyphosate acid also showed slightly poorer growth than the controls, with a maximum reduction of 8 % in week 51. However, this effect only achieved statistical significance on occasions during the study and is considered attributable to the poorer growth of two females and not an effect of glyphosate acid, since there was no effect at 15000 ppm.

Table 5.3.2-118: Glyphosate Acid: 1 Year Dietary Toxicity Study in Dogs ([redacted] 1996): Intergroup comparison of body weights [kg] (selected time points; adjusted mean values shown for weeks 2-53)

Week	Dietary Concentration of Glyphosate acid [ppm]							
	Males				Females			
	0	3000	15000	30000	0	3000	15000	30000
1	11.40 ± 1.19	↑11.53 ± 0.83	↓11.33 ± 0.95	↑11.45 ± 0.83	9.60 ± 0.80	↓9.55 ± 0.69	↓9.48 ± 0.55	↓9.58 ± 0.99
8	12.66 ± 0.92	↓12.40 ± 0.49	↓12.48 ± 0.82	↓12.37 ± 0.88	10.74 ± 0.88	**↓10.40* ± 0.66**	↓10.68 ± 0.73	**↓10.42* ± 0.81**
16	13.35 ± 0.99	↓12.97 ± 0.92	↓13.28 ± 0.83	↓12.95 ± 0.79	11.46 ± 0.94	**↓11.03* ± 0.61**	↑11.50 ± 0.96	**↓10.99* ± 0.87**
32	14.19 ± 0.94	↓13.69 ± 1.48	↓13.93 ± 1.15	↓13.69 ± 0.85	12.28 ± 0.80	**↓11.63* ± 0.49**	↑12.59 ± 1.35	**↓11.46** ± 1.17**
53	14.57 ± 0.77	↓14.24 ± 1.72	↓14.24 ± 1.75	↓13.85 ± 0.96	13.10 ± 0.92	↓12.25 ± 0.51	↓12.94 ± 1.17	**↓11.76** ± 1.48**

* Statistically significant difference from control group mean ($p < 0.05$; Student's t-test, 2-sided);
** Statistically significant difference from control group mean ($p < 0.01$ Student's t-test, 2-sided)

D. FOOD CONSUMPTION AND COMPOUND INTAKE
There was no effect on food consumption but 3 dogs (one male mid dose and 2 females top dose) left food on occasions which affected the group mean values. Dose rates (based on nominal dietary levels of glyphosate acid) were calculated in terms of mg/kg bw/day. Mean values are shown below:

Table 5.3.2-119: Glyphosate Acid: 1 Year Dietary Toxicity Study in Dogs ([redacted], 1996): Mean Dose Received [mg/kg bw/day]

Glyphosate acid [ppm]	3000	15000	30000
Males	90.9	440.3	906.5
Females	92.1	447.8	926.2

E. OPHTHALMOSCOPIC EXAMINATION

There was a very low incidence of corneal or lenticular opacities but these were seen both in control animals as well as those fed glyphosate acid. There were no treatment related abnormalities.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

There were no differences in haematological parameters which were considered to be related to treatment.

Blood clinical chemistry

There were no toxicologically significant findings.

Plasma cholesterol levels were increased slightly in the treated groups of both sexes at weeks 26 and 52 but there was no evidence of any dose relationship.

Plasma phosphorus levels were lower in the male treated groups at week 52 but this was due, in part, to slightly higher individual control values. Similarly, the reduced sodium value in males fed 30000 ppm at week 52 was due solely to one male.

Various animals in all groups (including controls) showed evidence of higher plasma alanine aminotransferase, aspartate aminotransferase and creatine kinase activities throughout the study as well as pre-experimentally, but there was little evidence of any conclusive group effects.

Other statistically significant differences were minor and/or not dose related and were considered to be of no toxicological significance.

Table 5.3.2-120: Glyphosate Acid: 1 Year Dietary Toxicity Study in Dogs ([redacted], 1996): Intergroup comparison of blood clinical chemistry selected parameters and time points (adjusted mean values)

Parameter	Week	Dietary Concentration of Glyphosate acid [ppm]							
		Males				Females			
		0	3000	15000	30000	0	3000	15000	30000
Cholesterol [mmol/L]	26	3.78 ± 0.34	↑**4.48* ± 0.31**	↑3.96 ± 0.26	↑**4.40* ± 0.65**	4.28 ± 0.96	↑4.59 ± 0.70	↑**4.88* ± 0.60**	↑4.86 ± 0.36
	52	3.42 ± 0.10	↑**4.25* ± 0.42**	↑4.12 ± 0.39	↑**4.33* ± 0.58**	4.15 ± 0.97	↑4.32 ± 0.54	↑**5.08* ± 0.69**	↑**4.94* ± 0.61**
Phosphorus [mmol/L]	52	1.29 ± 0.23	↓**0.99* ± 0.15**	↓**0.89** ± 0.04**	↓**0.80** ± 0.16**	0.91 ± 0.16	↑1.12 ± 0.15	↑1.05 ± 0.07	↓0.76 ± 0.27
Sodium [mmol/L]	52	147.5 ± 1.5	↓146.2 ± 1.5	↑148.2 ± 1.3	↓**142.6* ± 6.9**	147.5 ± 1.0	↓147.3 ± 1.0	↓147.3 ± 1.7	↑148.1 ± 1.2

* Statistically significant from controls ($p < 0.05$);
** Statistically significant from controls ($p < 0.01$)

G. URINALYSIS

There were no differences in urine clinical chemistry parameters which were considered to be related to treatment.

H. SACRIFICE AND PATHOLOGY

Organ weights

There were no treatment related effects on any organ weights. Adrenal weights were slightly raised in the male 3000 ppm group but this was exaggerated by a low value for one male in the control group.

Macroscopic findings

Several treated females showed red areas in or diffuse reddening of the urinary bladder mucosa. The incidence was not clearly related to dose and in the absence of a similar effect in males it was considered unlikely that the lesion is related to the administration of glyphosate acid.

Microscopic findings

It was considered unlikely that any of the lesions confined to the treated groups were related to the administration of glyphosate acid as they were either of low incidence or the incidence was not related to dose. The pathological no-effect level for glyphosate acid was 30000 ppm.

III. CONCLUSIONS

Mild toxicity was evident at 30000 ppm glyphosate acid, as a slight reduction in body weight in females throughout the latter half of the study. This reduction was generally independent of any reduction in food consumption and does not, therefore, reflect a palatability effect. There were no other toxicologically significant effects and the pathological no-effect level was 30000 ppm glyphosate acid.

Oral administration of 0, 3000, 15000 or 30000 ppm glyphosate acid in the diet for 52 weeks caused minimal toxicity at 30000 ppm, evident as a slight reduction in body weight in females. This dose level was equivalent to an overall mean dose of 906 mg/kg bw/day for males and 926 mg/kg bw/day for females.

There were no other treatment related findings and the pathological no-effect level was 30000 ppm glyphosate acid.

The no-observed adverse effect level (NOAEL) for toxicity over 1 year for females was 15000 ppm glyphosate acid (equivalent to an overall mean dose of 447 mg/kg bw/day). The no-observed adverse effect level (NOAEL) for toxicity over 1 year for males was 30000 ppm glyphosate acid (equivalent to an overall mean dose of 906 mg/kg bw/day).

3. Assessment and conclusion

Assessment and conclusion by applicant:

In this toxicity study, groups of four male and four female Beagle dogs were fed diets containing 0 (control), 3000, 15000 or 30000 ppm glyphosate acid (equivalent to 0, 90.9, 440.3 or 906.5 mg/kg bw/day for males and 0, 92.1, 447.8 or 926.2 mg/kg bw/day for females) for a period of at least 1 year. The study was conducted according to OECD 452 (1981) and in compliance with GLP (no certificate of the competent authority was provided).

Oral administration of glyphosate acid in the diet for 52 weeks caused minimal toxicity at 30000 ppm, evident as a slight reduction in body weight in females. This dose level was equivalent to an overall mean dose of 906 mg/kg bw/day for males and 926 mg/kg/ bw/day for females.

There were no other treatment-related findings and the pathological no-effect level was 30000 ppm glyphosate acid.

The NOAEL for toxicity over 1 year for females was 15000 ppm glyphosate acid (equivalent to an overall mean dose of 447 mg/kg bw/day). The NOAEL for toxicity over 1 year for males was 30000 ppm glyphosate acid (equivalent to an overall mean dose of 906 mg/kg bw/day).

Assessment and conclusion by RMS:
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1. Information on the study

Data point	CA 5.3.2/035
Report author	[redacted]
Report year	1990
Report title	Glyphosate: 52 Week Oral Toxicity Study in Dogs
Report No	7502
Document No	Not reported
Guidelines followed in study	OECD 409 (1981), FIFRA 83-1, in general compliance with OECD 452 (1981)
Deviations from current test guideline (OECD 409, 1998; OECD 452, 2018)	Activated partial thromboplastin time not measured. Clinical signs poorly reported in the report. Deviations from the current versions of OECD 409 (1998) and OECD 452 (2018) are basically due to the fact that the study was aligned to older versions of the OECD test guidelines.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary
In a 52-week oral study, groups of four male and four female Beagle dogs were administered glyphosate daily via capsule at dose levels of 0, 30, 300 or 1000 mg/kg bw/day.

Clinical observations were made daily. Body weights and food consumption were assessed in weekly intervals. Ophthalmoscopic examination was performed pre-test and during weeks 13, 29, 39 and 51. Haematological, blood biochemistry parameters, as well as urine and faecal analysis for occult blood were conducted prior to start of treatment and at weeks 13, 26, 39 and 51 of treatment. Plasma levels of glyphosate were determined after weeks 1, 12 and 51 of treatment. Red blood cell and plasma cholinesterase activity were measured at weeks 13, 26, 39 and 51 and brain cholinesterase measured at termination. At the end of the scheduled period, the animals were sacrificed and subjected to a full examination *post mortem*. Selected organs were weighed and tissues were taken for subsequent histopathology examination.

There were no mortalities in any of the dose groups. Changes in faecal consistency (soft/loose/liquid) were recorded more frequently for the animals in the high dose group throughout the dosing period. This finding was observed 4 – 6 h after dosing and was also recorded on isolated occasions for a few animals of the intermediate dose group. A reduction in body weight gain was recorded for all treated groups males (low

dose: approximately 17 % of control, intermediate and high dose: approximately 25 % of control). In the females, only the high dose group showed a reduction in body weight gain of approximately 19 % of control. These body weight differences did not achieve statistical significance and may have been a chance effect. The mean food consumption in treatment groups was not significantly different from controls. Ocular examinations revealed no treatment related abnormalities. There were no haematological or clinical chemistry changes observed that could be associated with treatment with glyphosate. Red blood cell, plasma, and brain cholinesterase activity was similar between all groups. There were no changes in any urinalysis parameters examined to indicate a treatment-related effect. Occult blood determinations performed on faecal samples obtained at the time of urine collections were negative for all animals throughout the study. There were no significant in absolute or relative (to body weight) organ weight differences between groups for either sex attributable to treatment with glyphosate. No pathologically significant gross or microscopic findings in any group could be related to treatment.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate technical
Description:	Not reported
Lot/Batch #:	206-Jak-25-1; 206-Jak-59-3; 229-Jak-5-1;
Purity:	98.6 %; 99.5 %; 98.9 %
Stability of test compound:	Shown stable in capsule for at least 7 days

2. Vehicle and/or positive control: Clear gelatine capsule (empty)

3. Test animals:

Species:	Dog
Strain:	Beagle
Source:	[redacted]
Age:	Approx. 5 – 6 months
Sex:	Male and female
Weight at dosing:	♂ 8.2 – 12.1 kg; ♀ 6.7 – 11.7 kg
Acclimation period:	3-weeks
Diet/Food:	SDS Dog Diet A, 400 g/day.
Water:	Tap water, *ad libitum*
Housing:	Paired in custom-designed pens
Environmental conditions:	Temperature: ~19 °C (12 – 25 °C) Humidity: ~50 % (30 – 83 %) Air changes: Not reported 12 hours light/dark cycle

B: Study design and methods

In life dates: 1989-08-29 to 1990-08-30

Animal assignment and treatment:
Four male and four female dogs per dose level received glyphosate technical (source Cheminova A/S, Lemvig, Denmark) orally, by capsule administration, once daily for 52 consecutive weeks. Three batches

of the test material (206-Jak-25-1, purity: 98.6 %; 206-Jak-59-5, purity: 99.5 % and 229-Jak-5-1, purity: 98.9 %) were used during the course of the study. The bulk powder was encapsulated in hard, clear gelatin capsules. The individual test substance amount to be given was calculated weekly on the basis of each dog's most recently recorded body weight. Multiple-capsule administration was necessary in the high dose groups and, to ensure equal conditions, in the controls. The dose levels of 0 (vehicle control group receiving empty capsules), 30, 300 and 1000 mg/kg bw/day were selected on the basis of the results from a previous maximum tolerated dose study ([redacted], 1989). Regular analyses of the capsule preparations performed at 3 monthly intervals revealed deviations of the actual from the nominal compound weight varying within tolerable limits (<5 %). Encapsulated Glyphosate was shown to be stable for at least 7 days in the capsules.

Table 5.3.2-121: Glyphosate: 52 Week Oral Toxicity Study in Dogs ([redacted], 1990): Study design

Test group	Dose Level [mg/kg bw/day]	Males	Females
Control	0	4	4
Low	30	4	4
Intermediate	300	4	4
High	1000	4	4

Mortality
Each animal was checked for mortality or signs of morbidity during the daily clinical observations.

Clinical observations
A check for clinical signs of toxicity was made once daily.

Body weight
The body weight of each animal was recorded weekly starting two weeks before the start of treatment.

Food consumption and utilisation
Individual food consumption was recorded daily starting two weeks before the start of treatment.

Ophthalmoscopic examination
Ophthalmoscopy was performed at pre-test and again during weeks 13, 29, 39 and 51 of treatment.

Haematology and clinical chemistry
Laboratory investigations of haematology and clinical chemistry were performed on all the dogs before dosing started and again during weeks 13, 26, 39 and 51 of treatment. The blood samples were taken from the jugular vein after the dogs had been fasted overnight, any food residues being withdrawn at approximately 1400 hours on the day before sampling.

EDTA was used as an anti-coagulant for evaluation of all parameters with the exception of prothrombin time for which citrate was used. The following haematological parameters were measured: Haemoglobin, haematocrit, red blood cell count, mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), reticulocyte count, total white blood cell and differential counts, platelet count and prothrombin time. Femoral bone marrow smears were taken at necropsy, air-dried and methanol-fixed but were not evaluated as there were no changes in peripheral blood or red cell morphology.

For clinical chemistry evaluations, heparin was used as an anti-coagulant and plasma analysed for the following parameters: Blood urea nitrogen, glucose, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, sodium, potassium, calcium, chloride, creatinine, total protein, protein electrophoresis, albumin, albumin/globulin ratio, cholesterol, triglycerides, alkaline phosphatase, gamma

glutamyl transpeptidase, phosphate, total bilirubin, red blood cell, plasma and brain cholinesterase.

Whole blood was collected from all the animals via the jugular vein into lithium heparinised tubes after 1, 12 and 51 weeks of treatment. Immediately after collection, the samples were centrifuged, the plasma separated and stored deep frozen (-20 °C) until subsequent analysis for plasma levels of glyphosate.

Urinalysis/Faecal analysis
Urine samples were collected from all dogs housed in metabolism cages over the final 17 hours of a 21 hour period of water deprivation during weeks 13, 26, 39 and 51 of treatment. The following parameters were measured: Appearance, volume, pH, specific gravity, proteins, glucose, ketones, blood pigments, bilirubin and urobilinogen. Microscopic examination of the spun urine deposit was performed for the presence of epithelial cells, white blood cells, red blood cells, crystals, organisms and abnormal constituents.
Faecal analysis for occult blood was performed at the time of the urine collections.

Sacrifice and pathology
After 52 weeks of consecutive treatment, all surviving animals were sacrificed by intravenous pentobarbitone followed by exsanguination and subjected to a gross pathological examination. Any macroscopic findings were recorded. Terminal body weights were recorded immediately after sacrifice.
The following organ weights were determined: Adrenals, thyroids with parathyroids, pituitary, brain, heart, liver and gallbladder (drained), kidneys, lungs, spleen, pancreas, thymus, testes with epididymides, prostate, uterus, ovaries and salivary gland (submaxillary, sublingual and parotid).

Tissue samples were taken from the following organs and preserved in buffered formalin: All gross lesions, adrenals, aortic arch, brain (3 sections), caecum, colon, duodenum, epididymides, eyes, heart, ileum, jejunum, kidneys, liver and gall bladder, lungs, lymph nodes (mandibular and mesenteric), mammary gland, oesophagus, ovaries, pancreas, pituitary gland, prostrate, rectum, salivary gland (submaxillary, sublingual and parotid), spinal cord, sciatic nerve, skeletal muscle, skin, spleen, stomach, sternum, testes, thymus, thyroid/parathyroids, tongue, trachea, urinary bladder and uterus. All tissues above from all groups were examined histopathologically.

Statistics
Haematology, clinical chemistry and body weight gain data were statistically analysed for homogeneity of variance using the 'F-max' test. If the group variance appeared homogeneous a parametric ANOVA was used and pairwise comparisons made via Student's t-test using Fisher's F-protected LSD. If the variance were heterogeneous, log or square root transformations were used in an attempt to stabilise the variances. If the variances remained heterogeneous then a non-parametric test such as Kruskal-Wallis ANOVA was used and pairwise comparisons made via the Dunn Z test where considered appropriate. Histological findings were analysed using Fisher's Exact Probability test. Organ weights were analysed relative to body weight.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities.

B. CLINICAL OBSERVATIONS
Changes in faecal consistency (soft/loose/liquid) were recorded more frequently for the animals in the high dose group throughout the dosing period. This finding was observed 4 – 6 h after dosing and was also recorded on isolated occasions for a few animals of the intermediate dose group. There were no other clinical signs related to treatment with Glyphosate. Numerical incidence and frequency are not provided in the study report.

C. BODY WEIGHT
A lower body weight gain was recorded for males in all treated groups (low dose: approximately 17 % of control, intermediate and high dose: approximately 25 % of control). In the females, only the high dose

group showed a lower body weight gain of approximately 19 % of control. These differences did not achieve statistical significance and may have been a chance effect.

Table 5.3.2-122: Glyphosate: 52 Week Oral Toxicity Study in Dogs ([redacted], 1990): Intergroup comparison of mean body weights and body weight gain – selected time points from start of study

Dose [mg/kg bw/day]	Initial body weight [kg]	Final body weight [kg]	Total weight gain [kg]	Total weight gain [% of controls]
Males				
0	10.1	14.9	4.8	-
10	↑10.3	↓14.3	↓4.0	83 %
300	↑10.2	↓13.8	↓3.6	75 %
1000	↑10.3	↓13.9	↓3.6	75 %
Females				
0	8.8	12.4	3.6	-
10	↓8.7	↑12.6	↑3.9	108 %
300	↑9.0	↑13.1	↑4.1	114 %
1000	↓8.7	↓11.6	↓2.9	81 %

D. FOOD CONSUMPTION AND TEST SUBSTANCE INTAKE

The mean food consumption in treatment groups was not significantly different from controls.

Analysis of glyphosate in the hard gelatin capsules showed an acceptable degree of accuracy in capsule preparation (<5 % deviation from nominal weight). There was no indication of degradation of encapsulated glyphosate over 7 days under the storage conditions employed. Plasma analysis indicated good proof of absorption and the levels of glyphosate in plasma were dose related. Plasma concentrations of glyphosate at week 1 ranged from 0.24 – 0.38, 1.29 – 2.17 and 5.44 – 10.23 µg/mL for low-, intermediate- and high dose males, respectively. Plasma concentrations of glyphosate at week 1 for females ranged from 0.16 – 1.01, 1.06 – 1.80 and 2.13 – 6.86 µg/mL for low-, intermediate- and high dose females, respectively. Plasma concentrations of glyphosate at week 52 ranged from 0.14 – 0.47, 0.99 – 2.64 and 3.18 – 9.49 µg/mL for low-, intermediate- and high dose males, respectively. Plasma concentrations of glyphosate at week 52 for females ranged from 0.24 – 0.63, 1.07 – 2.75 and 6.07 – 10.50 µg/mL for low-, intermediate- and high dose females, respectively.

E. OPHTHALMOSCOPIC EXAMINATION

Ocular examinations revealed no treatment-related abnormalities. There were incidental background observations which are commonly seen in dogs that were considered to be unrelated to treatment.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

All haematological parameters assessed gave values that were generally within the normal reference ranges for the species. There were no haematological changes observed that could be associated with treatment with glyphosate.

Blood clinical chemistry

There were no changes in the clinical chemistry parameters indicative of any treatment-related effects.

Red blood cell, plasma and brain cholinesterase activity was similar between all groups.

G. URINALYSIS AND FAECAL ANALYSIS

There were no changes in any of the parameters examined to indicate a treatment-related effect. Only the normal variations such as a few positive results for blood pigments and red blood cells were identified at pretrial and during the treatment period. In some instances, these positive results were attributable to the

animals being in oestrous.

Occult blood determinations performed on faecal samples obtained at the time of urine collections were negative for all animals throughout the study.

H. NECROPSY

Organ weights
There were no significant absolute or relative (to body weight) organ weight differences between groups for either sex attributable to treatment with glyphosate.

Gross pathology
Very few findings were noted and there were no significant differences between controls and glyphosate-treated animals.

Histopathology
No pathologically significant findings were recorded in any group and there were no differences in incidences which could be related to treatment.

III. CONCLUSIONS

There were no mortalities in any of the dose groups. Changes in faecal consistency (soft/loose/liquid) were recorded more frequently for the animals in the high dose group throughout the dosing period. This finding was observed 4 – 6 h after dosing and was also recorded on isolated occasions for a few animals of the intermediate dose group. A reduction in body weight gain was recorded for all treated groups males (low dose: approximately 17 % of control, intermediate and high dose: approximately 25 % of control). In the females, only the high dose group showed a reduction in body weight gain of approximately 19 % of control. These body weight differences did not achieve statistical significance and may have been a chance effect. The mean food consumption in treatment groups was not significantly different from controls. Ocular examinations revealed no treatment related abnormalities. There were no haematological or clinical chemistry changes observed that could be associated with treatment with glyphosate. Red blood cell, plasma, and brain cholinesterase activity was similar between all groups. There were no changes in any urinalysis parameters examined to indicate a treatment-related effect. Occult blood determinations performed on faecal samples obtained at the time of urine collections were negative for all animals throughout the study. There were no significant in absolute or relative (to body weight) organ weight differences between groups for either sex attributable to treatment with glyphosate. No pathologically significant gross or microscopic findings in any group could be related to treatment.

Chronic oral administration of glyphosate at dose levels up to 1000 mg/kg bw/day in Beagle dogs did not cause systemic organ toxicity. However, the 1000 mg/kg bw/day dose level (highest dose tested) was considered to be the maximum tolerated dose in view of the effect on faecal consistency at this dose level.

The no-effect level (NOEL) was judged to be 300 mg glyphosate/kg bw/day.

3. Assessment and conclusion

Assessment and conclusion by applicant:
In this 52-week oral study, groups of four male and four female Beagle dogs were administered glyphosate daily via capsule at dose levels of 0, 30, 300 or 1000 mg/kg bw/day. The study was conducted according to OECD 409 (1981) and in general compliance with OECD 452 (1981).
Chronic oral administration of glyphosate over a period of 52 weeks at dose levels up to 1000 mg/kg bw/day in Beagle dogs was free of systemic organ toxicity. However, the 1000 mg/kg bw/day highest dose level tested was considered to be the maximum tolerated dose in view of an effect on faecal consistency at this dose level. Therefore, the NOAEL was judged to be the intermediate dose of 300 mg/kg bw/day.

Assessment and conclusion by RMS:
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1. Information on the study

Data point	CA 5.3.2/036
Report author	[redacted]
Report year	1985
Report title	Twelve Month Study of Glyphosate Administered by Gelatin Capsule to Beagle Dogs
Report No	[redacted]-4965
Document No	Not reported
Guidelines followed in study	No guideline statement, but in general accordance with OECD 452 (1981)
Deviations from current test guideline (OECD 452, 2018)	Urine volume not measured, spleen and uterus not weighed, unclear of number and location of brain sections observed microscopically. Deviations from the current version of OECD 452 (2018) are basically due to the fact that the study was aligned to an older version of the OECD test guideline 452.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary
Glyphosate was administered orally by gelatin capsule to groups of six male and six female Beagle dogs at daily doses of 0, 20, 100 or 500 mg/kg bw/day for approximately twelve months.

Clinical observations were done twice daily. Weekly determinations of individual body weight were performed for fourteen weeks and bi-weekly determinations were made thereafter. Ophthalmologic examinations were performed at pretest and prior to final necropsy on all animals. Haematological, blood biochemistry parameters, as well as urine analysis, were conducted prior to start of treatment and during

months 3, 6, and 12 of treatment. At the end of the scheduled dosing period, the animals were sacrificed and subjected to a full examination post mortem. Selected organs were weighed and specified tissues were taken for subsequent histopathology examination.

There were no mortalities in any of the dose groups. Slightly higher incidences of abnormal excrement (bloody stool, yellow mucoid stool, diarrhoea, and emesis) were observed in low and high level females when compared to their control group. Approximately one-half of these observations were attributable to only one female dog from each of these groups of 6 animals per group. Females at the middle exposure level and males at all treatment levels had incidences of abnormal excrement similar to that of the control groups. One mid dose and one high dose female had localised skin redness with slight alopecia throughout most of the study. Body weight, food consumption and ophthalmologic findings were normal throughout the test duration. Haematology, clinical chemistry and urine pathologic parameters plus gross and microscopic findings revealed no changes attributable to glyphosate exposure.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate
Description:	White granular solid
Lot/Batch #:	NBP 2472136
Purity:	96.17 %
Stability of test compound:	Shown stable during the dosing period

2. Vehicle and/or positive control: Empty gelatin capsule

3. Test animals:

Species:	Dog
Strain:	Beagle
Source:	[redacted]
Age:	Approx. 6 months
Sex:	Male and female
Weight at dosing:	♂ 6.2 – 9.0 kg; ♀ 5.5 – 7.5 kg
Acclimation period:	Approx. 5 weeks
Diet/Food:	Purina Certified Dog Chow® 5007 (400 g available over 2 – 3 hour period)
Water:	Tap water, *ad libitum*
Housing:	Individually in stainless steel dog cages
Environmental conditions:	Temperature: 20 – 22 °C (68 – 72 °F) Humidity: Not reported Air changes: Not reported

B: Study design and methods

In life dates: 1983-09-20 to 1984-09-24

Animal assignment and treatment:

Six male and six female dogs per dose level received glyphosate orally by gelatin capsule administration, once daily for approximately 12 consecutive months. Dogs were dosed approximately one to six hours after food was removed each day. Doses were adjusted to correspond with each animal's most recent body weight. Capsules were prepared each week. The dose levels were 0 (vehicle control group receiving one empty capsule), 20, 100 and 500 mg/kg bw/day. The high dose was set based on the maximum capsule size and number of capsules (two 1/8 oz. capsules) that could be reasonably administered daily to dogs of this size over one year. Each capsule could hold a maximum of approximately 3 grams of packed glyphosate. Analyses after the completion of the study indicated 97.04 % glyphosate compared to an assay (conducted prior to the study) provided by the sponsor of 96.17 % glyphosate. The ± 0.87 % variation of analyses was within analytical limits and no decomposition of glyphosate was demonstrated.

Table 5.3.2-123: Twelve Month Study of Glyphosate Administered by Gelatin Capsule to Beagle Dogs ([redacted], 1985): Study design

Test group	Dose Level [mg/kg bw/day]	Males	Females
Control	0	6	6
Low	20	6	6
Mid	100	6	6
High	500	6	6

Mortality
Each animal was checked for mortality or signs of morbidity at least twice daily during the treatment period.

Clinical observations
A check for clinical signs of toxicity was made at least twice daily (morning and afternoon) on all animals.

Body weight
Weekly determinations of individual body weight were performed for fourteen weeks. Bi-weekly determinations were made thereafter.

Food consumption and utilisation
Weekly determinations of daily food consumption were performed for fourteen weeks. Bi-weekly determinations were made thereafter.

Ophthalmoscopic examination
Ophthalmologic examinations were performed at pretest and prior to final necropsy on all animals.

Haematology and clinical chemistry
Laboratory investigations of haematology and clinical chemistry were performed on all the dogs before dosing started and again during months 3, 6, and 12 of treatment. The blood samples were taken from the jugular vein after the dogs had been fasted overnight.

EDTA was used as an anti-coagulant for evaluation of all parameters with the exception of prothrombin time for which citrate was used. The following haematological parameters were measured: Haemoglobin, haematocrit, red blood cell count, mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), reticulocyte count, total white blood cell and differential counts, platelet count and prothrombin time.

For clinical chemistry evaluations, serum was harvested from whole blood and analysed for the following parameters: Blood urea nitrogen, glucose, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, sodium, potassium, calcium, chloride, creatinine, total protein, albumin, globulin, cholesterol, alkaline phosphatase, gamma glutamyl transpeptidase, phosphorus, direct and total bilirubin.

Urinalysis
Urinalysis was performed on all the dogs before dosing started and again during months 3, 6, and 12 of treatment. Urine was collected using metabolism cages. The following parameters were measured: Appearance, pH, specific gravity, proteins, glucose, ketones, blood pigments, bilirubin and urobilinogen. Microscopic examination of the spun urine deposit was performed for the presence of bacteria, epithelial cells, white blood cells, red blood cells, crystals and abnormal constituents.

Sacrifice and pathology
After 12 months of consecutive treatment, all surviving animals were fasted overnight, sacrificed by intravenous sodium pentobarbital followed by exsanguination and subjected to a gross pathological examination. Terminal body weights were recorded immediately prior to sacrifice.

The following organ weights were determined: Adrenals, thyroids with parathyroids, pituitary, brain, heart, liver, kidneys, testes with epididymides and ovaries.

Tissue samples were taken from the following organs and preserved in buffered formalin: All gross lesions, adrenals, aorta, brain, caecum, colon, duodenum, epididymides, eyes, heart, ileum, jejunum, kidneys, liver, gallbladder, lungs, lymph nodes, mammary gland, oesophagus, ovaries, pancreas, pituitary gland, prostrate, rectum, rib, salivary gland (mandibular), spinal cord (cervical, mid-thoracic, lumbar), sciatic nerve, skeletal muscle, skin, spleen, stomach, testes, thymus, thyroid/parathyroids, trachea, urinary bladder and uterus. All tissues above from all groups were examined histopathologically.

Statistics
Non-categorical data from haematology, serum chemical and urinalysis were statistically examined by Dunnett's test for the comparison of multiple treatments with a control, and/or by inspection. Categorical data were examined to determine any remarkable group differences. Statistical evaluation of differences in body weights, food consumptions, terminal body weights and absolute organ weights between treated and control groups was accomplished by the use of Dunnett's test. The Mann-Whitney test with Bonferroni's inequality procedure was used to assess the organ/body weight ratios. Frequency of microscopic lesions between treated groups and controls was evaluated by the use of Fisher's Exact Test with Bonferroni's inequality procedure.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities.

B. CLINICAL OBSERVATIONS
Slightly higher incidences of abnormal excrement (bloody stool, yellow mucoid stool, diarrhoea, emesis) were observed in low and high level females when compared to their control group. Approximately one half of these observations were attributable to one female dog (approx. 17 %) from each of these groups. Females at the middle exposure level and males at all treatment levels had incidences of abnormal excrement similar to that of the control groups. One mid dose and one high dose female had localized skin redness with slight alopecia throughout most of the study. One low dose male had similarly reddened skin, but his condition lasted for a relatively short time. All other animals' skin was unaffected. These findings were considered of questionable relationship to Glyphosate administration due to a lack of apparent dose-relationship. The remaining observations were not unusual and were not attributed to glyphosate exposure.

C. BODY WEIGHT
No significant body weight changes occurred between any of the dose groups.

D. FOOD CONSUMPTION AND TEST SUBSTANCE INTAKE
There were no treatment-related effects. Food consumption in treatment groups was comparable to controls. Test compound was administered by gelatin capsule with dosages adjusted according to individual animal

body weight. Determination of the degree of absorption of the test item following dosing was not performed.

E. OPHTHALMOSCOPIC EXAMINATION

There were no test substance-related ophthalmological findings at the end of the treatment period.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

There were no changes in haematology parameters considered associated with glyphosate administration. Mild elevations in red blood cell counts (RBC), haemoglobin (HGB) and haematocrit (Hct) for low dose females at all test periods were similar to those observed at pretest. Mild changes in MCHC in males from the mid dose group (pretest and 12 months) and females from the low and mid dose groups (at 3 and 12 months) were not considered biologically significant.

Blood clinical chemistry

Phosphorus levels were statistically decreased in females from the low and high dose groups at 3 months and high dose group at 12 months when compared to controls. At 3 months, serum glutamic pyruvic transaminase (SGPT) values were significantly increased for high dose females. Sodium and potassium values were decreased in both sexes from high dose groups and for mid dose group males at 3 months, however this statistical difference was largely caused by slightly low control values at this time point. Statistically significant changes occurred in serum sodium (mid dose), glucose (mid dose) and calcium (low dose) values among males at 12 months. At 6 months, increased albumin values were statistically significant in females from the low dose group.

Decreased phosphorus levels, although statistically significant in females at 3 and 12 months, did not appear to be related to compound administration since the values were within the normal range. The changes in SGPT, sodium, potassium, calcium, glucose and albumin values were transient and/or within the normal range and were not considered an effect of glyphosate exposure.

Table 5.3.2-124: Twelve Month Study of Glyphosate Administered by Gelatin Capsule to Beagle Dogs [redacted], 1985): Intergroup comparison of selected clinical chemistry parameters (group means)

Time point	Dose Level [mg/kg bw/day]							
	Males				Females			
	0	20	100	500	0	20	100	500
Phosphorus [mg/dL)]								
Pre-test	6.5	↓6.4	↓6.4	↑6.6	6.6	↓6.3	↑6.7	↓6.0
3 months	5.4	↓5.3	↓5.1	↓5.1	5.2	↓**4.4***	↓4.5	↓**4.4***
6 months	3.8	↑4.0	↑4.3	↑3.9	4.1	↓3.8	↓3.8	↓3.7
12 months	3.2	↑3.4	↑3.3	↓2.8	3.8	↓3.5	↓3.5	↓**2.8***
SGPT [U/L]								
Pre-test	29.9	↑31.0	↓28.9	↑32.3	29.6	↑29.8	↑29.8	↑35.8
3 months	33.8	↓31.2	↑34.7	↑36.7	32.8	↑35.0	↑33.2	↑**42.8***
6 months	33.5	↑35.7	↓31.9	↑34.7	26.7	↑28.2	↑27.2	↑32.1
12 months	44.2	↓32.4	↓35.8	↓37.0	29.4	↑31.3	↑29.8	↑34.3
Sodium [mEq/L]								
Pre-test	146	↑147	146	146	144	↑145	↑146	144
3 months	150	↓149	↓**147****	↓**147****	151	↓150	↓149	↓**149***
6 months	147	↑149	↑148	↑149	142	142	↓141	142
12 months	148	↑149	↓**147***	148	147	147	↑148	147
Potassium [mEq/L]								
Pre-test	5.0	↓4.7	↓4.8	↓4.7	4.8	↓4.5	4.8	↓4.5
3 months	5.3	↓4.9	↓**4.7****	↓**4.5****	5.3	↓4.9	↓4.8	↓**4.5****
6 months	4.9	↓4.7	↓4.8	↓4.6	4.4	↓4.3	4.4	↓4.3

Table 5.3.2-124: Twelve Month Study of Glyphosate Administered by Gelatin Capsule to Beagle Dogs ([redacted], 1985): Intergroup comparison of selected clinical chemistry parameters (group means)

Time point	Dose Level [mg/kg bw/day]							
	Males				Females			
	0	20	100	500	0	20	100	500
12 months	4.7	↑4.9	4.7	4.7	4.7	↓4.5	↓4.4	↓4.4
Calcium [mg/dL]								
Pre-test	11.6	↓11.5	↓11.5	↓11.5	11.2	↑11.3	11.2	11.2
3 months	10.8	↑11.0	↓10.7	10.8	11.1	11.1	↓11.0	11.1
6 months	10.6	↓10.4	↓10.5	10.6	10.5	↑10.8	10.5	10.5
12 months	10.4	**↑10.9***	↓10.2	↓10.1	10.8	↑11.2	10.8	↓10.7
Glucose [mg/dL]								
Pre-test	119	↓117	↓112	↓116	111	↑124	↑112	↑115
3 months	108	↑113	↓102	↑112	106	106	↓103	106
6 months	94.8	↑100	↑97.7	↑101	103	↓101	↓99.7	↓101
12 months	108	↓101	**↓96.0***	↓102	106	↓103	↓99.2	↓98.5

* Statistically significant from controls ($p < 0.05$);
** Statistically significant from controls ($p < 0.01$)

G. URINALYSIS

There were no urine abnormalities detected at any of the sampling periods.

H. NECROPSY

Organ weights

Absolute and relative (to body weight) pituitary weights for males from mid and high dose groups and absolute brain weights for males from mid dose group were statistically lower than controls at the terminal sacrifice. Reductions in pituitary weights were not conclusively associated with compound administration. There were no microscopic changes correlated with these reductions. Reduced brain weights did not follow a dose-related trend and were not considered a treatment effect. No other significant differences were observed in absolute organ weights or organ to body weight ratios.

Table 5.3.2-125: Twelve Month Study of Glyphosate Administered by Gelatin Capsule to Beagle Dogs ([redacted], 1985): Results from select absolute and relative organ weight determination

	Dose Level [mg/kg bw/day]							
	Males				Females			
	0	20	100	500	0	20	100	500
Mean pituitary weight [g]	0.084 ± 0.003	↓0.080 ± 0.003	**↓0.062* *** ± 0.003	**↓0.068* *** ± 0.004	0.059 ± 0.010	↑0.075 ± 0.004	↑0.068 ± 0.008	↑0.068 ± 0.004
Relative pituitary weight [% bw]	0.001 ± 0.000	0.001 ± 0.000	**0.001* ± 0.000**	**0.001* ± 0.000**	0.001 ± 0.000	0.001 ± 0.000	0.001 ± 0.000	0.001 ± 0.000

* Statistically significant from controls ($p < 0.05$);
** Statistically significant from controls ($p < 0.01$)

Gross pathology

There were no compound-related or biologically significant differences in the incidence of gross lesions between the glyphosate treated animals and controls.

Histopathology

There were no histopathological findings related to treatment.

III. CONCLUSIONS

There were no mortalities in any of the dose groups. Slightly higher incidences of abnormal excrement (bloody stool, yellow mucoid stool, diarrhoea, and emesis) were observed in low and high level females when compared to their control group. Approximately one-half of these observations were attributable to only one female dog from each of these groups of 6 animals per group. Females at the middle exposure level and males at all treatment levels had incidences of abnormal excrement similar to that of the control groups. One mid dose and one high dose female had localised skin redness with slight alopecia throughout most of the study. Body weight, food consumption and ophthalmologic findings were normal throughout the test duration. Haematology, clinical chemistry and urine pathologic parameters plus gross and microscopic findings revealed no changes attributable to glyphosate exposure.

Since there was no conclusive evidence of toxicity in this study, the highest dosage (500 mg/kg bw/day) was considered a no-effect level (NOEL) by the study authors.

3. Assessment and conclusion

Assessment and conclusion by applicant:
In this study, glyphosate was administered orally by gelatin capsule to groups of six male and six female Beagle dogs at daily doses of 0, 20, 100 or 500 mg/kg bw/day for approximately twelve months. The study was conducted according to a testing regime in general accordance with OECD 452 (1981) and in compliance with GLP.

Although some unusual clinical observations (abnormal excrement, alopecia and skin redness) were noted among a few animals, these changes did not appear dose-dependent and were of questionable significance. Body weight, food consumption and ophthalmologic findings were normal throughout the test duration. All dogs survived the twelve months of testing. Clinical pathologic parameters plus gross and microscopic findings revealed no changes attributable to glyphosate exposure. Since there was no conclusive evidence of toxicity in this study, the highest dosage of 500 mg/kg bw/day is considered a no observed adverse effect level (NOAEL).

Assessment and conclusion by RMS:
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1. Information on the study

Data point:	CA 5.3.2/037
Report author	[redacted]
Report year	1982 (Revision/ English version 1992)
Report title	12 month dietary toxicity study with glyphosate in dogs
Report No	8012
Document No	Not reported
Guidelines followed in study	Not known
GLP	Not known, GLP not compulsory when study was conducted
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	Four Beagle dogs per sex and dose were fed diets for 12 months containing 0, 30, 100 or 300 ppm (equivalent to approx. 0, 0.75, 2.5 and 7.5 mg/kg bw/day based on application of diet conversion factor 40 for dogs published by Derelanko 2008) of glyphosate (purity and source not specified). Animals were observed for clinical signs. Body weights and food consumption were

	measured. Haematological and clinical chemistry evaluations were performed. The frequency of these observations and measurements are not known. All animals were subjected to gross pathological examination and histopathology. Organ weights were determined.
Short description of results:	There were no treatment-related clinical signs and no haematological, clinical chemistry, gross or histopathological changes with the possible exception of rounded hepatocytes and narrower sinusoids observed in the livers of some (2/4) high dose male dogs and mid (2/4) and high dose (3/4) females but not in the low dose and in the control groups. Since there was no further evidence of morphological or functional liver alterations, the reported findings while possibly treatment-related were not considered adverse effects. Thus, the NOAEL in this study was the highest dose of 300 ppm (approx. 8 mg/kg bw/day for the sexes combined).
Reasons for why the study is not considered relevant/reliable or not considered as key study:	Monograph (2000): The study was considered supplementary only due to reporting deficiencies (test substance purity missing, other deficiencies not specifically defined). The dates of the experimental work were not included in the report. RAR (2015): The study was considered unacceptable due to serious reporting deficiencies, e.g., absence of information on batch and purity of the test material. Therefore and since the study report is not available, this study is not considered invalid.
Reasons why the study report is not available for submission	The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL.
Category study in AIR 5 dossier (IUCLID docs)	Category 4b

CA 5.3.3 Other routes

No new subacute toxicity studies by the dermal and inhalation routes have been performed.

Table 5.3.3-1 summaries the studies performed in rats and rabbits by percutaneous application previously assessed in the 2001 and 2015 EU glyphosate evaluations.

Table 5.3.3-1: Studies on dermal 21-/28–day repeated-dose toxicity with glyphosate

Annex Point	Study	Species Study type	Substance(s)	Reference list-related category§	Result
CA 5.3.3/001 CA 5.3.3/002	[redacted], 1996 (Study Report) [redacted] 1996 (Appendix)	Alpk:AP$_f$SD (Wistar-derived) rat 21-day, dermal (0, 250, 500, 1000 mg/kg bw/day)	Glyphosate acid (Purity: 95.6 %)	Valid, Category 2a	NOAEL = 1000 mg/kg bw/day
CA 5.3.3/003	[redacted] 1993	Sprague-Dawley rat 21-day, dermal (1000 mg/kg	Glyphosate (Purity: 101.5 %)	Valid, Category 2a	NOAEL = 1000 mg/kg bw/day

Table 5.3.3-1: Studies on dermal 21-/28–day repeated-dose toxicity with glyphosate

		bw/day)			
CA 5.3.3/004 CA 5.3.3/005 CA 5.3.3/006	[redacted] 1994 (Part 1, Study Report) [redacted], 1994 (Part 2, Individual Data) [redacted], 1994 (Part 3, Individual Data)	New Zealand White rabbit 28–day, dermal (0, 500, 1000, 2000 mg/kg bw/day)	Glyphosate technical (Purity: 99.6 %)	Valid, Category 2a	NOAEL = 2000 mg/kg bw/day
CA 5.3.3/007	[redacted], 1985	New Zealand White rabbit 21-day, dermal (0, 500, 1000, 2000 mg/kg bw/day)	Glyphosate (Purity: not reported)	Invalid, Category 4b	NOAEL = 2000 mg/kg bw/day
CA 5.3.3/008	[redacted], 1982	New Zealand White rabbit 21-day, dermal (0, 100, 1000, 5000 mg/kg bw/day)	Glyphosate technical (Purity: not reported)	Valid, Category 2a	NOAEL = 5000 mg/kg bw/day

§: The category describes the acceptability/reliability of the study within the AIR 5 submission. This category does not describe hazard classification. (for details please refer to the Doc ID: 110054-B-GRG_Jun_2020)

The short-term percutaneous toxicity of glyphosate has been investigated in the rat and rabbit.

In both Sprague-Dawley ([redacted] 1993) and Alpk:APfSD (Wistar derived) ([redacted], 1996) rats, no signs of systemic toxicity were noted following dosing for 21 days at 1000 mg/kg bw/day, the limit dose for this study type. Therefore, in both studies the NOAEL was set at 1000 mg/kg bw/day.

Three studies were conducted in New Zealand White rabbits ([redacted] 1985, [redacted] 1982 and [redacted] 1994) and doses ranged from 100 mg/kg bw/day to 5000 mg/kg bw/day. The study by [redacted] (1985) was considered supportive due to serious reporting deficiencies in the first EU evaluation (2001) and not accepted in the RAR (2015). Therefore and since the study report is not available, this study is not considered to be reliable. The other two subacute dermal studies were well conducted, in general compliance with OECD 410 (1981) and GLP and thus, considered acceptable. No signs of treatment-related systemic toxicity were noted in either study, the highest NOAEL being 5000 mg/kg bw/day.

In both the rat and the rabbit no signs of systemic toxicity were evident following repeated application of glyphosate to the skin. The NOAEL for short term percutaneous toxicity was 1000 mg/kg bw/day in the rat and 5000 mg/kg bw/day in the rabbit as previously concluded in the 2001 EU glyphosate evaluation. Based on the [redacted] (2012) *in vitro* dermal absorption of glyphosate through rabbit skin (CA 5.8.2/014), with 2.66 % bioavailability, the systemic NOAEL for rabbits exposed dermally to glyphosate is determined to be 133 mg/kg bw/day. When compared with the previous AOEL point of departure, 50 mg/kg bw/day (oral dose in rabbits), the systemic oral NOAEL based on 20 % gastrointestinal absorption is 10 mg/kg bw/day. The systemic oral NOAEL is over 10-fold lower than the dermal value, which highlights that the previous AOEL is overly conservative and based on non-systemic effects (gastrointestinal effects).

Table 5.3.3-2 summaries the studies performed in rats by inhalation previously assessed in the 2001 and 2015 EU glyphosate evaluations.

Table 5.3.3-2: Studies on 14-day repeated-dose toxicity by inhalation of glyphosate

Annex Point	Study	Species Study type	Substance(s)	Reliability/ GLP	Result
CA 5.3.3/009	[redacted], 1985	Wistar rat 14-day, inhalation (aerosol) (0, 0.28, 0.90, 0.93, 3.8 mg/L)	Glyphosate technical (Purity: not reported)	Not valid, Category 4b	NOAEC = 3.8 mg/L (aerosol)

Only one study on subacute inhalative toxicity (14 days) in rodents ([redacted], 1985) is available and was reviewed in the 2001 EU evaluation. The previous review concluded no treatment-related effects were observed and the NOAEC for inhalation of an aerosol was 3.8 mg/L. However, compared to modern regulatory standards, the study was considered unacceptable due to serious reporting deficiencies in the last evaluation (2015) (e.g. missing details on test substance batch and purity). Therefore and since the study report is not available, this study is invalid.

Since glyphosate is non-volatile (VP = 1.31×10^{-5} Pa (25 °C)) the conduct of a 28–day inhalation study is not needed according to EU requirements. Therefore, no further inhalation studies were conducted.

90–day inhalation studies are only required for volatile substances (i.e. substances with a vapour pressure (VP) $>10^{-2}$ Pa). Since glyphosate is non-volatile (VP = 1.31×10^{-5} Pa (25 °C)), no 90–day inhalation toxicity study was conducted.

Publications on repeated-dose toxicity via other routes

A literature search for the active substance glyphosate was performed in accordance to the provisions of the EFSA Guidance "Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) 1107/2009" and updated Appendix to this Guidance document. The following publications were found relevant and reliable for this section and the summaries are thus presented below and are part of the general discussion at the beginning of the section.

Table 5.3.3-3 summarises the relevant publications.

Table 5.3.3-3: Publications on repeated-dose toxicity with glyphosate via other routes

Annex Point	Study	Species Study type	Substance(s)	Result	Comments
CA 5.3.3/010	Mesnage R. *et al.*, 2018	*In Vitro:* Differentiated HepaRG™ cells (HPR 116) (0.06 µM, 6 µM, 600 µM)	Glyphosate (Purity: ≥ 96 %)	No NOAEL derived Only weakly toxic *in vitro*	No positive control. No cytoxicity tests to determine appropriate concentration range tested.
CA 5.3.3/011	Kumar S. *et al.*, 2014	C57BL/6, TLR4–/–, IL-13–/– mouse Daily for 7 days or 3 times a week for 3 weeks, intranasal (100 ng, 1 µg, 100 µg)	Glyphosate reagent grade (Purity: not reported)	No NOAEL derived Pulmonary IL-13-dependent inflammation	Method for the collection and analysis of the air samples not validated.

In the study published by Mesnage *et al.* (2018), the effect of glyphosate on the transcriptome and metabolome profile of differentiated HepaRG cells was investigated at concentrations of 0.06, 6 and 600 µM *in vitro*. Glyphosate was found to be only weakly toxic inducing little change in transcriptome profiles when compared with the other herbicides tested in the same study.

The aim of the second study (Kumar S. *et al.*, 2014) was to explore the mechanisms of glyphosate-induced pulmonary pathology by utilizing murine models and real environmental samples. Mice of different strains inhaled extracts of glyphosate-rich air samples collected on farms during spraying of herbicides or inhaled different doses of glyphosate (100 ng, 1 µg, 100 µg applied intranasally to anesthetised mice). The cellular response, humoral response, and lung function of exposed mice were evaluated. Glyphosate-rich farm air samples as well as glyphosate alone were found to induce pulmonary IL-13-dependent inflammation and promote Th2 type cytokines, but not IL-4 for glyphosate alone. This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions since the method for the collection and analysis of the air samples was not validated.

Study summaries on repeated dose toxicity via other routes

1. Information on the study

Data point:	CA 5.3.3/001
Report author	[redacted]
Report year	1996 (Study report)
Report title	Glyphosate Acid: 21-Day Dermal Toxicity Study in Rats
Report No	[redacted]/P/4985
Document No	Not reported
Guidelines followed in study	OECD 410 (1981): OPPTS 870.3200 (1998): 87/302/EEC B.28 (1988)
Deviations from current test guideline (OECD 410, 1981)	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a
Data point:	CA 5.3.3/002
Report author	[redacted]
Report year	1996
Report title	Glyphosate Acid: 21-Day Dermal Toxicity Study in Rats - Appendix
Report No	[redacted]/P/4985
Document No	Not reported
Guidelines followed in study	OECD 410 (1981): OPPTS 870.3200 (1998): 87/302/EEC B.28 (1988)
Deviations from current test guideline (OECD 410, 1981)	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

In a dermal toxicity study groups of five male and five female Alpk:AP$_f$SD (Wistar-derived) rats received 6-hour dermal applications of 0 (control), 250, 500 or 1000 mg glyphosate acid/kg bw/day. Glyphosate acid was prepared as a paste using deionised water as the control substance and vehicle. A total of 15 applications were made over a 21 day period (5 applications per week).

Clinical observations were made and body weights and food consumption were measured, and at the end of the scheduled period, the animals were killed and subjected to an examination *post mortem*. Cardiac blood samples were taken for clinical pathology, selected organs were weighed and specified tissues were taken for subsequent histopathological examination.

There were no clinical signs of systemic toxicity at any dose level and no adverse compound related effects on body weight, food consumption, haematology, clinical chemistry or organ weights. There was no evidence of toxicity at histopathological examination.

There was no evidence of systemic toxicity or dermal irritation following 15 dermal applications over a 21 day period of up to 1000 mg glyphosate acid/kg bw/day.

I. MATERIALS AND METHODS

A: Materials

1. **Test material:** Glyphosate acid
   - Description: Technical, white solid
   - Lot/Batch number: P24
   - Purity: 95.6 % w/w a.s.
   - CAS#: Not reported
   - Stability of test compound: Not reported
2. **Vehicle and/or positive control:** Deionised water / none
3. **Test animals:**
   - Species: Rat
   - Strain: Alpk:APfSD
   - Age/weight at dosing: 6 – 8.5 weeks / males 214 – 249 g, females 193 – 227 g
   - Source: [redacted]
   - Housing: Individually, in cages on multiple rat racks suitable for animals of this strain and weight range expected during the course of the study.
   - Acclimatisation period: At least 5 days
   - Diet: Diet (PCD) supplied by Special Diet Services Limited, Witham, Essex, UK, *ad libitum*
   - Water: Mains water, *ad libitum*
   - Environmental conditions: Temperature: 21 ± 2 °C
     Humidity: 40 – 70 %
     Air changes: At least 15 changes / hour
     Photoperiod: 12 hours light / 12 hours dark

B: Study design and methods

In-life dates: 1996-01-10 to 1996-02-01

Animal assignment
The study was divided into ten (randomised blocks), each containing one cage per treatment group. The animals were randomly allocated to groups as shown below:

Table 5.3.3-4: Glyphosate Acid: 21 Day Dermal Toxicity Study in Rats ([redacted], 1996): Study design

Test group	Dose level of glyphosate acid [mg/kg bw/day]	Males	Females
Control	0	5	5
Low	250	5	5
Mid	500	5	5
High	1000	5	5

Preparation and treatment of animal skin
Sixteen to twenty-four hours before application of the test substance, the hair was removed with a pair of veterinary clippers from an area, approximately 10 cm × 5 cm, on the dorso-lumbar region of each animal. The rats were dosed dermally and the amount applied was calculated for each animal according to its weight at the time of dosing. The paste covered by a gauze patch (approximately 7 cm × 7 cm × 4-ply) was applied to the shorn back of each animal and was kept in contact with the skin for approximately 6 hours using an occlusive dressing. The gauze patch was covered by a patch of plastic film (7 cm × 7 cm) and was held in position using adhesive bandage (25 cm × 7.5 cm). This was secured by two pieces of PVC tape (approximately 2.5 cm × 20 cm) wrapped around the animal. The control animals were treated in a similar manner except that deionised water only was used. The rats were dosed sequentially in group order at approximately the same time each day.

At the end of each 6-hour contact period, the dressings were carefully removed. The skin, at the site of application, was cleansed using clean swabs of absorbent cotton wool soaked in clean warm water and was then dried gently with clean tissue paper.

A total of 15 six-hour applications were made during a period of 21 days. During this time there were three two-day periods when the animals were not dosed. Following each application there was an 18-hour 'rest' period during which the animals were fitted with plastic collars to prevent oral contamination.

Observations
Prior to the start of the study, all rats were examined to ensure that they were physically normal and exhibited normal activity. Detailed clinical observations were recorded daily and after decontamination. Cage-side observations were also made as soon as possible after dosing, and towards the end of the working day.

Body weight
The body weight of each rat was recorded daily, immediately prior to application of the test substance where applicable and prior to termination on day 22.

Food consumption and test substance intake
Food consumption was recorded continuously throughout the study for each rat and calculated as a weekly mean (g food/rat/day).

Haematology and clinical chemistry
Blood was collected at termination, by cardiac puncture and the following parameters were examined: Haemoglobin, haematocrit, red blood cell count, mean cell volume, mean cell haemoglobin, red cell distribution width, activated partial thromboplastin time, mean cell haemoglobin concentration, platelet count, total white cell count, differential white cell count, blood cell morphology and prothrombin time.

Clinical chemistry
Blood was collected at termination, by cardiac puncture and the following parameters were examined: Urea, creatinine, glucose, albumin, total protein, cholesterol, triglycerides, total bilirubin, creatine kinase activity, alkaline phosphatase activity, aspartate aminotransferase activity, alanine aminotransferase activity, gamma-glutamyl transferase activity, calcium, phosphorus (as phosphate), sodium, potassium and chloride.

Investigations *post mortem*
Macroscopic examination
All animals were examined *post mortem*. This involved an external observation and an internal examination of all organs and structures.

Organ weights
From all animals surviving to scheduled termination, the following organs were removed, trimmed free of extraneous tissue and weighed: Adrenal glands, kidneys, liver and testes.
Paired organs were weighed together.

Tissue submission
The following tissues were examined *in situ*, removed and examined and fixed in an appropriate fixative: Gross lesions including masses, testis*, kidney, liver, adrenal gland*, epididymis*, treated skin and untreated skin.
*: Tissues marked were stored and not examined microscopically

Microscopic examination
All selected tissues processed from the control and 1000 mg glyphosate acid/kg bw/day, together with macroscopic abnormalities from these groups, were examined by light microscopy.

Statistics
Haematology, clinical chemistry, organ weights and weekly food consumption were analysed using analysis of variance. Body weights, on initial (day 1) body weight, organ weights on final body weight were analysed using analysis of covariance. All data were analysed using *SAS (1989*).

II. RESULTS AND DISCUSSION

Mortality
There were no mortalities.

Clinical observations
There were no significant signs of toxicity at any dose level of glyphosate acid. Generally the clinical findings observed were consistent with those commonly seen in dermal studies as a consequence of bandaging and were considered not to be related to treatment with glyphosate acid.

Body weight and weight gain
There were no effects due to treatment with glyphosate acid on body weight at any dose level.

Food consumption
There were no effects due to treatment with glyphosate acid on body weight at any dose level.

Haematology
A minimal statistically significant increase in haemoglobin levels was observed in females dosed at 1000 mg glyphosate acid/kg bw/day. A statistically significant decrease compared with control was seen in red cell distribution width in females dosed at 250 and 1000 mg glyphosate acid/kg bw/day. In the absence of any adverse effects on the red cell parameters, these minor changes are considered not to be of toxicological significance.

Table 5.3.3-5: Glyphosate Acid: 21 Day Dermal Toxicity Study in Rats ([redacted], 1996): Intergroup comparison of selected haematology parameters

Parameter	Dose level of glyphosate acid [mg/kg bw/day]							
	Males				Females			
	0	250	500	1000	0	250	500	1000
Haemoglobin [g/dL]	15.2 ± 0.4	15.3 ± 0.6	15.3 ± 0.3	15.0 ± 0.4	13.9 ± 0.6	13.7 ± 0.8	14.1 ± 0.6	**14.6* ± 0.3**
Red cell distribution width [%]	13.1 ± 0.9	12.9 ± 0.9	12.6 ± 0.3	13.4 ± 0.6	13.8 ± 1.2	**12.4** ± 0.8**	13.0 ± 0.4	**12.6* ± 0.7**

* Statistically significant difference from control group mean ($p < 0.05$; Student's t-test, 2-sided);
** Statistically significant difference from control group mean ($p < 0.01$; Student's t-test, 2-sided)

Blood clinical chemistry

Females dosed at 1000 mg glyphosate acid/kg bw/day showed a minimal, but statistically significant increase in plasma urea levels, but there were no differences seen in the plasma creatinine levels. This minimal change in urea was considered not to be of toxicological significance. A minimal but statistically significant decrease in plasma triglycerides was observed in males dosed at 500 mg glyphosate acid/kg bw/day and as this did not form part of a dose response relationship was considered not to be treatment related.

Table 5.3.3-6: Glyphosate Acid: 21 Day Dermal Toxicity Study in Rats ([redacted], 1996): Intergroup comparison of selected clinical chemistry parameters (mean values)

Parameter	Dose level of glyphosate acid [mg/kg bw/day]							
	Males				Females			
	0	250	500	1000	0	250	500	1000
Plasma urea [mmol/L]	8.4 ± 0.5	8.2 ± 0.2	8.5 ± 0.2	8.1 ± 0.8	7.6 ± 0.5	7.7 ± 1.0	6.9 ± 0.5	**8.6* ± 1.1**
Plasma creatinine [µmol/L]	56.8 ± 2.2	57.2 ± 4.4	57.8 ± 4.1	54.4 ± 4.7	58.2 ± 2.3	58.6 ± 3.4	58.0 ± 6.6	58.2 ± 2.4
Plasma trigycerides [mmol/L]	1.27 ± 0.28	1.01 ± 0.17	**0.87* ± 0.22**	1.27 ± 0.34	0.70 ± 0.11	0.66 ± 0.21	0.69 ± 0.19	0.76 ± 0.35

* Statistically significant difference from control group mean ($p < 0.05$; Student's t-test, 2-sided)

Sacrifice and pathology

Organ weights

Testes weights were slightly, but statistically significantly decreased at 500 mg glyphosate acid/kg bw/day, due to one animal having a very low weight recorded. There were no effects due to treatment with glyphosate acid in the other organs weighed.

Macroscopic findings

A small number of lesions were observed in a few animals in the 0 and 500 mg/kg bw/day groups, none of which were related to treatment.

Microscopic findings

A small number of common spontaneous lesions were observed, none of which were related to treatment.

III. CONCLUSIONS

There were no clinical signs of systemic toxicity at any dose level and no adverse compound related effects on body weight, food consumption, haematology, clinical chemistry or organ weights. There was no evidence of toxicity at histopathological examination.

There was no evidence of systemic toxicity or dermal irritation following 15 dermal applications over a 21 day period of up to 1000 mg glyphosate acid/kg bw/day.

The no-effect level (NOEL) for systemic toxicity and dermal irritation was considered to be 1000 mg glyphosate acid/kg bw/day in both sexes.

3. Assessment and conclusion

Assessment and conclusion by applicant:
In this dermal toxicity study, groups of five male and five female Alpk:AP$_f$SD (Wistar-derived) rats received 6 hour dermal applications of 0 (control), 250, 500 or 1000 mg glyphosate acid/kg bw/day. The study was conducted according to OECD 410 (1981) and in compliance with GLP.
There was no evidence of systemic effects of toxicological significance or dermal irritation following 15 dermal applications over a 21 day period of up to 1000 mg glyphosate acid/kg bw/day.
The NOAEL for systemic toxicity and dermal irritation was considered to be 1000 mg glyphosate acid/kg bw/day in both sexes.

Assessment and conclusion by RMS:
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1. Information on the study

Data point:	CA 5.3.3/003
Report author	[redacted] *et al.*
Report year	1993
Report title	Glyphosate: 3 Week Toxicity Study in Rats with Dermal Administration
Report No	7839
Document No	Not reported
Guidelines followed in study	US EPA 82-2 (Pesticide Assessment Guidelines, Subdivision F); in general compliance with OECD 410 (1981)
Deviations from current test guideline (OECD 410, 1981)	Mean weight of the female rats were slightly lighter than requested (195 g instead of 200 – 300 g), organ weights of the adrenals were not determined.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

A group of 5 male and 5 female Sprague-Dawley rats were dosed daily with glyphosate via the dermal route of application, for a period of ca 6 h per day for 3 weeks. The group was dosed at a constant volume of 3 mL/kg body weight at a dose level of 1000 mg glyphosate/kg bw/day. A further group of 5 males and 5 females received vehicle only (diethylphthalate) dermally at the same dose volume to act as a control. Blood samples were collected for haematology and clinical chemistry screens during week 3. After 3 weeks of dosing all rats were killed and necropsied and selected organs weighed. All control and high dose rats underwent a limited histological examination.

No effect on mortality or clinical signs could be observed. Findings in body weight changes or food/water consumption were not consistent and considered to be not treatment-related. The skin assessment showed a minor reaction to the treatment of glyphosate. Haematology, clinical chemistry or organ weights revealed no notable intergroup differences in either sex. Histopathological examination did not reveal any finding that could be attributed to dosing with glyphosate.

In conclusion, following dermal administration of glyphosate to Sprague-Dawley rats for 3 weeks at a dose level of 1000 mg/kg bw/day, there was no evidence of systemic toxicity. A mild transitory irritant effect was noted at the dosing site.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate
Description:	White crystalline solid
Lot/Batch #:	229-Jak-142-6
Purity:	101.5 %
Stability of test compound:	The article is stable at least 2 years from date of analysis when stored at ambient temperature in the dark

2. Vehicle and/or positive control: Diethylphthalate / none

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	Ca. 8 weeks
Sex:	Male and female
Weight at dosing:	♂ 295 g; ♀ 195 g
Acclimation period:	25 days
Diet/Food:	SDS Rat and Mouse (modified) No. 1 Diet SQC (supplied by Special Diets Services Limited, Witham, Essex, CM8 3AD), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in polypropylene cages (over all dimensions ca 420 × 270 × 200 mm) with stainless steel wire grid tops and bottoms.

Environmental conditions: Temperature: 20 ± 2 °C
Humidity: 50 ± 15 %
Air changes: 15 air changes per hour
12 hours light/dark cycle

B: Study design and methods

In life dates: 1992-04-06 to 1992-04-27

Animal assignment and treatment:
A group of 5 male and 5 female Sprague-Dawley rats were dosed daily with glyphosate via the dermal route of application, for a period of ca 6 h per day for 3 weeks. The group was dosed at a constant volume of 3 mL/kg body weight at a dose level of 1000 mg/kg bw/day (high limit dose). A further group of 5 males and 5 females received vehicle only (diethylphthalate) dermally at the same dose volume to act as a Control.

Table 5.3.3-7: Glyphosate – 3 Week Toxicity Study in Rats with Dermal Administration ([redacted] 1993): Study design

Test group	Treatment [mg/kg bw/day]	Males	Females
Control (1)	0	5	5
High (2)	1000	5	5

The hair on the animals' backs was clipped as necessary (once during Week 1, twice during Week 3 and not clipped at all during Week 2) and the test material applied daily at the constant volume of 3 mL/kg bw, over an area of approximately 10 % of the total body surface area. After application of the test material the dosing site was covered by a piece of gauze with a silver foil back (approximately 4 cm diameter). The treated area was protected by a semi-occlusive dressing (Micropore, 3M) held in place by means of a non-irritating tape (Blenderm, 3M). Dermal exposure was ca 6 h per day, the site of exposure being cleaned of test material with a soft cloth soaked in diethylphthalate immediately after removal of the dressing. If the animals managed to remove the dressing during the dosing period it was re-applied for the remainder of the 6 h period. A record of animals which managed to remove the dressing was maintained and is shown below.

Table 5.3.3-8: Glyphosate – 3 Week Toxicity Study in Rats with Dermal Administration ([redacted], 1993): Dress removal of tested animals

Timepoint	Group/dose level [mg/kg bw/day]							
	Animals found without dressing				Dressing chewed			
	1/0 ♂	2/1000 ♂	1/0 ♀	2/1000 ♀	1/0 ♂	2/1000 ♂	1/0 ♀	2/1000 ♀
Week 1	0	0	No. 14 on one occasions	0	0	0	0	0
Week 2	0	0	No. 13 on two occasions No. 14 on five occasion	0	0	0	No. 14 on one occasion	0
Week 3	0	0	No. 14 on one	0	0	0	No. 13 on one	0

Table 5.3.3-8: Glyphosate – 3 Week Toxicity Study in Rats with Dermal Administration ([redacted], 1993): Dress removal of tested animals

Timepoint	Group/dose level [mg/kg bw/day]							
	Animals found without dressing				Dressing chewed			
	1/0 ♂	2/1000 ♂	1/0 ♀	2/1000 ♀	1/0 ♂	2/1000 ♂	1/0 ♀	2/1000 ♀
			occasion				occasion No. 14 on one occasion	

Preparation of Dosing Suspensions
Dosing suspensions were prepared daily, using diethylphthalate (DEP) as vehicle. Water was originally intended as the vehicle for the study. However, this formed a very poor suspension with glyphosate and allowed a constant dose volume of only 2 mL/kg bw. Therefore, after experimentation at IRI, the vehicle was changed to diethylphthalate. This has been used successfully at IRI as a vehicle in previous dermal studies; it formed a far more homogeneous suspension with glyphosate, and allowed a constant dose volume of 3 mL/kg bw.

Analysis of the dosing suspensions
Analysis of the dosing suspension was conducted in another study and not reported.

Mortality
Animals were checked twice daily for mortality.

Clinical observations
All animals were examined for reaction to treatment during the day. The onset, intensity and duration of all signs were recorded.
In addition, all animals received a detailed clinical examination once each week.

Skin Assessment
Once each week all animals received a detailed examination of skin for erythema, eschar formation and oedema formation.

Table 5.3.3-9: Glyphosate – 3 Week Toxicity Study in Rats with Dermal Administration ([redacted], 1993): Grading system for the skin assessment

Erythema and eschar formation	No erythema	0
	Very slight erythema (barely perceptible)	1
	Well defined erythema	2
	Moderate to severe erythema	3
	Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Oedema formation	No oedema	0
	Very slight oedema (barely perceptible)	1
	Slight oedema (edges of area well defined by definite raising)	2
	Moderate oedema (edges raised approximately 1 mm)	3
	Severe oedema (raised by more than 1 mm and extending beyond the area of exposure)	4
Skin thickening	Normal	0
	Thicker than normal	1
Desquamation	No desquamation	0
	Mild desquamation (dry skin)	1
	Moderate desquamation (flaky skin)	2
	Severe desquamation (skin cracking)	3

Body weight
The weight of each animal was recorded twice during the week before the start of treatment, on five occasions during Week 1 and twice each week thereafter.

Food and water consumption
The quantity of food consumed by each animal was recorded twice each week starting 1 week pre-trial up until the end of the study.
Water consumption was monitored by visual inspection throughout the treatment period.

Haematology and clinical chemistry
Samples were taken from all animals during Week 3. Blood samples were collected from the orbital sinus under light ether anaesthesia (except Hepato Quick which was taken by tail snip) with overnight food deprivation.

Haematology:
The following parameters were determined: Haematocrit, haemoglobin, total red blood cell count, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration, Hepato Quick (clotting time), total white blood cell count and differential white blood cell count.

Clinical chemistry
The following parameters were determined: Aspartate amino transferase (AST), alanine aminotransferase (ALT), creatinine (Crea), total protein (TP), albumin (Alb), AG ratio (AG-R), blood urea nitrogen (BUN), glucose (Glu), total bilirubin (T Bi), sodium (Na), potassium (K), chloride (Cl), calcium (Ca), inorganic phosphorus (P).

Sacrifice and pathology
All animals were killed and necropsied after 3 weeks dosing. Method of killing was by carbon dioxide asphyxiation followed by exsanguination. The gross dissection and necropsy were performed under the supervision of a pathologist.

Organ weights
The following organs were weighed: Kidneys, liver and testes (with epididymides).

Histopathology
Histopathological examinations of the following organs were performed: Abnormal tissue, dosing site, kidneys, liver, lungs, normal skin, ovaries, spleen and testes (with epididymides).

Statistics
Body weight, haematology and clinical chemistry data were statistically analysed for homogeneity of variance using the 'F-max' test. If the group variances appeared homogeneous a parametric ANOVA was used and pairwise comparisons made via Student's t-test using Fisher's F-protected LSD. If the variances were heterogeneous, log or square root transformations were used in an attempt to stabilise the variances. If the variances remained heterogeneous, then a non-parametric test such as Kruskal-Wallis ANOVA was used. Individual between group comparisons were made using Fisher's F-protected LSD method.

Organ weights were analysed as above and conditional on body weight (i.e. analysis of covariance).
Histology data were analysed using Fisher's Exact Probability test.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF THE FORMULATED DOSING SUSPENSIONS

Analysis of the formulated dosing suspension was conducted in another study and not reported.

B. MORTALITY
There were no premature deaths.

C. CLINICAL OBSERVATIONS
There were no clinical signs observed which were considered to be related to treatment with glyphosate.

D. SKIN ASSESSMENT
During Week 2, 2/5 male animals of the high dose group and 3/5 female animals of the high dose group showed very slight erythema, however, during Week 3, this finding was only apparent in 1/5 of the high dose females.

Also during Week 2, desquamation was apparent in 3/5 male animals of the high dose group (ranging from moderate to severe) and in all female high dose animals (ranging from mild to severe), however, during Week 3, only mild desquamation was apparent in 1/5 high dose males and thickening and severe desquamation were apparent in only 1/5 high dose females.

No findings were noted in Control males or females at any time during the study.

Table 5.3.3-10: Glyphosate – 3 Week Toxicity Study in Rats with Dermal Administration ([redacted], 1993): Skin assessment

Group / Dose level [mg/kg bw/day]							
Group 1/0 ♂		Group 1/0 ♀		Group 2/1000 ♂		Group 2/1000 ♀	
Animal #	Assessment	Animal #	Assessment	Animal #	Assessment	Animal #	Assessment
1	No reaction	11	No reaction	6	No reaction	16	Week 2: severe desquamation
2	No reaction	12	No reaction	7	Week 2: slight erythema and moderate desquamation	17	Week 2: slight erythema and moderate desquamation
3	No reaction	13	No reaction	8	Week 2: slight erythema and severe desquamation	18	Week 2: slight erythema and severe desquamation Week 3: slight erythema, thickened skin and severe desquamation
4	No reaction	14	No reaction	9	No reaction	19	Week 2: slight erythema and moderate desquamation
5	No reaction	15	No reaction	10	Week 2: moderate desquamation Week 3: mild desquamation	20	Week 2: mild desquamation

E. BODY WEIGHT
The high dose males showed a moderate reduction in body weight gain (17 %) and the high dose females

showed a large increase in body weight gain (58 %) when compared to their respective controls.

In absolute terms, these changes did not reach statistical significance at all in males, and only once in females (during week 3, $p \leq 0.05$). This lack of statistical difference along with the fact that there was such a wide variation between the sexes and that similar changes have not been observed in previous studies using glyphosate led to the suggestion by the study authors that these changes are not related to treatment with glyphosate.

There were no other notable intergroup differences.

Table 5.3.3-11: Glyphosate – 3 Week Toxicity Study in Rats with Dermal Administration ([redacted], 1993): Group Mean Body weight data [g]

Treatment period [days]	Group/Dose level [mg/kg bw/day]			
	1/0 ♂	2/1000 ♂	1/0 ♀	2/1000 ♀
-7	241	↑249	172	172
-4	258	↑265	181	↑185
0	294	↑295	193	↑200
3	307	↓304	198	↑205
7	326	↓322	206	↑222
10	338	↓331	216	↑229
14	354	↓348	224	↑243
17	363	↓354	229	↑255
21	364	↓353	231	↑**260***
Body weight gain from day 0 – 21 [g]	70	↓58	38	↑60
% of control	-	83	-	158

* Significantly different from control, $p \leq 0.05$

F. FOOD AND WATER CONSUMPTION

There were no notable intergroup differences for food consumption in males. In females, there was a slight increase in food consumption in the high dose females (14 %) when compared to controls. With the lack of a similar effect in males, and as this finding has not been seen in previous studies using glyphosate, this increase was considered by the study authors a chance effect and not of toxicological importance.

There were no other notable intergroup differences.

Table 5.3.3-12: Glyphosate – 3 Week Toxicity Study in Rats with Dermal Administration ([redacted], 1993): Summary of food consumption data [g/rat/time period]

Treatment period [days]	Group/Dose level [mg/kg bw/day]			
	1/0 ♂	2/1000 ♂	1/0 ♀	2/1000 ♀
-7 to -4	28.9	↑31.2	23.1	↑25.2
-4 to 0	32.1	↓31.8	22.3	↑25.5
0-3	33.5	↓31.1	21.9	↑25.1
3-7	35.5	↓33.2	23.5	↑26.8
7-10	33.3	↓32.9	24.5	↑28.0
10-14	34.8	↓33.7	25.9	↑30.5
14-17	34.7	↓34.0	26.5	↑29.5
17-21	38.3	↑39.2	31.1	↑34.7
Total food consumed from day 0 – 21 [g]	701	↓679	510	↑581
% of control	-	97	-	114

There were no visual intergroup differences in either sex noted for water consumption.

G. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

Males: There was a decrease in monocytes (44 %, $p \leq 0.05$) and large unstained cells (62 %, $p \leq 0.05$) in the high dose males compared to Controls.

The study authors stated that these findings have not been seen in previous studies using glyphosate and the decrease in monocytes may partly be due to higher than normal values in Control animals (IRI historical data show a mean of 0.226 ± 0.126, number of samples being 74).

There were no other notable intergroup differences.

Females: There was an increase in MCH (4 %, $p \leq 0.05$) and MCV (4 %, $p \leq 0.01$) in the high dose females compared to Controls.

There was a reduction in neutrophils (31 %, $p \leq 0.05$) in the high dose females compared to Controls.

Again according to the study authors, these findings have not been seen in previous studies using glyphosate.

There were no other notable intergroup differences.

Table 5.3.3-13: Glyphosate – 3 Week Toxicity Study in Rats with Dermal Administration ([redacted], 1993): Summary of selected group mean haematology data

Parameter	Group/Dose level [mg/kg bw/day]			
	1/0 ♂	2/1000 ♂	1/0 ♀	2/1000 ♀
Monocytes [$\times 10^9$/L]	0.57 ±0.17	**↓0.32* ± 0.07**	0.23 ± 0.09	↓0.22 ± 0.11
Large unstained cells [$\times 10^9$/L]	0.29 ± 0.14	**↓0.11* ± 0.02**	0.06 ± 0.01	↑0.08 ± 0.04
Mean cell haemoglobin [pg]	19.6 ± 1.0	↓19.4 ± 0.4	19.9 ± 0.2	**↑20.7* ± 0.6**
Mean cell volume [fL]	54.6 ± 1.9	↓54.0 ± 1.3	53.5 ± 0.9	**↑55.8** ± 1.3**
Neutrophils [$\times 10^9$/L]	3.34 ± 1.65	↓1.99 ± 0.62	1.68 ± 0.30	**↓1.16* ± 0.41**

* Significantly different from Control, $p \leq 0.05$;
** Significantly different from Control, $p \leq 0.01$

Blood clinical chemistry

There were no notable intergroup differences in either sex.

H. NECROPSY

Organ weights

There were no notable intergroup differences in either sex.

Gross pathology

Unilateral dilatation of the kidneys was seen in 2/5 high dose males compared to 0/5 Controls. This is a common finding in rats of this age and not considered related to treatment with glyphosate.

There were no notable intergroup differences for females.

Histopathology

Unilateral papillary necrosis was seen in the kidney in 1/5 high dose males and urothelial hyperplasia was seen in the kidney in 2/5 high dose males, both compared to 0/5 in Controls. These lesions are relatively common in rats of this age and strain and are not considered to be related to treatment with glyphosate.

Pelvic dilation was seen in 3/5 high dose males compared to 0/5 in Controls.

There were no other notable intergroup differences for males or females.

Table 5.3.3-14: Glyphosate – 3 Week Toxicity Study in Rats with Dermal Administration ([redacted], 1993): Summary of selected haematology data

Parameter		Group/Dose level [mg/kg bw/day]			
		1/0 ♂	2/1000 ♂	1/0 ♀	2/1000 ♀
Kidneys	No abnormality detected	3	1	4	5
	Localised urothelial hyperplasia (Grade +/-)	0	2	0	0
	Total incidence for score expanded finding	0	2	0	0
	Basophilic tubules (Grade +/-)	0	1	0	0
	Total incidence for score expanded finding	0	1	0	0
	Pelvic dilatation (Grade +/-) (Grade ++) (Grade ++1-)	0 0 0	1 1 1	1 0 0	0 0 0
	Total incidence for score expanded finding	0	3	1	0
	Localised papillary necrosis	0	1	0	0
	Nephropathy (Grade +/-) (Grade +)	1 1	1 0	0 0	0 0
	Total incidence for score expanded finding	2	1	0	0

III. CONCLUSIONS

No effect on mortality or clinical signs could be observed. Findings in body weight changes or food/water consumption were not consistent and considered to be not treatment-related. The skin assessment showed a minor reaction to the treatment of glyphosate. Haematology, clinical chemistry or organ weights revealed no notable intergroup differences in either sex. Histopathological examination did not reveal any finding that could be attributed to dosing with glyphosate.

In conclusion, following dermal administration of glyphosate to Sprague-Dawley rats for 3 weeks at a dose level of 1000 mg/kg bw/day, there was no evidence of systemic toxicity. A mild transitory irritant effect was noted at the dosing site.

3. Assessment and conclusion

Assessment and conclusion by applicant:
In this study, a group of 5 male and 5 female Sprague-Dawley rats were dosed daily with glyphosate via the dermal route of application, for a period of ca 6 h per day for 3 weeks. The study was in general compliance with OECD 410 (1981) and GLP.

In conclusion, following dermal administration of glyphosate to Sprague-Dawley rats for 3 weeks at a limit dose level of 1000 mg/kg bw/day, there was no evidence of systemic toxicity. A mild irritant effect was noted at the dosing site.

Therefore, the NOAEL for dermal administration in rats under the conditions of this study can be set at 1000 mg/kg bw/day.

Assessment and conclusion by RMS:
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1. Information on the study

Data point	CA 5.3.3/004
Report author	[redacted]
Report year	1994
Report title	Glyphosate technical (Alkaloida, Tiszavasvári): Repeated dose twenty-eight- Day dermal toxicity study in rabbits (Part 1, Study Report)
Report No	MÜF 214/94
Document No	Not reported
Guidelines followed in study	No guideline followed; in general compliance with OECD guideline 410 (1981)
Deviations from current test guideline (OECD 410, 1981)	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a
Data point	CA 5.3.3/005
Report author	[redacted]
Report year	1994
Report title	Glyphosate technical (Alkaloida, Tiszavasvári): Repeated dose twenty-eight- Day dermal toxicity study in rabbits (Part 2, Individual data)
Report No	MÜF 214/94
Document No	Not reported
Guidelines followed in study	No guideline followed; in general compliance with OECD guideline 410 (1981)
Deviations from current test guideline (OECD 410, 1981)	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a
Data point	CA 5.3.3/006
Report author	[redacted]
Report year	1994
Report title	Glyphosate technical (Alkaloida, Tiszavasvári): Repeated dose twenty-eight- Day dermal toxicity study in rabbits (Part 3, Individual data)

Report No	MÜF 214/94
Document No	Not reported
Guidelines followed in study	No guideline followed; in general compliance with OECD guideline 410 (1981)
Deviations from current test guideline (OECD 410, 1981)	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The toxicity potential of glyphosate technical was assessed after repeated dermal application to groups of male and female New Zealand white rabbits. Doses of 0, 500, 1000 or 2000 mg/kg bw/day were applied for a 6-hour period on five consecutive days per week over 4 weeks. For application the solid test substance was mixed with water resulting in a 50 % (w/v) solution, and spread evenly over the application site.

There were no mortalities and no treatment-related signs of systemic toxicity. Very slight erythema was noted in one high-dose male and one low-dose female. However, this effect is not considered biologically significant and was not seen in the histopathological examination.
There were no treatment-related effects on body weight, food consumption, haematological and clinical chemistry parameters observed in any of the dose groups. The macroscopic and histopathological findings observed at necropsy were considered incidental and unrelated to the test substance.

Repeated dermal administration of glyphosate technical to rabbits for a period of 28 consecutive days at doses of up to 2000 mg/kg bw/day resulted only in slight dermal irritation in one high-dose male and one low-dose female. There were no treatment-related systemic signs of toxicity.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate technical
Description:	White powder
Lot/Batch #:	39730494
Purity:	99.6 %
Stability of test compound:	Not reported

2. Vehicle and/or positive control: Water

3. Test animals:

Species: Rabbit

Strain:	New Zealand
Source:	[redacted]
Age:	Young, adult
Sex:	Male and female
Weight at dosing:	♂ 2200 – 2800 g; ♀ 2100 – 2500 g
Acclimation period:	7 days
Diet/Food:	Altromin Rabbit Chow, *ad libitum*
Water:	Water, *ad libitum*
Housing:	Individually in wire mesh cages
Environmental conditions:	Temperature: 18 ± 2 °C Humidity: Not reported Air changes: 10 / hour 12 hours light / dark cycle

B: Study design and methods

In life dates: 1994-05-23 to 1994-06-20

Animal assignment and treatment:
The potential dermal toxicity of glyphosate technical after repeated exposure was assessed using young adult New Zealand albino rabbits (males and females). Five rabbits per sex per dose received daily dermal applications of 0, 500, 1000 or 2000 mg/kg bw/day, five days per week for a total of 20 applications.

Two days prior to the first application about 15 % of the skin of the dorsal back of the animals was clipped free of hair. The clipping was repeated weekly thereafter.

For each application the test substance was mixed with water to give a final concentration of 50 % (w/v) of glyphosate. Each dose was spread evenly over about 10 % of the body surface area and covered with a occlusive dressing made of polyethylene material that was secured by hypoallergenic Leucoplast tape. After an exposure period of six hours the dressings were removed and the application site was cleaned with hand soap, water and clean, absorbent paper pads. Applications were performed once daily, five days per week for a total of 28 days.

Clinical observations
A check for mortality, clinical signs of toxicity, and general appearance and behaviour, as well as a quantitative assessment of food and water intake was made twice daily. The applications sites were assessed for signs of irritation once daily.

Body weight
Individual body weights were recorded at weekly intervals during the pre-test and study periods and before sacrifice.

Food consumption
Food consumption was assessed at weekly intervals during the pre-test and study periods and before sacrifice.

Haematology and clinical chemistry
Haematological and blood chemical investigations were performed on all rabbits at termination.

The following parameters were measured: Haematocrit, haemoglobin, erythrocyte count, platelet count, total leukocyte count, differential leukocyte count, mean cell volume (MCV), mean cell haemoglobin

(MCH), mean cell haemoglobin concentration (MCHC), coefficient of variation of erythrocyte volume (RDW), platetelet volume distribution (PDW), mean platelet volume (MPV), thrombocrit (volume % of platelets), aspartate amino transferase (AST), alanine aminotransferase (ALT), blood urea nitrogen, total protein, glucose, albumin, total bilirubin, creatinine, inorganic phosphorus, calcium, sodium, potassium and chloride.

Sacrifice and pathology
All animals sacrificed at scheduled termination were subjected to a gross pathological examination. Any macroscopic findings were recorded.

The following organ weights were determined: Adrenals, brain, heart, kidneys, liver, lung, spleen, stomach, thymus, and testes. The organ-to-brain weight ratios were calculated.

Tissue samples were taken from the following organs and preserved in buffered formalin: Treated and untreated skin, adrenals, brain, heart, kidneys, liver, lungs, ovaries, spleen, stomach gl., testes, and thyroid. Histopathological examinations were performed on all collected tissues from the control and high-dose animals, as well as from abnormal tissues of animals from the low- and mid-dose groups.

Statistics
Body weights, haematological and clinical chemistry parameters, absolute and relative organ weights and histopathology data of treated animals were compared with control animals. Body weight, food consumption and haematology and clinical chemistry parameters were analysed by t-test. Histopathology data were analysed by Fisher´s exact test.

II. RESULTS AND DISCUSSION

A. MORTALITY
No deaths occurred during the study.

B. CLINICAL OBSERVATIONS
There were no signs of systemic toxicity noted in any animal of any dose group.

Signs of dermal irritation consistent of very slight erythema without oedema were observed in one high-dose male and one low dose female. The erythema lasted from day 7 to 20 for the male, and for 5 days for the female.

C. BODY WEIGHT
There were no statistically significant differences observed in body weights or body weight gains between the control and treated groups.

D. FOOD CONSUMPTION
There were statistically significant differences in food consumption between the control and the treated groups. Observed differences were unrelated to treatment. See Table below:

Table 5.3.3-15: Glyphosate technical (Alkaloida, Tiszavasvári): Repeated Dose Twenty-eight-Day Dermal Toxicity Study in Rabbits ([redacted], 1994): Group mean food consumption values [g] and standard deviations (SD)

Dose level [mg/kg bw/day]	Week 1	Week 2	Week 3	Week 4
Males				
0	311.4 ± 38.0	306.8 ± 20.9	286.8 ± 5.8	303.8 ± 28.5

Table 5.3.3-15: Glyphosate technical (Alkaloida, Tiszavasvári): Repeated Dose Twenty-eight-Day Dermal Toxicity Study in Rabbits ([redacted], 1994): Group mean food consumption values [g] and standard deviations (SD)

Dose level [mg/kg bw/day]	Week 1	Week 2	Week 3	Week 4
500	319.8 ± 23.7	278.2 ± 30.5	280.8 ± 35.2	267.6 ± 33.3
1000	↓292.0 ± 44.2	↓261.6 ± 48.3	**↓244.6* ± 25.3**	**↓240.0** ± 25.8**
2000	↓279.4 ± 42.2	↓269.0 ± 40.3	↓260.2 ± 35.4	↓276.8 ± 30.5
Females				
0	301.8 ± 51.8	289.8 ± 44.8	271.8 ± 33.5	272.2 ± 39.9
500	↑317.4 ± 26.1	↑303.8 ± 19.6	↑289.6 ± 19.2	↑291.6 ± 25.5
1000	↑323.6 ± 16.0	↑317.4 ± 33.2	↑311.4 ± 23.4	↑292.2 ± 17.1
2000	↑315.2 ± 41.7	↑297.4 ± 43.1	↑295.0 ± 32.3	↑289.6 ± 43.6

* Significantly different from control group ($p < 0.05$);
** Significantly different from control group ($p < 0.01$)

E. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

No treatment-related effects were detected in the haematological parameters measured.

In females of the mid-and high-dose group Mean Corpuscular Volume of Platelets (MPV) and Coefficient of Variation of Platelets Volume (PDW) values were significantly lower when compared to controls. However, the values were within the historical control range for female NZW rabbits of this age. Thus, these changes were not considered treatment-related (see **Error! Reference source not found.** below).

Blood chemistry

There were no treatment-related effects. The incidental significant changes observed with regard to urea level in high dose males were within the historical control range (not shown in this study report) of the testing facility (see table below, Table 5.3.3-16).

Table 5.3.3-16: Glyphosate technical (Alkaloida, Tiszavasvári): Repeated Dose Twenty-eight-Day Dermal Toxicity Study in Rabbits ([redacted], 1994): Group mean haematological and blood chemical values and standard deviations (SD)

Dose level [mg/kg bw/day]	MPV [fL]	PDW [fL]	Urea [mmol/L]
Males			
0	7.48 ± 0.28	5.80 ± 1.10	7.80 ± 0.66
500	↑7.94 ± 0.62	↑6.80 ± 1.57	↑8.72 ± 1.68
1000	7.64 ± 0.42	↑6.20 ± 1.04	7.64 ± 0.50
2000	7.48 ± 0.04	5.60 ± 0.42	**↑9.82** ± 1.02**

Table 5.3.3-16: Glyphosate technical (Alkaloida, Tiszavasvári): Repeated Dose Twenty-eight-Day Dermal Toxicity Study in Rabbits ([redacted], 1994): Group mean haematological and blood chemical values and standard deviations (SD)

Dose level [mg/kg bw/day]	MPV [fL]	PDW [fL]	Urea [mmol/L]
Females			
0	8.96 ± 0.85	9.70 ± 1.86	10.24 ± 1.09
500	↓8.15 ± 0.99	↓7.50 ± 2.71	↓9.70 ± 0.53
1000	**↓7.62* ± 0.13**	**↓6.20* ± 0.57**	10.58 ± 0.39
2000	**↓7.46* ± 0.27**	**↓5.10*** ± 0.65**	↓9.96 ± 1.31

MPV: Mean platelet volume; PDW: Platelet volume distribution (coefficient of variance of platelets volume);
* Significantly different from control group ($p < 0.05$);
** Significantly different from control group ($p < 0.01$);
*** Significantly different from control group ($p < 0.001$)

I. NECROPSY

Organ weights

Absolute but not relative testes to brain weights were statistically increased in high dose males but this was not considered treatment-related.

Table 5.3.3-17: Glyphosate technical (Alkaloida, Tiszavasvári): Repeated Dose Twenty-eight-Day Dermal Toxicity Study in Rabbits ([redacted] 1994): Group mean absolute and relative to brain testes weight values and standard deviations (SD)

Dose level [mg/kg bw/day]	Absolute weight [g]	Relative weight [g/1g brain wt.]
0	3.88 ± 0.327	0.4310 ± 0.0215
500	3.70 ± 1.255	0.3744 ± 0.1211
1000	↑4.36 ± 0.658	0.4692 ± 0.0730
2000	**↑4.52* ± 0.497**	0.4618 ± 0.0773

* Significantly different from control group ($p < 0.05$)

Gross pathology

There were no treatment-related macroscopic abnormalities observed in the treated skin or any other tissues in any group.

Histopathology

There was an increased incidence in high dose males versus control animals of erosions in the stomach (4/5 high dose versus 1/5 control) and interstitial nephritis (3/5 high dose versus 1/5 control) but these findings were not considered treatment-related lesions.

III. CONCLUSIONS

There were no treatment-related effects on body weight, food consumption, haematological and clinical chemistry parameters observed in any of the dose groups. The macroscopic and histopathological findings observed at necropsy were considered incidental and unrelated to the test substance.

Repeated dermal administration of glyphosate technical to rabbits for a period of 28 consecutive days at doses of up to 2000 mg/kg bw/day resulted only in slight dermal irritation in one high-dose male and one

low-dose female. There were no compound-related gross and microscopic findings noted.

3. Assessment and conclusion

Assessment and conclusion by applicant:
In this study, the toxicity potential of glyphosate technical was assessed after repeated dermal application to groups of male and female New Zealand white rabbits. Doses of 0, 500, 1000 or 2000 mg/kg bw/day were applied for a period of 6 hours a day, five days per week for 4 weeks. The study was conducted according to OECD 410 (1981) and in compliance with GLP.
Repeated dermal administration of glyphosate technical to rabbits for a period of 28 consecutive days at doses of up to 2000 mg/kg bw/day resulted only in slight dermal irritation in one of five high-dose males and one low-dose female. There were no treatment-related systemic signs of toxicity. Thus, the NOAEL is considered to be 2000 mg/kg bw/day.

Assessment and conclusion by RMS:
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1. Information on the study

Data point:	CA 5.3.3/007
Report author	[redacted]
Report year	1985
Report title	Subacute dermal toxicity (for 21 days in rabbits) of glyphosate (technical)
Report No	Not reported
Document No	Not reported
Guidelines followed in study	Not reported, comparable to OECD 410 (1981)
GLP	No
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	In a 21-day dermal toxicity study, glyphosate (purity not reported) was dermally applied to the intact skin of New Zealand White rabbits for 6 hours per day. The dose levels were 0, 500, 1000, and 2000 mg/kg bw/day and the groups consisted of 3 male and 3 female rabbits per group. Treatment was performed 5 days per week for 3 consecutive weeks. Treatment was followed by a 14-day recovery period prior to sacrifice. Animals were observed daily for clinical signs of toxicity and irritation. Food consumption was calculated daily and body weights were determined weekly. Laboratory investigations (haematology, clinical chemistry, and urinalysis) were performed on days 0, 21, and 35. At study termination, all animals were subjected to a gross and histopathological examination.
Short description of results:	There were no mortalities and no treatment-related signs of clinical toxicity. There were no signs of dermal irritation. Body weight and food consumption were not affected by treatment. Laboratory investigations and gross and histopathological examinations did not reveal any treatment-related effects. The No Observed Adverse Effect Level (NOAEL) was established at 2000 mg/kg bw/day based on the lack of effects.

Reasons for why the study is not considered relevant/reliable or not considered as key study:	The study was considered supportive due to serious reporting deficiencies in the Monograph (2000) and not accepted in the RAR (2015). Test substance purity and batch number or study details like study number were not reported. Additionally, a statistical analysis of the results was not reported. Therefore and since the study report is not available, this study is not considered to be reliable.
Reasons why the study report is not available for submission	The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL.
Category study in AIR 5 dossier (L docs)	Category 4b

1. Information on the study

Data point	CA 5.3.3/008
Report author	[redacted]
Report year	1982
Report title	21-Day Dermal Toxicity Study in Rabbits
Report No	[redacted]-81-195
Document No	Not reported
Guidelines followed in study	No guideline followed; in general compliance with OECD 410 (1981)
Deviations from current test guideline (OECD 410, 1981)	The application area in the high-dose group was about 1.5 – 2 times higher than the recommended 10 % of the body surface area.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The toxicity potential of glyphosate technical was assessed after repeated dermal application to groups of 5 male and 5 female New Zealand white rabbits on intact and on abraded skin. Doses of 0, 100, 1000 or 5000 mg/kg bw/day were applied five days per week for three consecutive weeks. The abrading was accomplished by producing shallow incisions (not deep enough to cause bleeding) with the blunt end of a scalpel. For application the solid test substance was moistened with an appropriate amount of water, and spread evenly over the application site. It has to be noted that the surface areas covered (i.e. 1 – 2 %, 5 – 10 % and 15 – 20 % body surface area for the low, mid- and high-dose group, respectively) were below and above the area of 10 % recommended by actual guidelines. Due to the higher exposed surface area in the high dose group, it has to be considered that more test substance can be absorbed through the skin and could be therefore systemically available.

There were no mortalities and no treatment-related signs of systemic toxicity. There were also no signs of dermal irritation observed in the control, low- and mid-dose group. At 5000 mg/kg bw/day slight dermal

irritation consisting of barely perceptible to slight erythema and oedema was noted. However, this effect is considered not to be of biological significance and no signs of irritation were seen in the histopathological examination.

There were no treatment-related effects on body weight, food consumption, haematological and clinical chemistry parameters observed in any of the dose groups. The macroscopic and histopathological findings observed at necropsy were considered incidental and unrelated to the test substance.

Repeated dermal administration of glyphosate technical to rabbits for a period of 21 consecutive days at doses of up to 5000 mg/kg bw/day resulted only in slight dermal irritation at 5000 mg/kg bw/day. No such effects were observed in the 0, 100 and 1000 mg/kg bw/day treatment groups. There were no treatment-related systemic signs of toxicity.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate technical
Description:	White powder
Lot/Batch #:	NBP 1992026
Purity:	Not reported
Stability of test compound:	Not reported

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rabbit
Strain:	New Zealand
Source:	[redacted]
Age:	Young, adult
Sex:	Male and female
Weight at dosing:	♂ 2359 – 2883 g; ♀ 2344 – 2955 g
Acclimation period:	14 – 16 days
Diet/Food:	Purina Certified Rabbit Chow #5322 (Ralston Purina Company, Missouri, USA), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in wire mesh cages
Environmental conditions:	Temperature: Not reported Humidity: Not reported Air changes: Not reported 12 hours light / dark cycle

B: Study design and methods

In life dates: 1981-07-28 to 1981-08-19

Animal assignment and treatment:
The potential dermal toxicity of glyphosate technical after repeated exposure was assessed using young

adult New Zealand albino rabbits (males and females). Ten rabbits per sex per dose received daily dermal applications of 0, 100, 1000 or 5000 mg/kg bw. The dose groups were further divided in halves. One half received the treatment on intact skin, the other half on abraded skin. Abrasion was done twice per week immediately prior to test substance application by producing shallow incisions (not deep enough to cause bleeding) with the blunt end of a scalpel blade.

The day prior to the first application about 30 % of the skin of the back of the animals was clipped free of hair. During the study rabbits were shaved as needed.

For each application the test substance was moistened with an appropriate amount of physiological saline. Each dose was spread evenly over the maximum body surface area possible (see **Error! Reference source not found.** table below) covered with a semi-occlusive dressing. After an exposure period of six hours the dressings were removed and the application site was cleaned with tepid tap water and dried with paper towels. Applications were performed once daily, five days per week for three consecutive weeks. Individual doses were adjusted weekly based on the body weight determined at the beginning of each study week.

Table 5.3.3-18: 21-Day Dermal Toxicity Study in Rabbits ([redacted], 1982): Application details for repeated dermal applications

Dose group [mg/kg bw/day]	Number of animals				Volume of physiological saline used for moistening [mL]	Total percent of the body surface covered by test substance [%]
	Intact skin		Abraded skin			
	♂	♀	♂	♀		
0	5	5	5	5		-
100	5	5	5	5	0.2	1 – 2
1000	5	5	5	5	1.5 – 2.0	5 – 10
5000	5	5	5	5	8.0 – 9.0	15 – 20

It has to be noted that the application area according to current guidelines (OECD and EC) should be about 10 % of the body surface. Thus, the body surface covered with test material in the 100 mg/kg bw/day dose group is lower than recommended, whereas the treatment-area in the high dose group is about 1.5 – 2 times higher than recommended. Due to the higher exposed surface area in the high-dose group a higher amount of test substance can be absorbed and therefore potentially be systemically available.

Clinical observations
A check for mortality was made twice daily. Observations for clinical signs of toxicity and behavioural changes were made once daily on all animals. The applications sites were assessed for signs of irritation.

Body weight
Individual body weights were recorded at weekly intervals during the pre-test and study periods and before sacrifice.

Food consumption
Food consumption was assessed daily for each individual animal by visual inspection.

Haematology and clinical chemistry
Haematological and blood chemical investigations were performed on 5 rabbits per sex and dose group with intact and abraded skin on day 21 after an overnight fast.

The following parameters were measured: Haematocrit, haemoglobin, erythrocyte count, reticulocyte count, platelet count, total leukocyte count, differential leukocyte count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), alkaline phosphatase, aspartate amino transferase (AST), alanine aminotransferase (ALT), γ-glutamyl-transferase,

creatine kinase, creatinine, blood urea nitrogen, total protein, glucose, albumin, globulin (calculated), total bilirubin, creatinine, lactate dehydrogenase, total cholesterol, inorganic phosphorus, calcium, sodium, potassium and chloride.

Sacrifice and pathology
All animals sacrificed at scheduled termination were subjected to a gross pathological examination. Any macroscopic findings were recorded.

The following organ weights were determined: Adrenals, gonads, heart, kidneys, liver, pituitary and thyroid (with parathyroid).

Tissue samples were taken from the following organs and preserved in buffered formalin: Treated and untreated skin (3 samples/each), adrenals, bone & bone marrow (sternum), brain (at three levels), colon, duodenum, eyes with Harderian gland, gross lesions, heart, ileum, jejunum, kidneys, liver, lungs with main stem bronchi, mammary gland, lymph nodes (mediastinal, mesenteric and regional when applicable), muscle (skeletal), oesophagus, ovaries, pancreas, pituitary, prostrate, salivary glands, sciatic nerve, seminal vesicles, spinal cord (cervical), spinal cord and vertebrae (lumbar), spleen, stomach, testes with epididymis, thymus, thyroid/parathyroid, trachea, urinary bladder, uterus and vagina.

Histopathological examinations were performed on the following tissues: Treated and untreated skin, liver, kidney, gonads and any gross lesions.

Statistics
Terminal body weights, haematological and clinical chemistry parameters, absolute and relative organ weights were analysed by one-way analysis of variance, Bartlett´s test for homogeneity of variance and appropriate t-test.

II. RESULTS AND DISCUSSION

A. MORTALITY
No deaths occurred during the study.

B. CLINICAL OBSERVATIONS
A number of incidental findings were observed in some animals in all dose groups. The most frequent signs were soft stool, diarrhoea, mucoid diarrhoea and ocular and nasal discharge.

No signs of dermal irritation were observed in the control, low- and mid-dose group. In the high-dose group at 5000 mg/kg bw/day doubtful or barely perceptible to very slight erythema and doubtful or barely perceptible oedema were noted. Dermal effects were observed as early day 2 with increasing number of animals showing the effects after additional exposures. There were no differences between the animals with intact and abraded skin.

C. BODY WEIGHT
There were no statistical significant differences observed in body weights or body weight gains between the control and treated groups (with abraded and intact skin).

D. FOOD CONSUMPTION
There were no major differences in food consumption between the control and the treated groups.

E. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology
No treatment-related effects were detected in the haematological parameters measured.
There were some statistical significance differences in some parameters. However, these were incidental and considered to be biologically insignificant (see table below**Error! Reference source not found.** Table 5.3.3-19).

Blood chemistry
There were no treatment-related effects. The incidental significant changes observed were considered not to be biologically significant (see Table 5.3.3-19).

Table 5.3.3-19: 21-Day Dermal Toxicity Study in Rabbits ([redacted], 1982): Group mean haematological and blood chemical values and (± SD)

Dose level [mg/kg bw/day]	Hb [g/dL]	Haematocrit [%]	Sodium [mEq/L]	Glucose (mg/dL)	LDH (IU/L)
Males					
0	11.3 ± 0.68	34.1 ± 1.78	143 ± 1.3	121 ± 21.6	258 ± 154.5
100	↑**12.5* ± 0.63**	↑**37.3* ± 1.92**	↑144 ± 2.1	↑134 ± 14.1	↓169 ± 115.9
1000	↑11.4 ± 0.46	↑34.2 ± 1.41	↑145 ± 5.1	↑149 ± 34.7	↑291 ± 198.7
5000	↑11.7 ± 0.61	↑35.5 ± 1.13	↑**146* ± 2.5**	↑125 ± 6.1	↓70 ± 72
Females					
0	11.5 ± 0.43	35.4 ± 2.14	142 ± 2.7	102 ± 16.2	189 ± 125.9
100	↓11.4 ± 0.50	↓34.3 ± 0.85	142 ± 1.5	↑**137** ± 18.7**	↓149 ± 109.1
1000	11.5 ± 0.50	↓34.5 ± 1.28	↑143 ± 1.9	↑123 ± 17.1	↑258 ± 204.4
5000	↓11.7 ± 0.42	↓34.4 ± 1.55	↑144 ± 1.6	↑**129* ± 4.7**	↓**28* ± 6.2**

LDH: Lactate dehydrogenase;
* Significantly different from control group ($p < 0.05$);
** Significantly different from control group ($p < 0.01$)

I. NECROPSY

Organ weights
Except for the statistically increased relative kidney weight observed in females at 5000 mg/kg bw/day (see table below), there were no treatment-related effects on absolute and relative organ weights noted. Since no histopathological changes were observed in the kidneys of high-dose females, the increase in relative kidney weights is considered to be of no toxicological relevance.

Table 5.3.3-20: 21-Day Dermal Toxicity Study in Rabbits ([redacted], 1982): Group mean absolute and relative kidney weights and standard variations (± SD)

Dose level [mg/kg bw/day]	Absolute organ weight [g]		Relative organ weight [%]	
	Kidney		Kidney	
	♂	♀	♂	♀
0	17.00 ± 2.281	16.41 ± 2.037	0.58 ± 0.078	0.55 ± 0.040
100	↓16.38 ± 1.371	↓15.26 ± 2.260	↓0.57 ± 0.067	↓0.54 ± 0.069
1000	↑18.15 ± 2.653	↓16.16 ± 2.449	↑0.59 ± 0.067	↓0.54 ± 0.048

Table 5.3.3-20: 21-Day Dermal Toxicity Study in Rabbits ([redacted], 1982): Group mean absolute and relative kidney weights and standard variations (± SD)

Dose level [mg/kg bw/day]	Absolute organ weight [g]		Relative organ weight [%]	
	Kidney		Kidney	
	♂	♀	♂	♀
5000	↓16.77 ± 2.016	↑18.14 ± 1.757	↑0.60 ± 0.097	**↑0.63* ± 0.072**

* Significantly different from control group ($p < 0.05$)

Gross pathology
There were no treatment-related macroscopic abnormalities observed in the treated skin or any other tissues in any group.

Histopathology
There were no treatment-related lesions observed in any dose group.

Microscopic evaluation of treated skin samples demonstrated only mild inflammatory cell infiltration and trace necrosis in the 1000 mg/kg bw/day group. However, in untreated skin samples of three rabbits from the 1000 mg/kg bw/day group from one rabbit of the high-dose group there was also mild necrosis, indicating that this lesion was incidental and unrelated to treatment. The lesions in treated and untreated skins of the control and test substance groups were similar indicating that the effects were not related to glyphosate treatment.

Trace/mild seminiferous tubule degeneration observed in the testis was not dose related in either incidence or severity and was considered unrelated to treatment. Other lesions observed in kidney, liver, lung, ovary, lymph node, salivary gland and skin (non-application site) were considered incidental or spontaneous (see tables below). In general there were no major differences between the treatment groups of intact and abraded skin.

Table 5.3.3-21: 21-Day Dermal Toxicity Study in Rabbits ([redacted], 1982): Histopathological findings in rabbits treated dermally on intact skin*

Dose level [mg/kg bw/day]	0		100		1000		5000	
Effect/Lesion	♂	♀	♂	♀	♂	♀	♂	♀
Kidney								
Cytoplasmic vacuolation (mild)	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5
Interstitial lymphocytic infiltrates (mild)	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Interstitial inflammation (trace)	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5
Interstitial inflammation (mild)	0/5	1/5	1/5	0/5	0/5	0/5	2/5	0/5
Infarct (mild)	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5
Mineralisation (trace)	0/5	0/5	0/5	0/5	2/5	0/5	0/5	0/5
Mineralisation (mild)	0/5	0/5	0/5	0/5	1/5	0/5	1/5	1/5
Liver								
Granuloma (moderate)	0/5	0/5	1/5	1/5	1/5	0/5	0/5	0/5
Mononuclear cell infiltration (trace)	1/5	0/5	0/5	0/5	0/5	1/5	1/5	0/5
Mononuclear cell infiltration (mild)	0/5	1/5	0/5	0/5	0/5	0/5	1/5	0/5
Mononuclear cell infiltration (moderate)	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5
Necrosis (mild)	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5
Lung								

Table 5.3.3-21: 21-Day Dermal Toxicity Study in Rabbits ([redacted], 1982): Histopathological findings in rabbits treated dermally on intact skin*

Dose level [mg/kg bw/day]	0		100		1000		5000	
Effect/Lesion	♂	♀	♂	♀	♂	♀	♂	♀
Abscess (moderate)	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5
Lymphocytic infiltration (mild)	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5
Pneumonia (mild)	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5
Pneumonia (moderate)	1/5	1/5	1/5	0/5	0/5	0/5	0/5	0/5
Congestion (mild)	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5
Congestion (moderate)	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5
Oedema (moderate)	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5
Testis								
Seminiferous tubule degeneration (trace)	1/5	--	3/5	--	2/5	--	3/5	--
Seminiferous tubule degeneration (mild)	2/5	--	2/5	--	0/5	--	1/5	--
Dilated tubules (moderate)	0/5	--	1/5	--	0/5	--	0/5	--
Ovaries								
Mineralisation (trace)	--	1/5	--	0/5	--	0/5	--	0/5
Salivary gland								
Abscess (moderate)	--	1/5	--	0/5	--	0/5	--	0/5
Skin (non-application site)								
Dermatitis (moderate)	0/5	--	0/5	--	1/5	--	0/5	--
Skin, treated								
Inflammation (trace)	1/5	3/5	2/5	3/5	2/5	5/5	3/5	2/5
Inflammation (mild)	2/5	1/5	2/5	1/5	1/5	0/5	0/5	3/5
Necrosis (trace)	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5
Skin, untreated								
Inflammation (trace)	4/5	1/5	4/5	2/5	3/5	2/5	3/5	2/5
Inflammation (mild)	1/5	3/5	0/5	3/5	1/5	2/5	0/5	3/5
Necrosis (mild)	0/5	0/5	0/5	0/5	3/5	0/5	1/5	0/5

* Number of animals affected / total number of animals;
- Not applicable

Table 5.3.3-22: 21-Day Dermal Toxicity Study in Rabbits ([redacted], 1982): Histopathological findings in rabbits treated dermally on abraded skin*

Dose level [mg/kg bw/day]	0		100		1000		5000	
Effect/Lesion	♂	♀	♂	♀	♂	♀	♂	♀
Kidney								
Interstitial inflammation (trace)	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5
Interstitial inflammation (mild)	1/5	0/5	1/5	1/5	1/5	0/5	0/5	1/5
Interstitial inflammation (moderate)	0/5	0/5	1/5	0/5	0/5	0/5	1/5	0/5
Infarct (moderate)	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5
Mineralisation (mild)	0/5	0/5	0/5	0/5	2/5	1/5	0/5	0/5
Liver								
Mononuclear cell infiltration (trace)	0/5	1/5	1/5	0/5	0/5	0/5	0/5	0/5
Mononuclear cell infiltration (mild)	1/5	0/5	1/5	2/5	4/5	1/5	1/5	1/5

Table 5.3.3-22: 21-Day Dermal Toxicity Study in Rabbits ([redacted], 1982): Histopathological findings in rabbits treated dermally on abraded skin*

Dose level [mg/kg bw/day]	0		100		1000		5000	
Effect/Lesion	♂	♀	♂	♀	♂	♀	♂	♀
Necrosis (mild)	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5
Lung								
Pneumonia (moderate)	0/5	--	1/5	--	1/5	--	0/5	--
Lymph node, prefemoral								
Lymphadenitis (mild)	--	0/5	--	0/5	--	0/5	--	1/5
Testis								
Seminiferous tubule degeneration (trace)	2/5	--	1/5	--	2/5	--	2/5	--
Seminiferous tubule degeneration (mild)	2/5	--	3/5	--	2/5	--	2/5	--
Ovaries								
Mineralisation (trace)	--	0/5	--	0/5	--	1/5	--	0/5
Mineralisation (mild)	--	0/5	--	0/5	--	1/5	--	0/5
Atretic follicles (mild)	--	0/5	--	1/5	--	0/5	--	0/5
Skin, treated								
Inflammation (trace)	2/5	2/5	3/5	2/5	4/5	2/5	2/5	1/5
Inflammation (mild)	0/5	3/5	2/5	1/5	0/5	3/5	3/5	4/5
Skin, untreated								
Inflammation (trace)	3/5	3/5	2/5	4/5	2/5	2/5	1/5	3/5
Inflammation (mild)	1/5	1/5	3/5	1/5	3/5	2/5	4/5	2/5

* Number of animals affected / total number of animals

III. CONCLUSIONS

There were no mortalities and no treatment-related signs of systemic toxicity. There were also no signs of dermal irritation observed in the control, low- and mid-dose group. At 5000 mg/kg bw/day slight dermal irritation consisting of barely perceptible to slight erythema and oedema was noted. However, this effect is considered not to be of biological significance and no signs of irritation were seen in the histopathological examination.

There were no treatment-related effects on body weight, food consumption, haematological and clinical chemistry parameters observed in any of the dose groups. The macroscopic and histopathological findings observed at necropsy were considered incidental and unrelated to the test substance.

Repeated dermal administration of glyphosate technical to rabbits for a period of 21 consecutive days at doses of up to 5000 mg/kg bw/day resulted only in slight dermal irritation at 5000 mg/kg bw/day. No such effects were observed in the 0, 100 and 1000 mg/kg bw/day treatment groups. There were no treatment-related systemic signs of toxicity. No test article-related macroscopic or microscopic lesions were observed.

3. Assessment and conclusion

Assessment and conclusion by applicant:
In this study, the toxicity potential of glyphosate technical was assessed after repeated dermal application to groups of male and female New Zealand white rabbits on intact and on abraded skin. Doses of 0, 100, 1000 or 5000 mg/kg bw/day were applied five days per week for three consecutive weeks. The study was conducted following a testing regime in general compliance with OECD 410 (1981) and with GLP.

Repeated dermal administration of glyphosate technical to rabbits for a period of 21 consecutive days at doses of up to 5000 mg/kg bw/day resulted only in slight dermal irritation at 5000 mg/kg bw/day. No such effects were observed in the 0, 100 and 1000 mg/kg bw/day treatment groups. There were no treatment-related systemic signs of toxicity of biological significance. Thus, the NOAEL is considered to be 5000 mg/kg bw/day.

In light of an *in vitro* dermal absorption of glyphosate with rabbit skin following OECD 428 (Hadfield 2012; CA 5.8.2/014), demonstrating 2.66 % bioavailability via the skin exposure, the systemic NOAEL in this study is 133 mg/kg bw/day, de

Assessment and conclusion by RMS:
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1. Information on the study

Data point:	CA 5.3.3/009
Report author	[redacted]
Report year	1985
Report title	Report on subacute inhalation toxicity in rats (14 days) of glyphosate (technical)
Report No	Not reported
Document No	Not reported
Guidelines followed in study	Not applicable
GLP	No, pre-GLP
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	Four groups of 5 male and 5 females Wister rats (bred at [redacted]) were exposed to an atmosphere containing glyphosate (purity not stated) in propylene glycol for 6 hours per day, 5 days per week for two weeks. There were one low and one high dose group and two intermediate dose groups. One of the latter groups was sacrificed 14 days after the treatment period had been finished (reversal group). Two control groups of the same size were also included, one of them being exposed to filtered air only and the other to an atmosphere containing the vehicle propylene glycol. Glyphosate was mixed with the vehicle and nebulised by using compressed air. The animals were exposed in a dynamic inhalation chamber by mouth and nose route by restraining them in polypropylene tubes. The target, the nominal and the actually measured mean concentrations for the various groups are given in the table below.

Table 5.3.3-23: Report on subacute inhalation toxicity in rats (14 days) of glyphosate (technical) [redacted] 1985): Mean concentrations [mg/L air] of the test material or the vehicle

Dose group	Target concentration	Nominal concentration	Measured concentration
Control (filtered air)	0	0	0
Vehicle control	5.0	16.5	5.6
Low dose	0.25	0.90	0.28
Intermediate	1.0	3.01	0.93

	dose: —; —; — Intermediate dose (reversal): 1.0; 3.10; 0.90 High dose: 4.0; 11.7; 3.8 With the exception of the exposure periods, animals were housed five per cage and had free access to food and drinking water. The rats were examined individually before, during and after exposure for any signs of toxicity. Food consumption was recorded daily and body weight was determined at pretest and then on every 4th day during the study and on the day of termination. Blood samples for haematological (red and white blood cell parameters, thrombocyte count and prothrombin time) and clinical chemistry (total serum protein, blood urea nitrogen, glucose, alanine aminotransferase (ALAT) and alkaline phosphatase (AP)) investigations were taken before first exposure and after one week from some animals of each group and from all animals (except the recovery group) after the last exposure. From reversal group rats, blood was obtained at termination. Urinalysis was also performed. All rats were necropsied and the following organs were removed and weighed: adrenals, gonads, heart, kidneys, liver, and spleen. These organs as well as aorta, brain, eyes, intestines, larynx, lungs, lymph nodes (axillary and mesenteric), nose, oesophagus, pancreas, pituitary, seminal vesicle, stomach, thyroid, trachea, urinary bladder and uterus were examined microscopically.
Short description of results:	Mass median aerodynamic diameters of all atmospheres were within the respirable range of 0 - 7 µm on all occasions. The temperature, humidity, oxygen concentration and air flow were similar for all groups. There was no mortality during the treatment or the recovery periods. Neither treatment-related clinical signs nor effects on body weight or food consumption were observed. In the vehicle control group and in all groups exposed to glyphosate, salivation, lacrimation, redness to nose and urination were observed during exposure. No change in the rate of respiration was seen. No adverse effects of treatment on haematology, clinical chemistry and urinalysis were noted in any of the groups. No significant changes in organ weights or upon gross examination were seen. Histopathology did not reveal evidence of adverse effects. Up to the highest concentration tested of approx. 3.8 mg/L air (mean measured concentration) repeated inhalative exposure of Wistar rats to an aerosol containing glyphosate did not lead to any local (respiratory) or systemic toxicity. Therefore, the NOAEC for rats under the conditions of this study was 3.8 mg/L.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	Monograph (2000): The study was considered supportive since an effect dose was lacking. In addition, there were some reporting deficiencies. Furthermore, statistical analysis of the results obtained was either not performed or not reported. RAR (2015): The study was considered unacceptable due to serious reporting deficiencies, e.g., absence of information on batch and purity of the test material. Therefore and since the study report is not available, this study is

	considered invalid.
Reasons why the study report is not available for submission	The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL.
Category study in AIR 5 dossier (L docs)	Category 4b

Publications on repeated-dose toxicity via other routes

1. Information on the study

Data point:	CA 5.3.3/010
Report author	Mesnage, R. *et al.*
Report year	2018
Report title	Comparison of transcriptome responses to glyphosate, isoxaflutole, quizalofop-p-ethyl and mesotrione in the HepaRG cell line
Document No	doi.org/10.1016/j.toxrep.2018.08.005 E-ISSN: 2214-7500
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary of the study according to OECD format

Together with three other herbicide active substances (quizalofop-p-ethyl, isoxaflutole and mesotrione) the effect of glyphosate on the transcriptome and metabolome profile of differentiated HepaRG cells was investigated.

Materials and methods
Chemicals - Glyphosate (purity ≥ 96 %) was purchased from Sigma-Aldrich, Gillingham, Dorset, UK.

HepaRG cell culture - Differentiated HepaRG™ cells (HPR 116) were purchased from Biopredic International (Rennes, France). Cells were thawed, suspended and plated in general purpose medium (Williams' E medium + GlutaMAX™) containing the ADD670 supplement. Cells were kept in general purpose medium until day 8, when the culture becomes well organized and includes well-delineated trabeculae and many canaliculi-like structures. At this time, the culture is composed of primitive biliary epithelial cells and mature hepatocytes with basal metabolic activities similar to freshly isolated primary cells. From day 8 to day 14, cells were switched to the test medium composed of Williams' E medium + GlutaMAX™ supplemented with 2 % foetal bovine serum and 1 % DMSO, as well as different concentrations of glyphosate or solvent as a control. Glyphosate was tested at 0.06 µM (concentration representative of low environmental exposure), 6 µM, and 600 µM.

Library generation and RNA-sequencing - A 100 ng aliquot of total RNA from each sample was used to prepare total RNA libraries using the KAPA Stranded RNA-Seq Kit with RiboErase, and samples were randomised before preparation. Polymerase chain reaction (PCR) was performed for 14 cycles for final

library amplification. Resulting libraries were quantified using the Qubit 2.0 spectrophotometer and average fragment size assessed using the Agilent 2200 Tapestation. The transcriptome of HepaRG cells exposed to glyphosate was sequenced employing this strategy, except that the libraries were prepared as previously described. A total of 3 separate sequencing pools were created using equimolar quantities of each sample with compatible indexes: 2 with 17 samples each, and one with 16 samples. Paired-end reads of 75bp were generated for each library using the Illumina NextSeq®500 in con-junction with the NextSeq®500 v2 high-output 150-cycle kit.

Mass spectrometry-based metabolomics - Approximately 5,000,000 hepaRG cells per sample were harvested from the 6 well-plates to obtain a sufficient quantity of material to perform the metabolomics experiment. Cells were detached using 0.05 % trypsin EDTA, and centrifuged to eliminate trypsin residues. Finally, cell pellets were frozen at -80 °C pending analysis. Metabolomics analysis of the frozen cell pellets was conducted by Metabolon Inc. The sample extracts were stored overnight under nitrogen before preparation for analysis. The resulting extract was analysed on four independent instrument platforms: two different separate RP/UPLC-MS/MS with positive ion mode electrospray ionisation (ESI), a RP/UPLC-MS/MS with negative ion mode ESI, as well as by hydrophilic-interaction chromatography(HILIC)/UPLC-MS/MS with negative ion mode ESI as previously described. Compounds were identified by comparison to library entries of purified standards or recurrent unknown entities. Biochemical identifications are based on 3 criteria: retention index within a narrow retention time/index (RI) window of the proposed identification, accurate mass match to the library +/- 10 ppm, and the MS/MS forward and reverse scores between the experimental data and authentic standards. The use of all three data points can be utilised to distinguish and differentiate biochemicals. Peaks were quantified using the area-under-the-curve method.

Statistics - Metabolome data analysis was performed using in-house services of Metabolon Inc. Biochemical data were normalized with respect to protein concentration as determined by the Bradford assay. The Welch's two-sample t-test was used to test whether control and treatment group means are different from two independent populations. This version of the two-sample t-test allows for unequal variances. FDR methods and estimated q-values were used to account for the highest number of false positive results caused by the high number of statistical tests. The RNA-seq data analysis was performed using the new version of the Tuxedo protocol with HISAT2, StringTie and Ballgown. A standard linear model-based comparison of transcript abundance was performed without adjusting for other covariates to identify differentially expressed transcripts for each group. Although 3 concentrations were tested for glyphosate, no multigroup comparisons were used because dose spacing was too large to allow reliable conclusions to be drawn from these methods. Instead, pairwise comparisons were used.

Results

Control, untreated cell cultures presented no visible signs of aging after a 6-day exposure. Transcriptome profiles of HepaRG cells were then determined using the Illumina-based RNA sequencing platform. The highest concentration of glyphosate tested caused significant changes in transcriptome profiles. Alterations in gene expression caused by the 2 lowest concentrations (0.06 and 6 µM) failed to pass the statistical threshold that took into account the high number of tests performed. A total of 7 transcripts had their levels altered ($p < 0.05$) with glyphosate at 600 µM. The number of genes disturbed by the exposure to glyphosate was insufficient to use a functional annotation tool for pathway enrichment analysis. It is not clear if glyphosate lacks hepatic toxic effects at these concentrations or if this experimental design lacks sensitivity to detect hepatic effects of weak toxicants. To further explore changes in liver metabolism caused by glyphosate in greater detail, a global metabolome profiling of HepaRG cells exposed to three concentrations of glyphosate was explored. The Metabolon HD4 platform detected 802 named biochemicals in the HepaRG samples. Overall, glyphosate did not cause significant alterations in metabolome composition. However, exposure did cause a significant decrease in long chain fatty acids (LCFAs) and polyunsaturated fatty acids (PUFAs). HepaRG cells exposed to the lowest concentration of glyphosate tested (0.06 µM) showed the most dramatic effects in the levels of these fatty acids as either significant or trends towards significant lower levels. At the higher glyphosate concentrations of 6 µM and 600 µM, lower lipid levels were also observed but these did not reach statistical significance.

Discussion and conclusion

An in-depth investigation was conducted of transcriptome profile alterations in HepaRG human liver cells caused by exposure to pesticide active substances. Glyphosate was found to be only weakly toxic inducing little change in transcriptome profiles. Interestingly, a follow-up metabolomics analysis of HepaRG cells treated with the lowest (0.06 µM) concentration of glyphosate revealed a significant decrease in the levels of LCFAs and PUFAs. Although these findings from an *in vitro* tissue culture model system cannot be readily translated to effects *in vivo*, they are nevertheless indicative of differences in toxicity potency between pesticide ingredients. The exact nature of this low dose effect of glyphosate cannot be determined from this single experiment, but it is possible that at higher concentrations, more overtly toxic mechanisms are masking the effects on lipids. Another possibility is that a saturation effect is occurring once the low dose is exceeded bearing in mind that glyphosate levels found in the HepaRG cells during the metabolomics analysis increased by 3.7-and 336.35-fold at the intermediate and highest concentrations tested compared to the negative controls. Glyphosate was the least toxic of the compounds tested in this study.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Together with three other herbicide active substances (quizalofop-p-ethyl, isoxaflutole and mesotrione) the effect of glyphosate on the transcriptome and metabolome profile of differentiated HepaRG cells was investigated at 0.06, 6 and 600 µM. Glyphosate was found to be only weakly toxic inducing little change in transcriptome profiles when compared with the other herbicides tested. A follow-up metabolomics analysis of HepaRG cells exposed to glyphosate at 0.06 µM revealed a significant decrease in the levels of long chain fatty acids (LCFAs) and polyunsaturated fatty acids (PUFAs). At the higher glyphosate concentrations of 6 and 600 µM, lower lipid levels were also observed but these did not reach statistical significance. It is not clear, however, how these findings from an *in vitro* tissue culture model can be translated to effects *in vivo*.N

Reliability criteria for *in vitro* toxicology studies

Publication: Mesnage *et al*., 2018.	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Glyphosate (purity ≥ 96 %). Source: Sigma-Aldrich, Gillingham, Dorset, UK.
Only glyphosate acid or one of its salts is the tested substance	N	Also three other pesticide active substances were tested (quizalofop-p-ethyl, isoxaflutole and mesotrione).
AMPA is the tested substance	N	
Test system clearly and completely described	Y	
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	N	Not relevant.

Test concentrations in physiologically acceptable range (< 1 mM)	Y	0.06, 6 and 600 µM.
Cytotoxicity tests reported	N	
Transcriptomics and metabolomics methods described	Y	
Positive and negative controls	N	No positive controls included.
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Reliability not assignable		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because no positive control was used and no cytoxicity tests were performed to optimise the concentration range to be explored.		

1. Information on the study

Data point:	CA 5.3.3/011
Report author	Kumar, S. *et al.*
Report year	2014
Report title	Glyphosate-rich air samples induce IL-33, TSLP and generate IL-13 dependent airway inflammation
Document No	doi.org/10.1016/j.tox.2014.08.008 E-ISSN: 1879-3185
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	None
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary of the study according to OECD format

The aim of this study was to explore the mechanisms of glyphosate-induced pulmonary pathology by utilizing murine models and real environmental samples. C57BL/6, TLR4–/–, and IL-13–/– mice inhaled extracts of glyphosate-rich air samples collected on farms during spraying of herbicides or inhaled different doses of glyphosate and ovalbumin. The cellular response, humoral response, and lung function of exposed mice were evaluated. Exposure to glyphosate-rich air samples as well as glyphosate alone to the lungs increased: eosinophil and neutrophil counts, mast cell degranulation, and production of IL-33, TSLP, IL-13, and IL-5. In contrast, in vivo systemic IL-4 production was not increased. Co-administration of ovalbumin with glyphosate did not substantially change the inflammatory immune response. However, IL-13-deficiency resulted in diminished inflammatory response but did not have a significant effect on airway resistance upon methacholine challenge after 7 or 21 days of glyphosate exposure. Glyphosate-rich farm air samples as well as glyphosate alone were found to induce pulmonary IL-13-dependent inflammation and promote Th2 type cytokines, but not IL-4 for glyphosate alone.

Materials and methods

Mice

C57BL/6 female (6–9 weeks) mice were purchased from Jackson Laboratory (Sacramento, CA). TLR4–/– mice (backcrossed 10 generations) were received from Cincinnati Children's Hospital Medical Center (CCHMC). Both strains were subsequently bred in house. Female mice of wild type and IL-13–/– BALB/c background were received from the laboratory of Dr. Fred Finkelman, CCHMC. Mice were housed in individually ventilated cages in a pathogen free facility at the Department of Environmental Health, University of Cincinnati (UC) following the UC Institutional Animal Care and Use Committee (IACUC) guidelines and all experiments were conducted following a UC IACUC – approved protocol.

Antibodies and reagents

The following antibodies for flow cytometry were purchased: Ly-6G (Gr-1) eFluor1 450 (RB6-8C5; Isotype Rat IgG2b) from eBioscience (San Diego, CA). CD16/CD32 (2.4G2; Isotype Rat IgG2b) and SiglecF-PE (E50-2440; Isotype Rat IgG2a) were purchased from BD PharMingen (San Jose, CA). A kit for measuring serum levels of MMCP1 was purchased from eBioscience.

Collection of farm air samples during summer pesticide spray seasons

Air samples were collected by three sets of total inhalable aerosol samplers (Button Inhalable Aerosol Sampler, SKC Inc., Eighty Four, PA) operated in parallel on three farms in Butler County, Ohio during summer glyphosate spray seasons. Samplers were installed at 1.5 m height at the edge of the field downwind from the spraying locations (sizes: approx. 5000 - 10,000 m^2). The sampling period was approximately 24 h starting from glyphosate spraying and air samples were collected at a flow rate of approximately 4 l/min on glass fiber filters. The filters from one set of samplers containing aerosolized glyphosate were eluted using PBS and the suspensions were filtered. A stock solution was prepared by pooling the samples collected from three farms (from now on referred as 'Real Env.') and used for intranasal treatment of mice. The filters from the other two sets of samplers were analysed for glyphosate and endotoxin to estimate the levels of glyphosate and endotoxin in 'Real Env.' samples.

Analysis of glyphosate in filter extracts

Glyphosate residues from filters were extracted using KH2PO4 buffer/1 M NaOH in an automatic shaker followed by freeze-drying. The freeze-dried samples were dissolved with deionised water and filtered through 0.45 mM Millipore filter. Glyphosate levels in the suspensions were determined by Abraxis ELISA Kit at 450 nm. The average amount of glyphosate per filter was 17.33 µg, which correspond to average airborne concentration of 22.59 ng/m^3.

Analysis of endotoxin in filter extracts

Endotoxin in filter extracts were analysed using the Limulus amebocyte lysate assay (Pyrochrome LAL; Associates of Cape Cod Inc., Falmouth, MA), as described previously. The samples were spiked with endotoxin standard of 0.50 EU/mL to assure that there was no inhibition or enhancement between the filter extracts and the reagents. The average amount of endotoxin per filter was 24.49 EU, which correspond to average airborne concentration of 4.87 EU/m^3.

Treatment of mice with farm-derived air samples, glyphosate and sensitization with OVA

PBS suspended farm air sample ('Real Env.'; estimated amount of glyphosate: 8.66 µg/mL) and reagent grade glyphosate (Sigma – Aldrich, St. Louis, MO) (100 ng, 1 µg or 100 µg) were delivered (in 30 µL) to the nose of anesthetised mice which were witnessed to aspirate the solution. Treatments were administered either: daily for 7 days or 3 times a week for 3 weeks. Same exposure schedule was followed for OVA alone (100 µg) and for OVA (100 µg) plus different dose of farm air sample and glyphosate. Mice were sacrificed 24 h after final airway treatment with sodium pentobarbital.

Histological analysis of lung

Formalin-fixed paraffin embedded lung sections (5 µm thick) were prepared for H&E and chloroacetate esterase (CAE) staining. The entire histological slide from each mouse was examined in blinded fashion and given a global categorical severity score based on infiltration of cells into parenchymal, peribronchial,

and perivascular regions of lungs.

Immunohistochemical staining
To analyse IL-33 and TSLP expression in the lungs section, the following antibodies were used for immunostaining: mouse IL-33 (0.2 mg/mL, AF3626, R&D Systems, Minneapolis, MN); mouse TSLP biotinylated (0.2 mg/ml, BAF555, R&D Systems) and respective isotype controls (R&D Systems). IL-33 and TSLP antibody–antigen complex were detected using Cy3 donkey anti-goat IgG (1:10,000) (Invitrogen/Molecular probes, Grand Island, NY). Slides were counterstained with DAPI (Vector Labs, Burlingame, CA). Images were obtained using a Nikon A1R Si microscope.

Isolation of lung inflammatory cells
Lungs were perfused with PBS, removed, manually minced into 1–2 mm fragments and then placed in Hank's Balanced Salt Solution (Sigma–Aldrich) containing Liberase TL (50 µg/mL; Roche Diagnostics, Indianapolis, IN) and DNase I (0.5 mg/mL; Sigma – Aldrich). Tissue was digested at 37 °C in a CO_2 incubator for 30 min. The tissue suspension was then passed through a 40 µm cell strainer. ACK lysis buffer (invitrogen) was used to clear red blood cells.

Flow cytometric analysis
Single cell suspensions from lungs (10^6 per mL) were blocked with anti-mouse CD16/CD32 antibodies before cell-surface staining. Cells were stained with fluorescently-labeled antibodies against SiglecF, Ly-6G/C (Gr-1), in different combinations according to the experiment. Analysis was performed using a FACSCanto II cytometer and FACSDIVA software (BD Biosciences). Eosinophils were defined as being $SiglecF^+Gr\text{-}1^+$ and neutrophils as $SiglecF^-$ $Gr\text{-}1^+$.

Cytokine measurement
IL-4, IL-10, IL-13, and IFN-γ production were measured by the in vivo cytokine capture assay (IVCCA). Briefly, biotinylated cytokine-specific mAbs were injected via tail vein immediately before the last airway treatment, and blood was collected 24 h later; sera or plasma were analysed with microtiter plates wells coated with corresponding anti-cytokine mAbs. Cytokine levels were also assessed in Bronchoalveolar lavage fluid (BALF) that was obtained 24 h after the last airway treatment. A kit for measuring in vivo IL-4 production by IVCCA, R46A2 and XMG1.2 anti-IFN-γ mAbs was purchased from Becton–Dickinson (Franklin Lakes, NJ); eBio1316H and eBio13A anti-IL-13 mAbs JES5-2A5 and JES5-16E3 anti-IL-10 mAbs ELISA Ready-SET-Go analysis kits for measurement IL-33 and IL-5 were purchased from eBioscience. Assays were performed according to the kit's manufacturer protocols.

Statistical analyses
Data were analysed with Sigma Plot 12.0 (Systat Software, Inc., San Jose, CA). Statistically significant differences in means were determined by one-way ANOVA followed by Bonferroni multiple comparison tests. Kruskal–Wallis tests were conducted if the data did not have a normal distribution. All the data are presented as mean ± SD for each group. Probability values of <0.05 were considered significant.

Results
Exposure of air samples collected during glyphosate spray on farms stimulates airway inflammation
Wild type C57BL/6 (WT) and TLR4–/– mice were intranasally exposed to 'Real Env.' samples (PBS suspended farm air samples) daily for 7 days. 'Real Env.' exposure was found to substantially increase the cell count in both the lungs and BAL fluid of WT and TLR4–/– mice. Additionally, the increase in pulmonary infiltrate in lungs was found to be higher in TLR4–/– than in WT mice (Fig. 1A and B). Similarly, an increase in eosinophil and neutrophil levels in 'Real Env.' treated mice (Fig. 1C–F) were observed. This inflammation was also confirmed by histological examinations (Fig. 1G) and elevated IgG1 and IgG2a levels. Additional experiments were conducted using reagent grade glyphosate of different doses. Administration of reagent grade glyphosate to the airway of mice produced substantial pulmonary inflammation whether the daily dose given was 100 ng, 1 µg or 100 µg for 7 days.

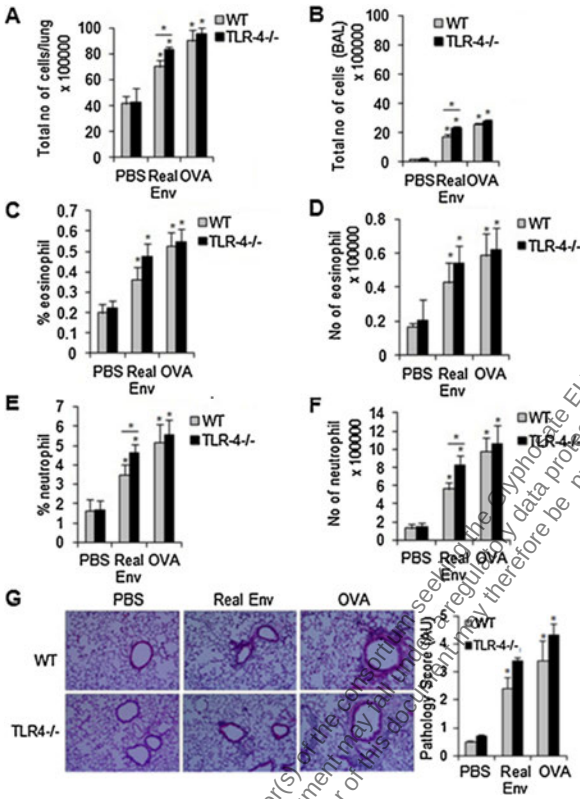


Figure 1: Increase in total number of cells, eosinophils, and neutrophils in lung and BAL fluids upon airway exposure to farm air samples ('Real Env.') and OVA for seven consecutive days (mean ± SD; *n* = 8). Increase in total number of cells in (A) lung and (B) BAL fluids. Increase in percentage (C) and total number (D) of eosinophils and neutrophils (E, F) per lung upon exposure to farm air samples ('Real Env.'). (G) Representative lung sections (H&E staining) and its pathology score from mice treated with PBS, farm air samples ('Real Env.') and OVA intranasally for 7 consecutive days (mean ± SD; *n* = 8); magnification 200x. * Indicates statistically significant differences (p <0.05) with respect to PBS treated control and in between WT and TLR4–/– mice group.

In the BALF and lung digests, a significant increase in the total cell count when treated with glyphosate at 1 µg or 100 µg (Fig. 2A and D) was found. Eosinophils (Fig. 2B and C), neutrophils, (Fig. 2E and F), and IgG1 and IgG2a levels) were also increased in glyphosate-treated mice compared to controls. However, No significant changes in the total cell count, eosinophils and neutrophils, IgG1 and IgG2a at glyphosate dose of 100 ng were found. Inflammation was confirmed by histological examination (Fig. 2M). Mice treated with both reagent grade glyphosate and OVA demonstrated significantly higher cell count (Fig. 2G and J), eosinophils (Fig. 2H and K), neutrophils (Fig. 2I and L), IgG1, and IgG2a compared to PBS treated mice.

Because pulmonary mastocytosis is typically observed in protein-allergen-induced experimental asthma, the pulmonary mast cell burden in the mice were assessed.

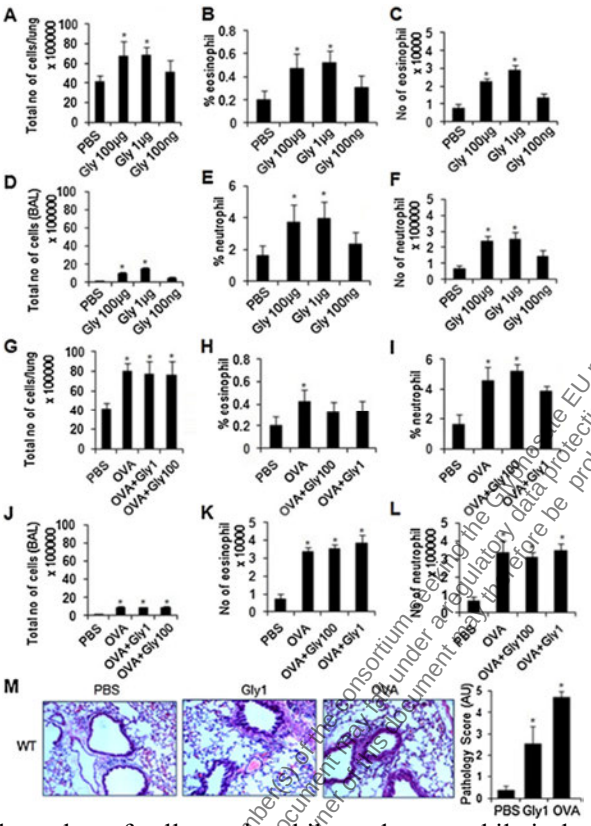


Figure 2: Increase in total number of cells, eosinophils, and neutrophils in lung and BAL fluids of WT mice upon airway exposure to glyphosate and combinations of glyphosate and OVA for seven consecutive days (mean ± SD; $n = 8$). Increase in total number of cells in (A) lungs and (D) BAL fluids upon exposure to different doses of glyphosate (100 ng, 1 µg or 100 µg). Increase in percentage (B) and total number (C) of eosinophils and neutrophils (E and F) per lung upon exposure to two doses of glyphosate. Increase in total number of cells in (G) lungs and (J) BAL fluids upon exposure to combination of glyphosate (1 µg or 100 µg) with OVA (100 µg). Increase in percentage and total number of (H and K) eosinophils and (I and L) neutrophils per lung upon exposure to OVA and combination of glyphosate, respectively. (M) Representative lung sections (H&E staining) and its pathology score from WT mice treated with PBS, glyphosate (1 µg) and OVA (100 µg) intranasally for 7 consecutive days (mean ± SD; $n = 8$); magnification 200 x. * Indicates statistically significant differences ($p < 0.05$) with respect to PBS and treated WT mice group

A significant increase in mast cell number in lungs treated with the substances isolated from the air on active farms ('Real Env.') and reagent grade glyphosate (Fig. 3A and C) was not observed. However, the MCPT-1 levels were found to be substantially higher in both groups indicating increased mast cell degranulation in the treated mice (Fig. 3B and D).

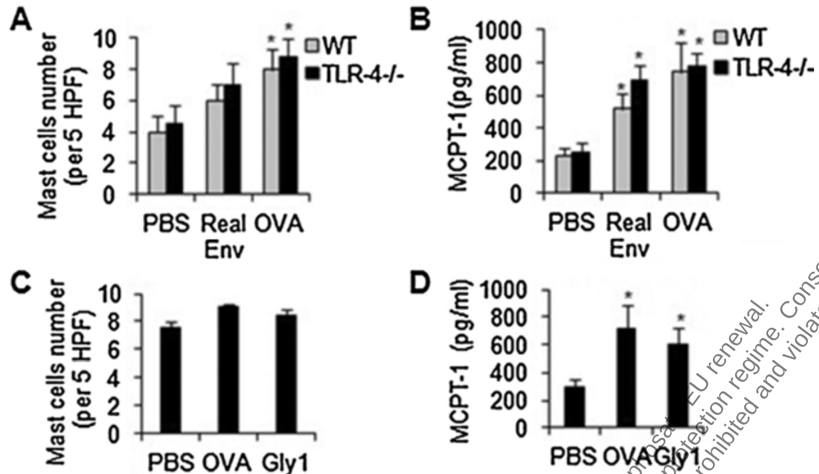


Figure 3: Farm air samples containing glyphosate as well as pure glyphosate alone induce increased mast cell degranulation but no increase in lung mast cell numbers upon airway exposure. (A) Mast cells number in CAE stained lung section and (B) serum MCPT-1 concentration in blood 4 h after last exposure of PBS, farm air samples ('Real Env.'), and ovalbumin (OVA). (C) Mast cells number in CAE stained lung section and (D) serum MCPT-1 concentration from mice treated with PBS, ovalbumin and 1 µg of glyphosate delivered to intranasally for 7 consecutive days (mean ± SD; n = 8). * Indicates statistically significant differences ($p < 0.05$) with respect to PBS and treated mice group.

Glyphosate-rich farm air samples induced airway inflammation and higher production of IL-10, IL-13, IL-5, IFN-γ and IL-4 but glyphosate alone failed to produce IL-4

To evaluate the glyphosate-induced inflammation, the systemic cytokine profile (Fig. 4A–E) of 'Real Env.' and glyphosate exposed mice were measured using IVCCA (Finkelman and Morris, 1999). Significantly higher levels of IL-5, IL-10, IL-13, and IL-4 were found upon treatment with 'Real Env.' alone in WT and TLR4–/– mice (Fig. 4A–D) approaching the levels induced by treating with OVA alone. The production of IL-5, IL-13 and IL-10 following 'Real Env.' exposures was higher in TLR4–/– than in WT mice. No significant difference in IL-4 production between TLR4–/– and WT mice were found (Fig. 4D). Production of these cytokines in mice given two different doses of glyphosate were then tested and found significantly higher levels of IL-5, IL-10, IL-13 and IFN-γ (Fig. 4F) that approached those levels induced by treating with OVA alone. Notably, there was no additional or synergistic effect when OVA was co-administered with glyphosate (Fig. 4G). Another interesting finding is that glyphosate alone was unable to induce significant levels of IL-4 while airway treatment with glyphosate with OVA did so.

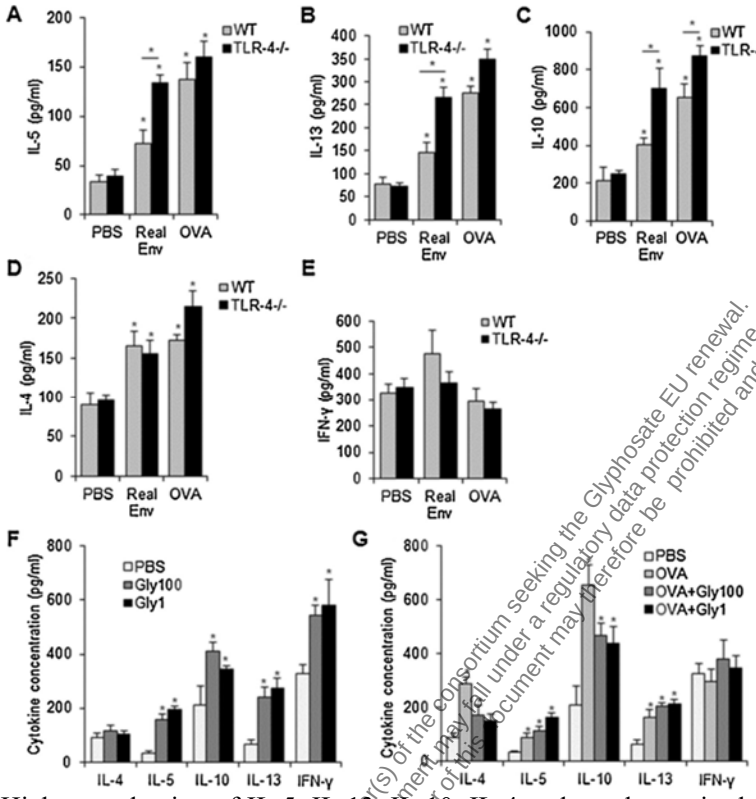


Figure 4: (A–E) Higher production of IL-5, IL-13, IL-10, IL-4 and no change in the IFN-γ levels upon exposure to farm air samples in WT and TLR4–/– mice. (F) The increased level of IL-5, IL-10, IL-13, IFN-γ and no change in the IL-4 levels upon glyphosate (1 or 100 µg) exposure to WT mice. (G) The increased level of IL-4, IL-5, IL-10, IL-13, and no change in IFN-γ levels upon combination of glyphosate (1 or 100 µg) and ovalbumin (100 µg) exposure to WT mice (mean ± SD; $n = 8$). Levels of cytokines were evaluated by IVCCA in serum of mice upon 7 consecutive days of intranasal treatment with farm air samples ('Real Env.') and glyphosate. Blood samples were collected 24 h after the last exposure. IL-5 was measured in the BAL fluids. * Indicates statistically significant differences ($p < 0.05$) with respect to PBS treated control and in between WT and TLR4–/– mice group.

IL-33 and TSLP in lungs are increased upon exposure to glyphosate-rich air samples as well as reagent grade glyphosate alone

As the cytokine profile of mice treated with 'Real Env.' and glyphosate approximated those treated with OVA, mediators known to promote type 2 pathology were examined. IL-33 and TSLP appeared to be logical choices because of their well-recognized effector functions, and due to their source – the respiratory epithelium cells which would be the first cells to encounter inhaled glyphosate. The IL-33 and TSLP content of BALF were measured directly and found an abundance of both cytokines in 'Real Env.' – treated WT and TLR4–/– mice (Fig. 5A and B). IL-33 production was observed to be significantly higher in TLR4–/– mice compared to WT mice. An abundance of both cytokines in glyphosate-treated mice were observed (Fig. 5C and D). This finding was confirmed by immunohistochemical staining of IL-33 and TSLP in lung sections of glyphosate-treated mice (Fig. 5E) and 'Real Env.' – treated WT and TLR4–/– mice, which demonstrated substantial production of both cytokines, which was limited to the respiratory epithelium after glyphosate exposure.

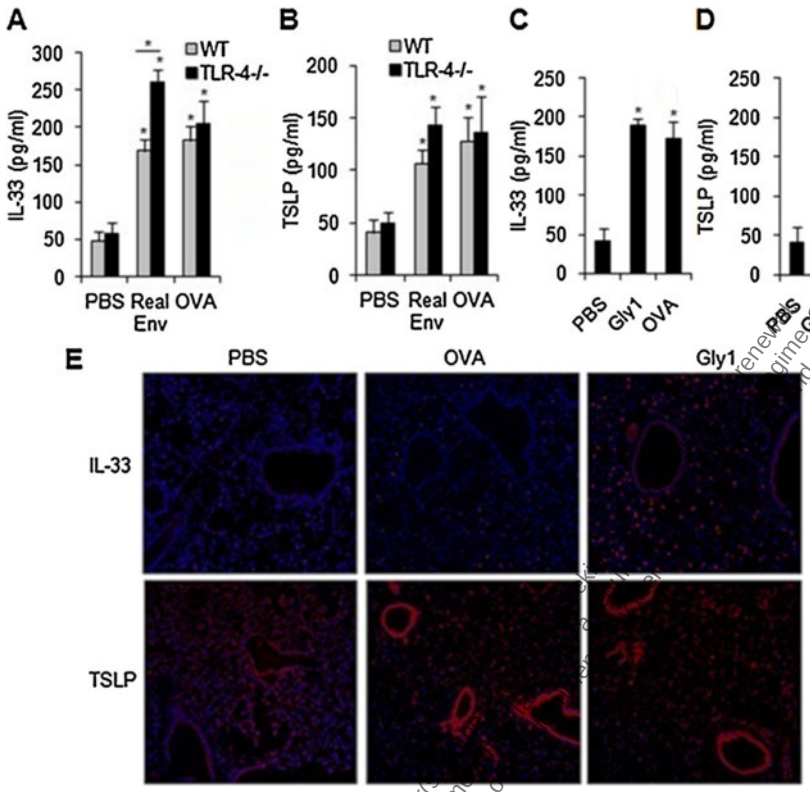


Figure 5: IL-33 and TSLP productions increased in the lung upon exposure to farm air samples and glyphosate. (A and B) ELISA based measurement of IL-33 and TSLP in BAL fluids of PBS, farm air samples and ovalbumin (100 µg) treated WT and TLR4–/– mice, respectively (mean ± SD; $n = 8$). (C and D) ELISA based measurement of IL-33 and TSLP in BAL fluids of PBS, OVA and pure glyphosate (1 µg) treated WT mice, respectively (mean ± SD; $n = 8$). (E) Immunofluorescence staining of IL-33 and TSLP in the lung sections of the glyphosate treated WT mice, magnification 200x. * Indicates statistically significant differences ($p < 0.05$) with respect to PBS treated control and in between WT and TLR4–/– mice group.

Glyphosate-induced pulmonary inflammation is attenuated in IL-13 –/– mice

Glyphosate as a small molecule may not be efficiently presented to conventional T cells by antigen-presenting cells. The involvement of innate pathways upon glyphosate exposure, as hypothesized, was supported by the absence of an increased production of IL-4. This absence would have been expected if type 2 innate lymphoid cells (ILC2s) were the primary source of the IL-5 and IL-13 detected. IL-33 and TSLP have been well described to induce ILC2s, which in turn causes lung pathology particularly via IL-13-dependent mechanism. To test this hypothesis, IL-13 deficient mice were exposed to glyphosate for 7 and 21 days and assessed lung inflammation. While there was no change in IL-4 levels, the inability to produce IL-13 prevented the rise in IL-5 production, but not the rise in IL-10 production, at both time points during glyphosate treatment. Deficiency in IL-13 also prevented a significant rise in IL-33 and TSLP levels at the early time point but not the latter one (Fig. 6A–D). Lack of IL-13 production was also associated with significantly less ($p < 0.05$) severe cellular infiltration noted on histology (Fig. 6E). Despite significant inflammation, no airway hyper responsiveness was found in glyphosate-treated wild type and IL-13–/– mice.

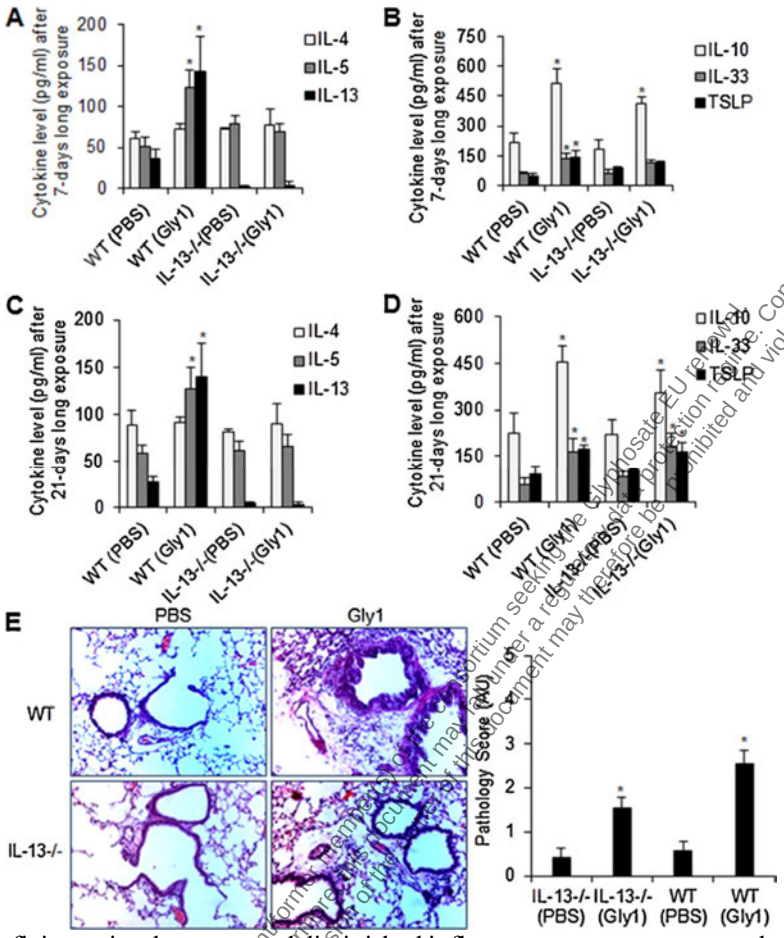


Figure 6: IL-13-deficient mice demonstrated diminished inflammatory response upon glyphosate exposure. (A, C) Diminished production of IL-5 but no change in IL-4 level, and (B, D) diminished production of TSLP, IL-33, IL-10 levels, between IL-13-deficient mice and WT mice upon glyphosate exposure (1 µg) for 7 or 21 days, respectively (mean ± SD; *n*=8). (E) Representative lung sections (H&E staining) from mice treated with PBS and glyphosate (1 µg) intranasally 3 times a week for 21 days; magnification 200 x (left panel). Arbitrary scores were based on inflammatory cells infiltration in lungs parenchyma, peribronchial, and perivascular regions. Analysis was performed in a double-blinded manner (right panel). * Indicates statistically significant differences (p <0.05) with respect to PBS treated control group.

Conclusion

The results demonstrate the capacity of glyphosate-rich air samples from farms as well as pure glyphosate to induce type 2 airway inflammation, over both short and longer time courses. Furthermore, glyphosate induced inflammation was found to be associated with induction of IL-33 and TSLP. This work also highlights the production of IL-13 as well as modulation of innate immune system by glyphosate, which may play an important role in exacerbation of airway inflammation by this low molecular weight chemical.

3. Assessment and conclusion

Assessment and conclusion by applicant:

This study evaluated nose-only exposure to glyphosate and collected farm air samples containing glyphosate and evaluated the immune response in the lungs. This is not a guideline study nor an endpoint used in risk assessment. This study in not usable for risk assessment in terms of hazard assessment. In terms of exposure, the study determined that average amount of glyphosate per filter from environmental samples after spray application to fields was 17.33 µg, which correspond to average airborne concentration of 22.59 ng/m^3. The method for the collection and analysis of the air samples was not

validated and the assumptions and calculations used in the determination of the average airborne concentration were not provided, therefore the results cannot be verified. While the study itself is acceptable, it is unreliable in terms of usable endpoints for risk assessment.

Assessment and conclusion by RMS:

Reliability criteria for *in vivo* toxicology studies

Publication: Kumar et al., 2014.	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity is reagent grade. Source: Sigma - Aldrich, St. Louis, USA.
Only glyphosate acid or one of its salts is the tested substance	N	Sampling of aerosols from field spraying also contain co-formulants of the GBH applied.
AMPA is the tested substance	N	
Study		
Test species clearly and completely described	Y	Female mice of wild type and IL-13-/- BALB/c background.
Test conditions clearly and completely described	Y	
Route and mode of administration described	Y	Intranasal application of air samples taken during glyphosate field application (24 hours) and glyphosate.
Dose levels reported	Y	Field air sample and 100 ng, 1 µg or 100 µg of glyphosate delivered intranasally.
Number of animals used per dose level reported	Y	
Method of analysis described for analysis test media	Y	Glyphosate measured in air sample with ELISA kit.
Positive control	Y	Ovalbumin.
Validation of the analytical method	N	
Analytical verifications of test media	N	
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical control data of the laboratory reported	N	
Dose-effect relationship reported	Y	For glyphosate.
Overall assessment		
Reliable without restrictions		

Reliable with restrictions	Y	
Reliability not assignable		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the method for the collection and analysis of the air samples was not validated.		

Assessment and conclusion by RMS:

CA 5.4 Genotoxicity

In the 2001 EU evaluation glyphosate was examined for genotoxicity in a wide range of test systems covering all relevant endpoints *in vitro* (see CA 5.4.1) and *in vivo*, in both somatic (see CA 5.4.2) and germ cells (see CA 5.4.3). Additional studies have been conducted on glyphosate since the last EU review, however, all these studies were negative and are considered confirmatory data.

In addition to studies commissioned by representatives of the Glyphosate Renewal Group, several publications identified from the open literature have been submitted. The majority of these publications report *in vitro* comet assay data, although there are also data from two mouse bone marrow micronucleus tests and *in vitro* chromosome aberration, cytotoxicity and cytokinesis block micronucleus tests. It is noted that a common deficieny for many of these publications is a lack of adequate information concerning the characterisation of the glyphosate source used.

The two *in vivo* publications report negative results for six out of a total of seven different batches of glyphosate tested, thus confirming the findings of the Glyphosate Renewal Group studies. The one batch reported as positive is likely to have been compromised by the presence of 0.13 % formaldehyde.

Although a small number of publications reported negative *in vitro* findings (CA 5.4/003 and CA 5.4/007), the majority of studies are indicated to be positive for DNA damage. Due to inconsistencies in methodology (e.g. cell lines used, exposure conditions, toxicity meansurements, concentrations investigated etc) the genotoxicological relevance of the reported data is unclear. However, the majority of the publications indicate the observed DNA damage was frequently concomitant with high, confounding toxicity and markers of oxidative damage. When considered alongside the consistently negative findings in the regulatory mutagenicity studies (see Sections CA 5.4.1, CA 5.4.2 and CA 5.4.3), it is concluded that the results of these indicator tests (i.e. assays that do not measure an apical mutagenicity endpoint) confirm the lack of an ability for glyphosate to react directly with cellular DNA. Instead the findings are indicative of DNA damage occurring secondary to other toxic cellular events. Furthermore, as demonstrated by the consistent negative findings in *in vivo* models, provided that normal homeostasis mechanisms are not overwhelmed, glyphosate-induced secondary DNA damage does not occur *in vivo*.

The overwhelming weight of evidence gathered from all the submitted studies, is that glyphosate does not exhibit a genotoxic risk to humans.

Nota bene: In autumn of 2019 NGOs and media published accusations against the German based contract research organization "[redacted]" on animal welfare and GLP. In the frame of this glyphosate renewal dossier GRG is submitting 24 studies performed during the years 1995 – 2010 at [redacted]. All these studies were conducted during the validity periods of GLP certificates routinely renewed and re-confirming the GLP-standard every three years. Studies performed at this laboratory are clearly indicated in the overview table (Table 5.4.1-1, 5.4.2.-1) as well as in the respective study header. Due to the large number of corroborative studies available within the Glyphosate Renewal

Group, the overall endpoint conclusion of glyphosate is not reliant on the results of the studies performed at [redacted]

CA 5.4.1 *In vitro* studies

Gene mutation in bacteria

Twenty-four bacterial reverse mutation ("Ames") studies are available for glyphosate (Table 5.4.1-1). Although several of these have some deficiencies compared to OECD 471 (1997), and thus are considered as supportive or invalid, there are 12 valid studies (reliable with restrictions) that are either fully compliant with OECD 471 (1997) or have only minor deviations that do not affect the validity or integrity of the data. Overall, there are sufficient, reliable data to allow thorough evaluation of this data requirement. There is no evidence that glyphosate is able to cause gene mutations in bacterial systems.

Table 5.4.1-1: Summary of *in vitro* genotoxicity testing with glyphosate acid: Gene mutation in bacteria

Annex Point	Study (Data owner)	Study type Test system	Substance, Purity Dose levels Metabolic activation	Reference list-related category[2]	Result
CA 5.4.1/001	[redacted] 2014	Ames test *S. typhimurium* TA 98, TA 100, TA 1535 and TA 1537 *E. coli* WP2 uvrA	Glyphosate Purity: 85.79 % SPT: 1.5 – 5000 µg/plate ± S9 PIT: 5 – 5000 µg/plate ± S9	Valid, Category 2a	Negative
CA 5.4.1/002	[redacted] 2012	Ames test *S. typhimurium* TA 98, TA 100, TA 102, TA 1535 and TA 1537	Glyphosate technical Purity: 97 % SPT: 10 – 5000 µg/plate ± S9 PIT: 10 – 5000 µg/plate ± S9	Valid, Category 2a	Negative
CA 5.4.1/003	[redacted], 2010	Ames test *S. typhimurium* TA 98, TA 100, TA 102, TA 1535 and TA 1537	Glyphosate technical Purity: 95.23 % SPT: 31.6 – 3160 µg/plate ± S9 PIT: 31.6 – 3160 µg/plate ± S9	Valid, Category 2a	Negative
CA 5.4.1/004	[redacted] 2010	Ames test *S. typhimurium* TA 98, TA 100, TA 1535 and TA 1537 *E. coli* WP2 uvrA	Glyphosate technical Purity: 97.16 % (spiked with glyphosine) SPT: 3 – 5000 µg/plate ± S9 PIT: 3 – 5000 µg/plate ± S9	Valid, Category 2a	Negative
CA 5.4.1/005	[redacted], 2010	Ames test *S. typhimurium* TA 98, TA 100, TA 1535, TA 97a, and TA102	Glyphosate technical Purity: 98.2 % SPT: 31.6 – 5000 µg/plate ± S9 PIT: 31.6 – 5000 µg/plate ± S9	Valid, Category 2a	Negative
CA 5.4.1/006	[redacted], 2009[1]	Ames test *S. typhimurium* TA 98, TA 100, TA 102, TA 1535 and TA 1537	Glyphosate technical Purity: 98.8 % SPT: 31.6 – 3160 µg/plate ± S9 PIT: 31.6 – 3160 µg/plate ± S9	Valid, Category 2a	Negative
CA 5.4.1/007	[redacted] 2009	Ames test *S. typhimurium* TA 98, TA 100, TA 1535 and TA 1537 *E. coli* WP2 uvrA (pKM101) and	Glyphosate technical Purity: 96.3 % SPT: 3 – 5000 µg/plate ± S9 PIT: 33 – 5000 µg/plate ± S9	Valid, Category 2a	Negative

		WP2 pKM101			
CA 5.4.1/008	[redacted] 2008	*S. typhimurium* TA 98, TA 100, TA 1535, TA 97a, and TA102	Glyphosate Purity: 98.05 % SPT: 1 – 1000 µg/plate ± S9	Invalid, Category 3b	Negative
CA 5.4.1/009	[redacted], 2007	Ames test *S. typhimurium* TA 98, TA 100, TA 1535 and TA 1537 *E. coli* WP2 uvrA	Glyphosate technical Purity: 95.1 % SPT: 3 – 5000 µg/plate ± S9 PIT: 33 – 5000 µg/plate ± S9	Valid, Category 2a	Negative
CA 5.4.1/010	[redacted] 2007	Ames test *S. typhimurium* TA 98, TA 100, TA 1535 and TA 1537 *E. coli* WP2 uvrA	Glyphosate technical Purity: 97.7 % SPT: 3 – 5000 µg/plate ± S9 PIT: 33 – 5000 µg/plate ± S9	Valid, Category 2a	Negative
CA 5.4.1/011	[redacted] 2007	Ames test *S. typhimurium* TA 98, TA 100, TA 1535 and TA 1537 *E. coli* WP2 uvrA	Glyphosate technical Purity: 95.0 % SPT: 3 – 5000 µg/plate ± S9 PIT: 33 – 5000 µg/plate ± S9	Valid, Category 2a	Negative
CA 5.4.1/012	[redacted] 2007	Ames test *S. typhimurium* TA 98, TA 100, TA 1535, TA 1537 and TA 102	Glyphosate technical Purity: 98.01 % SPT: 648 – 5000 µg/plate ± S9	Supportive, Category 2a	Negative
CA 5.4.1/013	[redacted] 1996	Ames test *S. typhimurium* TA 98, TA 100, TA 1535 and TA 1537 *E. coli* WP2 P and WP2 uvrA	Glyphosate acid Purity: 95.6 % SPT: 100 – 5000 µg/plate ± S9 PIT: 100 – 5000 µg/plate ± S9	Valid, Category 2a	Negative
CA 5.4.1/014	[redacted] 1996	Ames test *S. typhimurium* TA 98, TA 100, TA 1535, TA 1537 *E. coli* WP2 uvrA	Technical glyphosate Purity: 95.3 % SPT: 50 – 5000 µg/plate ± S9	Valid, Category 2a	Negative
CA 5.4.1/015	[redacted] 1995	Ames test *S. typhimurium* TA 98, TA 100, TA 1535 and TA 1537 *E. coli* WP2 uvrA	Glyphosate technical Purity: 95.68 % PIT: 156 – 5000 µg/plate ± S9	Valid, Category 2a	Negative
CA 5.4.1/016	[redacted], 1995	Ames test *S. typhimurium* TA 98, TA 100, TA 102, TA 1535 and TA 1537	Glyphosate Purity: not reported 50 – 5000 µg/plate ± S9	Invalid, Category 4b	Negative
CA 5.4.1/017	[redacted], 1995	Ames test *S. typhimurium* TA 98, TA 100, TA 1535, TA 1537 and TA 1538	Glyphosate Purity: 95 % 8 – 5000 µg/plate ± S9	Invalid, Category 4b	Negative
CA 5.4.1/018	[redacted] 1993	Ames test *S. typhimurium* TA 98, TA 100, TA 1535, TA 1537 and TA1538	Glyphosate technical Purity: 96.0 % SPT: 1 – 1000 µg/plate ± S9	Supportive, Category 3a	Negative
CA 5.4.1/019	[redacted], 1993	Ames test *S. typhimurium* TA 98 and TA 100	Glyphosate isopropylamine salt Purity: 64.5 % SPT: 0.01 – 100 µg/plate ± S9	Invalid, Category 3b	Negative

CA 5.4.1/020	[redacted] 1991	Ames test *S. typhimurium* TA 98, TA 100, TA 1535 and TA 1537	Glyphosate Purity: 98.6 % SPT: 160 – 2500 µg/plate – S9 310 – 5000 µg/plate + S9 PIT: 160 – 2500 µg/plate - S9 310 – 5000 µg/plate + S9	Supportive, Category 2a	Negative
CA 5.4.1/021	[redacted] 1990	Ames test *S. typhimurium* TA 98, TA 100, TA 1535, TA 1537 and TA 1538	Glyphosate active Purity: not reported SPT: 8 – 5000 µg/plate ± S9 and 312.5 – 5000 µg/plate ± S9	Invalid, Category 3b	Negative
CA 5.4.1/022	[redacted], 1986	Ames test *S. typhimurium* TA 98, TA 100, TA 1535 and TA 1537 *E. coli* WP2 uvrA	Glyphosate technical Purity: not reported PIT: 10 – 1000 µg/plate ± S9	Invalid, Category 3b	Negative
CA 5.4.1/023	[redacted], 1981	Ames test *S. typhimurium* TA his-G46, TA 1537 and TA 1538	Glyphosate active principle Purity: not reported Spot test: 1 – 1000 µg/plate - S9	Invalid, Category 3b	Negative
CA 5.4.1/024	[redacted] 1978	Ames test *S. typhimurium* TA 98, TA 100, TA 1535, TA1537 and TA 1538 *E. coli* WP hcr	Glyphosate Purity: 98.4 % SPT: 10 – 5000 µg/plate ± S9	Supportive, Category 2a	Negative

SPT: Standard plate test (plate-incorporation method); PIT: Pre-incubation test

[1] Testing was performed at the Laboratory of Pharmacology and Toxicology (LPT), Hamburg, Germany.

[2] The category describes the acceptability/reliability of the study within the AIR 5 submission. This category does not describe hazard classification. (for details please refer to the Doc ID: 110054-B-GRG_Jun_2020)

1. Information on the study

Data point	CA 5.4.1/001
Report author	[redacted]
Report year	2014
Report title	Glyphosate: Reverse Mutation Assay 'Ames Test' using *Salmonella typhimurium* and *Escherichia coli*
Report No	41401854
Document No	Not reported
Guidelines followed in study	OECD 471 (1997), Commission Regulation (EC) no. 440/2008 Method B13/14 (2008), U.S. EPA OCSPP 870.5100, Japanese MAFF
Deviations from current test guideline OECD 471 (1997)	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Glyphosate (batch: 04062014, purity: 85.79 %) was assessed for it's potential to induce gene mutations in bacteria in an Ames test conducted with *S. typhimurium* strains TA 98, TA 100, TA 1535 and TA 1537 and *E. coli* strain WP2uvrA in the presence and absence of metabolic activation (phenobarbitone / β-naphthoflavone-induced rat liver S9 fraction). Two independent experiments were performed, using the plate-incorporation method (standard plate test, first experiment) and the pre-incubation method (second experiment). Triplicate cultures each were exposed to test item concentrations in the range of 1.5 – 5000 µg/plate (first experiment) and 5 – 5000 µg/plate (second experiment). Negative (untreated), vehicle (DMSO) and positive controls were included in each experiment. After an incubation period of 48 hours, the plates were inspected for a possible reduction in the bacterial background lawn and the number of revertant colonies were counted for each plate.

There was no precipitation observed in any tester strain up to the highest tested concentration, neither in the presence nor absence of S9 mix. Cytotoxicity, evident as a reduction in the bacterial background lawn, was evident in both experiments at 5000 µg/plate for all strains in the absence of metabolic activation. There was no reduction in bacterial growth observed in the presence of S9 mix, however, a reduced frequency in the number of revertant colonies was noted in tester strain TA100 in the first experiment at 5000 µg/plate and in tester strain WP2 uvrA in the second experiment at 5000 µg/plate.

There was no statistically significant increase in the frequency of revertant colonies noted for any of the bacterial strains at any dose level, either with or without S9 mix. The number of revertant colonies for the vehicle control and for negative (untreated) controls were considered acceptable. All of the positive controls induced marked increases in the frequency of revertant colonies, thus confirming the activity of the metabolic activation system and the sensitivity of the test itself.
Based on the experimental findings, the test item is not mutagenic in bacteria with and without metabolic activation.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material: Glyphosate

Identification:	Not specified
Description:	White crystalline solid
Lot/Batch #:	04062014
Purity:	85.79 %
Stability of test compound:	The stability of the test item under storage conditions (at room temperature in the dark) and in solvent (vehicle) was not specified. All formulations were used within 4 h of preparation and were assumed to be stable for this period.
Solvent (vehicle) used:	Dimethylsulfoxide (DMSO)

2. Control materials:

Negative control:	Untreated controls were included in each experiment.
Solvent (vehicle) control:	Dimethylsulfoxide (DMSO)
Solvent (vehicle)/final concentration:	0.1 mL per plate.
Positive controls:	Please refer to table below.

Strain	Metabolic activation	Mutagen	Conc. [µg/plate]
S. typhimurium strains			
TA 100	-S9	N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG)	3.0
	+S9	2-Aminoanthracene (2-AA)	1.0
TA 1535	-S9	N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG)	5.0
	+S9	2-Aminoanthracene (2-AA)	2.0
TA 98	-S9	4-Nitroquinoline-1-oxide (4-NQO)	0.2
	+S9	Benzo[a]pyrene (BP)	5.0
TA 1537	-S9	9-Aminoacrinidine (9AA)	80.0
	+S9	2-Aminoanthracene (2-AA)	2.0
E. coli strain(s)			
WP2 uvrA	-S9	N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG)	2.0
	+S9	2-Aminoanthracene (2-AA)	10.0

3. Metabolic activation:

S9 mix was produced from the livers of male rats, which were treated orally with 80 mg/kg bw/day phenobarbitone and 100 mg/kg bw/day β-naphthoflavone for three days. On Day 4, the livers were prepared and S9 homogenate was generated. The S9 mix was thawed prior to each experiment and co-factor was immediately added to prepare S9 mix containing the following components:

S9 mix component	Concentration	Unit
0.2 M Sodium phosphate buffer (pH 7.4)	25.0	mL
1.65 M KCl/0.4 M $MgCl_2$	1.0	mL
NADPH-generating system		
0.1 M Glucose 6-phosphate	2.5	mL
0.1 M NADP	2.0	mL
Sterile distilled water	14.5	mL
S9	5.0	mL

4. Test organisms:

Tester strains				Bacteria batch checked for	
S. typhimurium		***E.coli***			
TA 98	✓	WP2 uvrA	✓	Deep rough character (rfa)	✓
TA 100	✓	WP2 uvrA (pKM101)		Ampicillin resistance (R factor plasmid)	✓
TA 1535	✓			UV-light sensitivity (absence of uvrB and uvrA genes in *S. typhimurium* and *E. coli* strains, respectively)	✓
TA 1537	✓				
TA 1538				Histidine auxotrophy (automatically *via* the spontaneous rate)	✓

5. Test concentrations:

(a) Preliminary cytotoxicity assay:

Plate incorporation test ± S9 mix:	
Concentrations:	*1.5, 5, 15, 50, 150, 500, 1500 and 5000 µg/plate*
Tester strains:	TA 98, TA 100, TA 1535, TA 1537 and WP2 uvrA

Replicates:	*Triplicates*
Pre-incubation test ± S9 mix:	
Concentrations:	*5, 15, 50, 150, 500, 1500 and 5000 µg/plate*
Tester strains:	TA 98, TA 100, TA 1535, TA 1537 and WP2 uvrA
Replicates:	*Triplicates*

B: STUDY DESIGN AND METHODS

1. **Dates of experimental work:** 16 Jul – 31 Aug 2014
Finalisation date: 02 Sep 2014

2. **Standard plate test (plate-incorporation test, SPT):**
An aliquot of 0.1 mL test solution, vehicle or positive control, an aliquot of 0.1 mL fresh bacterial culture and 0.5 mL S9 mix (in tests with metabolic activation) or 0.5 mL phosphate buffer (in tests without metabolic activation) were added to 2 mL of molten trace amino acid supplemented medium (1.0 mM histidine + 1.0 mM biotin or 1.0 mM tryptophan). After mixing, the mixture was then overlayed onto a Vogel-Bonner agar plate. Negative (untreated) controls were included in each experiment. The plates were incubated at 37 ± 3 °C for approximately 48 hours and scored for the presence of revertant colonies and thinning of the bacterial background lawn.

3. **Pre-incubation test (PIT):**
0.1 mL of test solution, vehicle or positive control, 0.1 mL pre-cultured bacterial suspension and 0.5 mL of S9 mix (in tests with metabolic activation) or 0.5 mL phosphate buffer (in tests without metabolic activation) were mixed and pre-incubated for 20 minutes at 37 ± 3 °C while shaking. After pre-incubation, 2.0 mL of molten amino acid supplemented medium (1.0 mM histidine + 1.0 mM biotin or 1.0 mM tryptophan), the mixture was plated onto Vogel-Bonner agar plates. Negative (untreated) controls were included in each experiment. The plates were incubated at 37 ± 3 °C for approximately 48 hours and scored for the presence of revertant colonies and thinning of the bacterial background lawn.

4. **Cytotoxicity**
Toxicity was detected by a
   - reduction in the number of spontaneous revertants
   - clearing or diminution of the background lawn (= reduced his^- or trp^- background growth)

and recorded for all test groups both with and without S9 mix in all experiments.

5. **Statistics**
Statistical analysis was performed according to the UKEMS sub-committee on guidelines for mutagenicity testing[10].

6. **Acceptance criteria**
The test was considered valid if the following criteria were met:
   - All bacterial strains must have demonstrated the required characteristics as determined by their respective strain checks.

[10] Mahon G.A.T. (1989) Analysis of data from microbial colony assays. In: KIRKLAND D.J. (eds). Statistical Evaluation of Mutagenicity Test Data. Cambridge University Press Report, pp. 26-65

- All tester strains cultures should exhibit a characteristic number of spontaneous revertants per plate, which is in the range of historical control data.
- All tester strain cultures should be in the range of 0.9 – 9 x 10^9 bacteria per mL.
- Positive control chemicals should induce marked increases in the frequency of revertant colonies, both with or without metabolic activation, which are in the range of historical control data.
- There should be a minimum of 4 non-toxic test item concentrations.
- There should be no evidence of excessive contamination.

7. Evaluation criteria

A test item was considered positive for mutagenicity if the following criteria were met:

- There was a dose-related increase in mutant frequency over the dose range tested.
- There was a reproducible, statistically significant increase at one or more concentrations.
- There was a biological relevance against the laboratories historical control ranges.
- The increase fold was greater than two times the concurrent solvent control for any tester strain.

A test item was considered non-mutagenic (negative) in the test system if the above-mentioned criteria were not met.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Analytical determinations were not performed in the study, as not required by the test guideline

B. CYTOTOXICITY

Cytotoxicity, evident as a visible reduction in the growth of the bacterial background lawn was observed in the first experiment (plate incorporation test) for all tester strains at 5000 µg/plate in the absence of metabolic activation.

There was no reduction in the bacterial background lawn observed in the presence of S9 mix, however, when compared to solvent controls, strain TA 100 exhibited a lower number of revertant colonies at 5000 µg/plate.

In the second experiment (pre-incubation test), cytotoxicity results in the absence of S9 mix were identical to those of the first experiment, showing a reduced bacterial background lawn at 5000 µg/plate for all strains. In the presence of S9 mix, the bacterial growth was not impaired, but a lower frequency of revertant colonies was noted for tester strain WP2 uvrA at 5000 µg/plate.

C. SOLUBILITY

Precipitation of the test item was not observed on the plates at any of the dose levels tested, neither in the presence nor absence of S9 mix.

D. MUTATION ASSAY

There was no statistically significant increase in the frequency of revertant colonies recorded for any of the bacterial strains at any dose of the test item, either with or without metabolic activation. The number of revertant colonies for the vehicle control and for negative (untreated) controls were considered acceptable. In the first experiment, one value of the strain TA1535 was slightly outside of the historical control range, but as the count was only two colonies out of range and all other counts were within the range of historical controls, the value was considered acceptable. All of the positive controls induced marked increases in the frequency of revertant colonies, thus confirming the activity of the metabolic activation system and the sensitivity of the test itself.

Table 5.4.1-2: Glyphosate: Reverse Mutation Assay 'Ames Test' using Salmonella typhimurium and Escherichia coli ([redacted], 2014), first experiment

Experiment 1: Standard plate test (SPT)										
Strain	**TA 100**		**TA 1535**		**WP2 uvrA**		**TA 98**		**TA1537**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Negative control[$]										
mean	72		11		18		19		8	
± SD	± 14.2		± 7.4		± 6.2		± 4.0		± 3.5	
Vehicle control										
DMSO mean	70	82	16	9	18	25	19	22	12	12
± SD	± 5.1	± 9.3	± 8.3	± 3.2	± 6.2	± 3.8	± 5.5	± 9.0	± 4.2	± 3.6
HCD[#] mean	103	101	20	15	28	33	22	26	11	13
± SD	± 14.4	± 15.6	± 4.4	± 3.5	± 6.6	± 7.1	± 5.0	± 5.1	± 3.1	± 3.5
[range]	68 - 147	63 - 153	9 - 37	8 - 29	15 - 47	13 - 54	10 - 42	12 - 43	5 - 26	5 - 23
Test item [µg/plate]										
1.5 mean	71	73	12	13	21	21	14	21	9	10
± SD	± 8.3	± 8.1	± 7.6	± 4.0	± 5.5	± 7.9	± 3.1	± 8.7	± 7.2	± 2.1
5 mean	64	77	12	8	17	20	16	22	14	16
± SD	± 2.3	± 11.5	± 7.5	± 0.6	± 8.7	± 1.0	± 4.0	± 5.7	± 5.0	± 6.1
15 mean	69	90	19	12	21	14	19	20	15	11
± SD	± 6.8	± 7.2	± 5.3	± 2.3	± 4.9	± 2.1	± 6.0	± 8.1	± 5.9	± 3.5
50 mean	72	72	12	14	21	20	12	17	14	12
± SD	± 4.7	± 7.5	± 4.0	± 5.0	± 2.6	± 3.5	± 3.6	± 6.1	± 1.7	± 5.0
150 mean	74	73	16	10	20	22	16	20	8	10
± SD	± 9.5	± 11.0	± 7.2	± 2.9	± 3.5	± 4.9	± 6.1	± 4.2	± 2.3	± 4.0
500 mean	70	87	11	11	17	22	20	18	13	11
± SD	± 3.5	± 7.0	± 4.0	± 6.7	± 6.7	± 9.6	± 4.5	± 4.6	± 0.0	± 4.4
1500 mean	67	67	13	12	19	22	12	14	6	11
± SD	± 11.5	± 4.0	± 4.0	± 0.6	± 0.6	± 2.1	± 3.6	± 5.6	± 1.2	± 7.4
5000 mean	32[S]	48	7[S]	7	11[S]	25	9[S]	16	4[S]	11
± SD	± 8.0	± 14.4	± 2.3	± 2.1	± 3.8	± 4.0	± 7.1	± 1.2	± 1.2	± 2.5
Positive control										
[§] mean	830	1595	866	276	972	349	268	190	407	377
± SD	± 130.0	± 97.9	± 97.5	± 65.7	± 71.2	± 72.5	± 63.1	± 29.3	± 68.5	± 63.5
HCD[#] mean	543	1211	644	250	611	320	207	226	813	286
± SD	± 192.1	± 509.3	± 685.5	± 98.9	± 256.3	± 120.9	± 76.9	± 92.6	± 384.2	± 127.7
[range]	240 - 1429	349 - 3117	91 - 3750	103 - 1153	129 - 1275	101 - 733	102 - 783	84 - 669	113 - 2161	86 - 1238

[§] Information on respective positive control is reported in Material and Method section I.A.2

[$] Spontaneous mutation rates, untreated plates

[#] Historical control data from 2013, HCD of untreated and vehicle controls the values were combined

Table 5.4.1-2: Glyphosate: Reverse Mutation Assay 'Ames Test' using Salmonella typhimurium and Escherichia coli ([redacted], 2014), first experiment

Experiment 1: Standard plate test (SPT)										
Strain	**TA 100**		**TA 1535**		**WP2 uvrA**		**TA 98**		**TA1537**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**

[S] Sparse bacterial background lawn

Table 5.4.1-3: Glyphosate: Reverse Mutation Assay 'Ames Test' using *Salmonella typhimurium* and *Escherichia coli* ([redacted] 2014), second experiment

Experiment 2: Pre-incubation Test (PIT)										
Strain	**TA 100**		**TA 1535**		**WP2 uvrA**		**TA 98**		**TA 1537**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Negative control[$]										
mean ± *SD*	100 ± *10.6*		15 ± *6.2*		19 ± *4.5*		24 ± *7.0*		14 ± *2.3*	
Vehicle control										
DMSO mean ± *SD*	90 ± *4.6*	87 ± *18.4*	16 ± *3.5*	13 ± *2.1*	19 ± *1.7*	21 ± *5.1*	21 ± *4.0*	23 ± *5.3*	17 ± *3.2*	8 ± *3.1*
HCD[#] mean ± *SD*	103 ± *14.4*	101 ± *15.6*	20 ± *4.4*	15 ± *3.5*	28 ± *6.6*	33 ± *7.1*	22 ± *5.0*	26 ± *5.1*	11 ± *3.1*	13 ± *3.5*
[range]	*68 - 147*	*63 - 153*	*9 - 37*	*8 - 29*	*15 - 47*	*13 - 54*	*10 - 42*	*12 - 43*	*5 - 26*	*5 - 23*
Test item										
5 mean ± *SD*	91 ± *4.6*	76 ± *15.0*	9 ± *1.7*	16 ± *7.5*	17 ± *2.5*	21 ± *4.4*	22 ± *10.3*	18 ± *1.7*	13 ± *4.7*	9 ± *2.1*
15 mean ± *SD*	92 ± *8.7*	88 ± *9.2*	13 ± *6.5*	13 ± *4.4*	20 ± *3.1*	22 ± *1.7*	25 ± *3.8*	27 ± *6.2*	12 ± *3.1*	8 ± *0.6*
50 mean ± *SD*	99 ± *7.0*	72 ± *12.4*	13 ± *6.8*	8 ± *1.2*	22 ± *3.1*	20 ± *5.0*	18 ± *9.5*	22 ± *9.8*	14 ± *4.9*	13 ± *6.6*
150 mean ± *SD*	83 ± *7.0*	91 ± *10.8*	10 ± *2.3*	12 ± *0.6*	22 ± *10.5*	18 ± *4.2*	22 ± *6.7*	26 ± *6.0*	12 ± *4.0*	14 ± *1.2*
500 mean ± *SD*	62 ± *2.1*	79 ± *4.6*	11 ± *4.0*	14 ± *1.7*	17 ± *1.5*	21 ± *2.0*	21 ± *7.4*	17 ± *1.5*	12 ± *0.0*	13 ± *8.4*
1500 mean ± *SD*	70 ± *15.9*	79 ± *3.1*	11 ± *2.9*	8 ± *0.6*	16 ± *5.0*	14 ± *3.1*	20 ± *1.0*	19 ± *2.1*	8 ± *0.6*	7 ± *2.9*
5000 mean ± *SD*	65[S] ± *17.3*	94 ± *9.1*	6[S] ± *1.2*	9 ± *0.6*	12[S] ± *0.0*	11 ± *4.6*	16[S] ± *3.6*	20 ± *7.5*	6[S] ± *1.7*	9 ± *3.8*
Positive control										
[§] mean	1278	1279	2080	228	1183	298	407	163	977	545

± SD	*± 223.2*	*± 79.3*	*± 464.1*	*± 22.0*	*± 176.3*	*± 27.6*	*± 31.8*	*± 5.0*	*± 166.9*	*± 43.9*
HCD[#] mean	543	1211	644	250	611	320	207	226	813	286
± SD	*± 192.1*	*± 509.3*	*± 685.5*	*± 98.9*	*± 256.3*	*± 120.9*	*± 76.9*	*± 92.6*	*± 384.2*	*± 127.7*
[range]	*240 - 1429*	*349 - 3117*	*91 - 3750*	*103 - 1153*	*129 - 1275*	*101 - 733*	*102 - 783*	*84 - 669*	*113 - 2161*	*86 - 1238*

[§] Information on respective positive control is reported in Material and Method section I.A.2

[$] Spontaneous mutation rates, untreated plates

[#] Historical control data from 2013, HCD of untreated and vehicle controls the values were combined

[S] Sparse bacterial background lawn

III. CONCLUSION

Under the conditions of the present study, the test item is not mutagenic in the Ames test (standard plate and pre-incubation method) with and without metabolic activation.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Negative for gene mutation in bacteria (*S. typhimurium* TA100, TA98, TA1535 and TA1537 and *E. coli* WP2uvrA) in the presence and absence of metabolic activation.

The study was performed according to OECD guideline 471 (1997) and compliant with GLP. It is therefore considered valid and acceptable.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.4.1/002
Report author	[redacted]
Report year	2012
Report title	Reverse Mutation Assay using Bacteria (*Salmonella typhimurium*) with Glyphosate tech.
Report No	126159
Document No	Not reported
Guidelines followed in study	OECD 471 (1997), Commission Regulation (EC) No. 440/2008 B.13/14 (2008), US EPA OPPTS 870.5100 (1998)
Deviations from current test guideline OECD 471 (1997)	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Glyphosate technical (batch: 20110107-2, purity: 97 %) was assessed for its potential to induce gene mutations in bacteria in an Ames test conducted in *S. typhimurium* strains TA 98, TA 100, TA 102, TA 1535 and TA 1537. All strains were exposed, to the test item, solvent (DMSO), negative (water) and appropriate positive controls in the presence and absence of metabolic activation (phenobarbital and β-naphthoflavone-induced rat liver S9 fraction) for at least 48 h.

Concentrations were selected based on the results of a preliminary cytotoxicity test, in which the test item induced cytotoxicity in strain TA 100 at ≥ 2500 µg/plate in the presence of metabolic activation. Test item concentrations for the main mutagenicity study ranged from 10 to 5000 µg/plate. Two independent experiments were performed, using the plate incorporation method (standard plate test, experiment 1) and the pre-incubation method (experiment 2).

There was no precipitation observed up to the highest concentration tested, neither in the presence, nor in the absence of metabolic activation. Cytotoxicity was observed in the first experiment for strain TA98 at 5000 µg/plate in the presence of S9 mix, for strain TA 100 at ≥ 2500 µg/plate in the presence and absence of S9 mix and in strains TA 1535 and TA 102 at 5000 µg/plate in the absence of S9 mix.

In the second experiment, cytotoxicity was observed in strains TA 98 and TA 100 at 5000 µg/plate in the absence of S9 mix, in strain TA 1537 at ≥ 2500 µg/plate in the absence of S9 mix and in strain TA102 at 5000 µg/plate in the presence and absence of S9 mix.

Upon treatment with glyphosate technical, there was no statistically significant or biologically relevant increase in the number of revertant colonies in any of the five tester strains in any experiment up to the highest tested concentration, neither in the presence, nor in the absence of S9 mix.
The number of revertant colonies in the solvent, negative and positive control was within the range of the laboratories historical control data, demonstrating the functionality of the S9 mix and the validity of the test system.

Under the experimental conditions, glyphosate technical was considered negative for gene mutation in bacteria with and without metabolic activation.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material: Glyphosate Tech. (N-phosphonomethyl)glycine

Identification:	Not specified
Description:	Not specified
Lot/Batch #:	20110107-2
Purity:	97 %
Stability of test compound:	The stability of the test item under storage conditions (at room temperature, protected from light) was guaranteed until 01 Feb 2013. The stability of the test item in solvent (vehicle) was not specified.
Solvent (vehicle) used:	Dimethylsulfoxide (DMSO)

2. Control materials:

Negative control:	Distilled water
Solvent (vehicle) control:	Dimethylsulfoxide (DMSO)

Solvent (vehicle)/final concentration: 0.1 mL per plate.

Positive controls: Please refer to table below.

Strain	Metabolic activation	Mutagen	Solvent	Conc. [µg/plate]
S. typhimurium strains				
TA 100	-S9	Sodium azide (NaN_3)	Water	10.0
	+S9	2-Aminoanthracene (2-AA)[1]	DMSO	2.5
TA 1535	-S9	Sodium azide (NaN_3)	Water	10.0
	+S9	2-Aminoanthracene (2-AA)[1]	DMSO	2.5
TA 98	-S9	4-nitro-o-phenylene-diamine (4-NOPD)	DMSO	10
	+S9	2-Aminoanthracene (2-AA)[1]	DMSO	2.5
TA 1537	-S9	4-nitro-o-phenylene-diamine (4-NOPD)	DMSO	40.0
	+S9	2-Aminoanthracene (2-AA)[1]	DMSO	2.5
TA 102	-S9	Methylmethanesulfonate (MMS)	Water	1.0 µL/plate
	+S9	2-Aminoanthracene (2-AA)[1]	DMSO	10.0

[1] The functionality of the S9 mix batch used was checked with benzo(a)pyrene and showed the expected results

3. Metabolic activation:

S9 mix was prepared by [redacted] ([redacted], Experiment 1) and purchased from [redacted] ([redacted], Experiment 2). S9 mix from [redacted] was obtained from the livers of male Wistar rats, that received phenobarbital (80 mg/kg bw) and β-naphthoflavone (100 mg/kg bw) by oral treatment for three consecutive days. S9 mix from [redacted] was produced from male Sprague-Dawley rats, which were induced with phenobarbital / β-naphthoflavone.

Prior to each experiment, co-factor was added to the S9 mix containing the following components:

S9 mix component	Concentration	Unit
Sodium phosphate buffer (pH 7.4)	100	mM
KCl	33	mM
NADPH-generating system		
Glucose 6-phosphate	5	mM
NADP	4	mM
$MgCl_2$	8	mM
S9	5	% (v/v)

4. Test organisms:

Tester strains				Bacteria batch checked for	
S. typhimurium		***E.coli***			
TA 98	✓	WP2 uvrA		deep rough character (rfa)	✓
TA 100	✓	WP2 uvrA (pKM101)		ampicillin resistance (R factor plasmid)	✓
TA 1535	✓			UV-light sensitivity (absence of uvrB and uvrA genes in *S. typhimurium* and *E. coli* strains, respectively)	✓
TA 1537	✓				
TA 102	✓				
TA 1538				Histidine and tryptophan auxotrophy (automatically via the spontaneous rate)	✓

5. Test concentrations:

(a) Preliminary cytotoxicity assay:

Plate incorporation test ± S9 mix:	
Concentrations:	*3.16, 10, 31.6, 100, 316, 1000, 2500 and 5000 µg/plate*
Tester strains:	TA 98 and TA 100
Replicates:	*Triplicates*

(b) Mutation assays:

Plate incorporation test ± S9 mix:	
Concentrations:	*10, 31.6, 100, 316, 1000, 2500 and 5000 µg/plate*
Tester strains:	TA 1535, TA 1537, TA 98, TA 100 and TA 102
Replicates:	*Triplicates*
Pre-incubation test ± S9 mix:	
Concentrations:	*10, 31.6, 100, 316, 1000, 2500 and 5000 µg/plate*
Tester strains:	TA 1535, TA 1537, TA 98, TA 100 and TA 102
Replicates:	*Triplicates*

B: STUDY DESIGN AND METHODS

1. **Dates of experimental work:** 13 Nov - 10 Dec 2012
   Finalisation date: 17 Dec 2012

2. **Standard plate test (plate-incorporation test, SPT):**
An aliquot of 0.1 mL test solution, negative control (water), solvent control (DMSO) or positive control, an aliquot of 0.1 mL fresh bacterial culture and 0.5 mL S9 mix (in tests with metabolic activation) or 0.5 mL of S9 substitution buffer (in tests without metabolic activation) were added to 2 mL of molten overlay agar (supplemented with 10.5 mg/L L-histidine + 12.2 mg/L biotin). All components were mixed in a test tube and poured onto minimal agar plates. Each concentration and the controls were tested in triplicates. After incubation at 37 °C upside down for at least 48 h in the dark, the background bacterial lawn was examined and the bacterial colonies (his^+ revertants) were counted.

3. **Pre-incubation test (PIT):**
0.1 mL of test solution, negative control (water), solvent control (DMSO) or positive control, 0.1 mL pre-cultured bacterial suspension and 0.5 mL of S9 mix (in tests with metabolic activation) or 0.5 mL of S9 mix substitution buffer (in tests without metabolic activation) were pre-incubated at 37 °C for 60 minutes, followed by addition of 2 mL molten overlay agar. Each concentration and the controls was tested in triplicates. The mixture was poured onto the surface of a minimal agar plate and after solidification, the plates were incubated upside down for at least 48 h at 37°C in the dark. Subsequently, the number of bacterial colonies (his^+ revertants) was counted.

4. Cytotoxicity

Toxicity was detected by a

- reduction in the number of spontaneous revertants (approx. 50 % reduced in relation to the solvent control).
- clearing or diminution of the background lawn (= reduced background growth)

and recorded for all test groups both with and without S9 mix in all experiments.

5. Statistics

Results were judged without statistical analysis.

6. Acceptance criteria

The test was valid if the following criteria were met:

- The bacteria demonstrated their typical responses to ampicillin (TA 98, TA 100 and TA 102).
- The control plates with and without S9 mix were within the ranges of the laboratory's historical data.
- Corresponding background growth on negative control, solvent control and test plates was observed.
- The positive controls showed a distinct enhancement of revertant rates over the control plate.

7. Evaluation criteria

A test item was considered as mutagenic if the following criteria were met:

- A clear and dose-related increase in the number of revertants occurred and/or
- A biologically relevant positive response for at least one of the dose groups occurred in at least one tester strain with or without metabolic activation. A biologically relevant increase was obtained when the number of revertants was at least two-fold when compared to solvent controls for testers strains TA 98, TA 100 and TA 102 and at least three-fold when compared to solvent controls for tester strains TA 1535 and TA 1537.

A test item was considered non-mutagenic if it produced neither a dose-related increase in the number of revertants nor a reproducible biologically relevant positive response at any of the dose levels.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Analytical determinations were not performed in the study as not required by the test guideline.

B. CYTOTOXICITY

In the preliminary toxicity test, cytotoxicity was observed in strain TA 100 at ≥ 2500 µg/plate in the presence of metabolic activation.

In the main mutagenicity assay, cytotoxicity was observed in the first experiment (standard plate test = plate-incorporation method) for strain TA98 at 5000 µg/plate in the presence of S9 mix, for strain TA 100 at ≥ 2500 µg/plate in the presence and absence of S9 mix and in strains TA 1535 and TA 102 at 5000 µg/plate in the absence of S9 mix.

In the second experiment (pre-incubation test), cytotoxicity was observed in strains TA 98 and TA 100 at 5000 µg/plate in the absence of S9 mix, in strain TA 1537 at ≥ 2500 µg/plate in the absence of S9 mix and in strain TA 102 at 5000 µg/plate in the presence and absence of S9 mix.

The reduction in the number of revertant colonies in strain TA 1537 at 100 µg/plate in the absence of S9 mix was regarded as not biologically relevant, due to a lack of a dose-response relationship.

C. SOLUBILITY

There was no precipitation of the test substance observed up to the highest dose tested in any of the experiments, neither in the presence nor absence of S9 mix.

D. MUTATION ASSAY

There was no statistically significant or biologically relevant increase in the number of his$^+$ revertant colonies in any of the five tester strains in any of the two experiments up to the highest tested concentration, neither in the presence, nor in the absence of S9 mix.
The number of revertant colonies in the solvent, negative and positive control was within the range of the laboratories historical control data. The positive controls induced a marked increase in the number of revertant colonies, demonstrating the functionality of the S9 mix and the validity of the test system.

Table 5.4.1-4: Reverse Mutation Assay using Bacteria (*Salmonella typhimurium*) with Glyphosate Tech ([redacted], 2012), first experiment

Experiment 1: Standard plate test (SPT)										
Strain	**TA 98**		**TA 100**		**TA 1535**		**TA 1537**		**TA 102**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Vehicle control										
Water mean	22	26	88	115	23	14	6	9	232	333
± *SD*	*± 3.0*	*± 8.1*	*± 6.4*	*± 13.4*	*± 3.1*	*± 2.6*	*± 2.9*	*± 3.2*	*± 3.5*	*± 37.3*
DMSO mean	15	27	81	89	18	11	6	8	202	287
± *SD*	*± 4.7*	*± 2.6*	*± 6.6*	*± 11.8*	*± 4.0*	*± 5.6*	*± 2.6*	*± 2.1*	*± 10.7*	*± 18.0*
HCD# mean	23.8	30.6	113.3	114.8	10.0	8.7	8.8	9.1	252.1	288.0
± *SD*	*± 5.8*	*± 5.1*	*± 16.2*	*± 16.2*	*± 2.9*	*± 2.3*	*± 2.9*	*± 3.0*	*± 46.6*	*± 58.0*
[range]	*16 - 46*	*18 - 53*	*77 - 174*	*79 - 162*	*5 - 27*	*5 - 26*	*5 - 28*	*5 - 32*	*164 - 399*	*169 - 467*
Test item [µg/plate]										
10 mean	11	23	85	83	19	18	6	7	225	338
± *SD*	*± 1.0*	*± 3.1*	*± 13.0*	*± 4.6*	*± 3.8*	*± 3.1*	*± 3.5*	*± 3.1*	*± 20.4*	*± 16.0*
31.6 mean	20	25	77	90	21	11	7	6	219	314
± *SD*	*± 4.0*	*± 1.0*	*± 5.6*	*± 2.0*	*± 2.9*	*± 2.0*	*± 2.1*	*± 1.2*	*± 37.9*	*± 13.8*
100 mean	14	21	86	93	18	9	4	4	225	288
± *SD*	*± 1.5*	*± 0.6*	*± 8.2*	*± 9.0*	*± 2.1*	*± 1.5*	*± 2.9*	*± 1.2*	*± 14.2*	*± 8.7*
316 mean	15	24	75	103	15	11	7	7	197	251
± *SD*	*± 1.5*	*± 4.4*	*± 7.6*	*± 15.3*	*± 7.9*	*± 3.5*	*± 0.6*	*± 2.1*	*± 3.6*	*± 13.8*
1000 mean	14	21	73	88	16	11	6	6	187	254
± *SD*	*± 2.1*	*± 3.5*	*± 7.4*	*± 7.9*	*± 3.6*	*± 3.2*	*± 2.3*	*± 2.0*	*± 8.5*	*± 28.4*
2500 mean	10	20	49^B	85^B	18	14	6	7	203	331
± *SD*	*± 1.0*	*± 6.4*	*± 15.0*	*± 19.1*	*± 2.1*	*± 4.6*	*± 2.6*	*± 3.0*	*± 3.2*	*± 25.2*
5000 mean	12	15	19^B	53^B	9^B	12	4	6	77	255
± *SD*	*± 5.5*	*± 7.1*	*± 8.5*	*± 15.3*	*± 2.3*	*± 4.4*	*± 3.2*	*± 1.0*	*± 42.5*	*± 9.0*
Positive control										
§ mean	299	1906	390	1533	1423	203	52	130	1338	769
± *SD*	*± 25.5*	*± 219.0*	*± 73.4*	*± 369.8*	*± 27.1*	*± 13.7*	*± 8.5*	*± 27.6*	*± 263.3*	*± 66.4*
HCD# mean	491.8	2283.1	933.9	1759.0	1031.9	115.7	125.2	237.6	1516.9	1043.6
± *SD*	*± 154.9*	*± 651.4*	*± 263.7*	*± 508.4*	*± 287.1*	*± 59.4*	*± 30.6*	*± 91.7*	*± 335.0*	*± 305.9*

Table 5.4.1-4: Reverse Mutation Assay using Bacteria (*Salmonella typhimurium*) with Glyphosate Tech ([redacted], 2012), first experiment

Experiment 1: Standard plate test (SPT)										
Strain	**TA 98**		**TA 100**		**TA 1535**		**TA 1537**		**TA 102**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
[range]	*205 - 2613*	*313 - 3587*	*279 - 1876*	*462 - 3204*	*67 - 1850*	*27 - 732*	*34 - 275*	*32 - 474*	*391 - 2902*	*371 - 2422*

§ Information on respective positive control is reported in Material and Method section I.A.2

Historical control data generated from 2009 - 2011

B Background lawn reduced

Table 5.4.1-5: Reverse Mutation Assay using Bacteria (*Salmonella typhimurium*) with Glyphosate Tech ([redacted], 2012), second experiment

Experiment 2: Pre-incubation test (PIT)										
Strain	**TA 98**		**TA 100**		**TA 1535**		**TA 1537**		**TA 102**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Vehicle control										
Water mean	21	29	110	103	14	12	6	7	215	215
± *SD*	*± 2.0*	*± 9.2*	*± 3.5*	*± 20.7*	*± 3.1*	*± 6.2*	*± 3.2*	*± 1.5*	*± 15.8*	*± 4.4*
DMSO mean	20	23	104	94	11	12	8	5	171	184
± *SD*	*± 7.5*	*± 3.6*	*± 6.5*	*± 7.4*	*± 4.6*	*± 4.5*	*± 0.7*	*± 2.1*	*± 17.7*	*± 11.5*
HCD# mean	23.8	30.6	113.3	114.8	10.0	8.7	8.8	9.1	252.1	288.0
± *SD*	*± 5.8*	*± 5.1*	*± 16.2*	*± 16.2*	*± 2.9*	*± 2.3*	*± 2.9*	*± 3.0*	*± 46.6*	*± 58.0*
[range]	*16 - 46*	*18 - 53*	*77 - 174*	*79 - 162*	*5 - 27*	*5 - 26*	*5 - 28*	*5 - 32*	*164 - 399*	*169 - 467*
Test item										
10 mean	23	30	119	98	17	13	10	4	163	233
± *SD*	*± 8.0*	*± 7.5*	*± 18.6*	*± 16.6*	*± 2.5*	*± 2.5*	*± 3.2*	*± 2.0*	*± 10.6*	*± 7.0*
31.6 mean	24	29	114	115	17	12	6	5	167	233
± *SD*	*± 1.5*	*± 7.2*	*± 9.1*	*± 8.4*	*± 3.6*	*± 3.0*	*± 1.0*	*± 1.7*	*± 15.9*	*± 8.7*
100 mean	18	26	104	115	19	8	3	5	147	210
± *SD*	*± 5.5*	*± 3.2*	*± 4.7*	*± 6.7*	*± 0.0*	*± 2.1*	*± 2.1*	*± 2.0*	*± 12.4*	*± 14.0*
316 mean	20	22	113	102	15	11	4	6	155	210
± *SD*	*± 3.5*	*± 5.3*	*± 4.5*	*± 10.4*	*± 1.5*	*± 4.6*	*± 2.3*	*± 1.0*	*± 7.8*	*± 10.0*
1000 mean	25	32	131	108	13	13	6	3	170	230
± *SD*	*± 5.6*	*± 9.5*	*± 33.3*	*± 14.2*	*± 2.9*	*± 0.6*	*± 5.3*	*± 2.1*	*± 10.6*	*± 21.8*
2500 mean	22	26	114	89	15	11	4	11	178	229
± *SD*	*± 5.7*	*± 10.8*	*± 11.0*	*± 8.5*	*± 5.3*	*± 4.7*	*± 2.6*	*± 4.6*	*± 3.5*	*± 24.6*
5000 mean	7B	26	21	100	8	12	2	6	37	61
± *SD*	*± 3.2*	*± 3.8*	*± 16.6*	*± 2.1*	*± 2.1*	*± 2.5*	*± 0.6*	*± 4.0*	*± 16.5*	*± 25.3*

Table 5.4.1-5: Reverse Mutation Assay using Bacteria (*Salmonella typhimurium*) with Glyphosate Tech ([redacted], 2012), second experiment

Experiment 2: Pre-incubation test (PIT)										
Strain	**TA 98**		**TA 100**		**TA 1535**		**TA 1537**		**TA 102**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Positive control										
[§] mean	552	1958	1102	2002	1220	197	117	151	1972	530
± *SD*	± *119.9*	± *674.0*	± *74.8*	± *360.7*	± *114.0*	± *28.3*	± *25.0*	± *21.8*	± *201.8*	± *63.0*
HCD[#] mean	491.8	2283.1	933.9	1759.0	1031.9	115.7	125.2	237.6	1516.9	1043.6
± *SD*	± *154.9*	± *651.4*	± *263.7*	± *508.4*	± *287.1*	± *59.4*	± *30.6*	± *91.7*	± *335.0*	± *305.9*
[range]	*205 - 2613*	*313 - 3587*	*279 - 1876*	*462 - 3204*	*67 - 1850*	*27 - 732*	*34 - 275*	*32 - 474*	*391 - 2902*	*371 - 2422*

[§] Information on respective positive control is reported in Material and Method section 1.A.2

[#] Historical control data generated from 2009 - 2011

[B] Background lawn reduced

III. CONCLUSION

In conclusion, based on the experimental findings, glyphosate tech. did not induce gene mutations by base pair exchanges or frameshifts in the genome of the five tested *S. typhimurium* strains. Therefore, the test item was considered non-mutagenic in the bacterial reverse mutation test (Ames test).

3. Assessment and conclusion

Assessment and conclusion by applicant:

Negative for gene mutation in bacteria (*S. typhimurium* TA 98, TA 100, TA 102, TA 1535 and TA 1537) with and without metabolic activation.

The study was performed according to OECD guideline 471 (1997) and under GLP conditions. There were no deviations when compared to the guideline. The study is therefore considered valid and acceptable.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.4.1/003
Report author	[redacted]
Report year	2010
Report title	Mutagenicity study of Glyphosate TC in the *Salmonella typhimurium* Reverse Mutation Assay (*in vitro*)
Report No	24880
Document No	Not reported

Guidelines followed in study	OECD 471 (1997), Commission Regulation (EC) No. 440/2008 B.13/14 (2008), US EPA OPPTS 870.5100 (1998)
Deviations from current test guideline OECD 471 (1997)	Historical control data for the positive controls were not included in the study report.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid[1]
Category study in AIR 5 dossier (L docs)	Category 2a

[1]: Following the feedback from the Assessment Group Glyphosate in March 2020, the Notifier is aware of concerns regarding work conducted at this specific contract laboratory (LPT). For the sake of completeness, this study is included in the dossier submission since it was part of the data package of previous EU evaluation processes.

2. Full summary

Glyphosate technical (batch: 2009051501, purity 95.23 %) was investigated for its potential to induce gene mutation in bacteria in an Ames test. In the presence and absence of metabolic activation (Aroclor 1254-induced rat liver S9 fraction), *S. typhimurium* strains TA 98, TA 100, TA 102, TA 1535 and TA 1537 were exposed to the test item, vehicle (aqua ad iniectabilia) and positive controls for 48 – 72 hours at 37 °C.

Based on the results of a preliminary cytotoxicity test conducted in strain TA 100 in the absence of metabolic activation, test item concentrations in the main mutagenicity test ranged from 31.6 to 3160 µg/plate. Two independent experiments were performed, using the standard plate test (plate-incorporation method) and the pre-incubation test. After 48 to 72 hours of incubation, the number of revertant colonies was counted and the bacterial background lawn was examined.

Precipitation of the test item was observed in the preliminary toxicity study at 5000 µg/plate, but at no concentration used in the main mutagenicity test. For all tester strains in the presence and absence of metabolic activation, cytotoxicity was observed at 3160 µg/plate.

There was no increase in the number of revertant colonies when compared to control counts observed in any of the five tester strains following treatment with glyphosate technical tested up to 3160 µg/plate, neither in the presence nor in the absence of metabolic activation.

In both experiments, the number of revertants induced by the vehicle control was within the range of the historical control data for each strain. A distinct increase in the number of revertant colonies was observed for the positive controls, demonstrating the functionality of the S9 mix and the sensitivity of the test system.

Based on the result of the present study and under the experimental conditions of the test, glyphosate technical is negative for mutagenicity in bacteria in the Ames standard plate and pre-incubation test with and without metabolic activation.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material: Glyphosate TC

Identification: 37/206/09

Description:	White powder
Lot/Batch #:	2009051501
Purity:	95.23 %
Stability of test compound:	The stability of the test item under storage conditions (at room temperature) was guaranteed until the mentioned expiry date 15 May 2011. The stability of the test item in vehicle was not specified in the study report.
Solvent (vehicle) used:	Aqua ad iniectabilia

2. Control materials:

Negative control:	Sterility controls were not included in the present study
Solvent (vehicle) control:	Aqua ad iniectabilia
Solvent (vehicle) /final concentration:	1 mL per plate
Positive controls:	Please refer to the table below.

Strain	Metabolic activation	Mutagen	Solvent	Conc. [µg/plate]
S. typhimurium strains				
TA 100	-S9	Sodium azide	Water	10.0
	+S9	Cyclophosphamide	Water	1500.0
TA 1535	-S9	Sodium azide	Water	10.0
	+S9	Cyclophosphamide	Water	1500.0
TA 98	-S9	2-Nitrofluorene	DMSO	10
	+S9	2-Aminoanthracene	DMSO	2.0
TA 1537	-S9	9-Aminoacridine	Ethanol	100.0
	+S9	2-Aminoanthracene	DMSO	2.0
TA 102	-S9	Methylmethane sulfonate	DMSO	1300.0
	+S9	2-Aminoanthracene	DMSO	2.0

3. Metabolic activation:

S9 mix was obtained from the livers of 20 – 30 rats treated with Aroclor 1254. The pooled fraction exhibited a protein content of 26.6 mg/mL and a cytochrome P450 level of 0.21 nmol/protein. Aliquots of the S9 mix were thawed prior to each experiment and co-factor was immediately added to prepare S9 mix containing the following components:

S9 mix component	Concentration	Unit
Sodium phosphate buffer (pH 7.4)	100.0	mM
KCl	33.0	mM
NADPH-generating system		
Glucose 6-phosphate	5.4	mM
NADP	4.1	mM
$MgCl_2$	8.0	mM
S9	5.0	% (v/v)

4. Test organisms:

Tester strains				Bacteria batch checked for	
S. typhimurium		*E. coli*			
TA 98	✓	WP2 uvrA		deep rough character (rfa)	✓
TA 100	✓	WP2 uvrA (pKM101)		ampicillin resistance (R factor plasmid)	✓
TA 1535	✓			UV-light sensitivity (absence of uvrB genes)	✓
TA 1537	✓				
TA 102	✓				
TA 1538				Histidine auxotrophy (automatically via the spontaneous rate)	✓

5. Test concentrations:

(a) Preliminary cytotoxicity assay

Plate incorporation test - S9 mix:	
Concentrations:	*0.316, 1.0, 3.16, 10.0, 31.6, 100, 316, 1000, 3160 and 5000 µg/plate*
Tester strain:	TA 100
Replicates:	*Duplicates*

(b) Main mutation assay

Plate incorporation test ± S9 mix:	
Concentrations:	*31.6, 100, 316, 1000 and 3160 µg/plate*
Tester strains:	TA 98, TA 100, TA 102, TA 1535 and TA 1537
Replicates:	*Triplicates*
Pre-incubation test ± S9 mix:	
Concentrations:	*31.6, 100, 316, 1000 and 3160 µg/plate*
Tester strains:	TA 98, TA 100, TA 102, TA 1535 and TA 1537
Replicates:	*Triplicates*

B. STUDY DESIGN AND METHODS

1. **Dates of experimental work:** 15 Oct – 23 Nov 2009
   Finalisation date: 25 Jan 2010

2. Standard plate test (plate-incorporation test, SPT):
An aliquot of 1 mL test solution or vehicle or 0.1 mL positive control, an aliquot of 0.1 mL fresh bacterial culture and 0.5 mL S9 mix (in tests with metabolic activation) or 0.5 mL of S9 phosphate buffer (in tests without metabolic activation) were added to 2.0 mL of molten top agar (supplemented with 0.5 mM L-histidine + 0.5 mM biotin). The test components were mixed by vortexing at low speed, poured onto coded minimal glucose agar plates and the plates were quickly tilted and rotated for homogenous distribution. Each concentration and the controls were tested in triplicates. After solidification, the plates were incubated upside down for 48 to 72 hours at 37 °C in the dark. After incubation, the number of revertant colonies was counted and the presence of bacterial background lawn was confirmed.

3. Pre-incubation test (PIT):
1 mL of test solution or vehicle or 0.1 mL of positive control, 0.1 mL pre-cultured bacterial suspension and 0.5 mL of S9 mix (in tests with metabolic activation) or 0.5 mL of S9 mix phosphate buffer (in tests without metabolic activation) were pre-incubated for 20 minutes at 37 °C using a shaker.
Afterwards, 2.0 mL of molten top agar was added to each test tube and the mixture was poured on coded minimal glucose agar plates. Each concentration and the controls were tested in triplicates. After solidification, the plates were incubated upside down for 48 to 72 hours at 37 °C in the dark. After incubation, the number of revertant colonies was counted and the presence of bacterial background lawn was examined.

4. Cytotoxicity
Toxicity was detected by

- a reduction in the number of spontaneous revertants (below 50 % when compared to solvent controls)
- a clearing or diminution of the background lawn (= reduced his^- background growth)
- the degree of survival of the treated cultures

and recorded for all test groups both with and without S9 mix in all experiments.

5. Statistics
Statistical significance for an increased number of revertants compared to the solvent control was determined using the Mann-Whitney U-test. A concentration-related effect was identified using the Spearman's rank correlation coefficient.

6. Acceptance criteria
Acceptance criteria were not defined in the study report.

7. Evaluation criteria
A test item was considered to show a positive response if the following criteria were met:

- The number of revertant colonies was statistically significantly increased compared to the solvent control to at least 2-fold of the solvent control for TA 98, TA 100 and TA 102 and 3-fold of the solvent control for TA 1535 and TA 1537 in both experiments.
- There was a statistically significant concentration-related effect.
- Positive results were reproducible and the histidine independence of the revertants was confirmed by streaking random samples on histidine-free agar plates

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS
Analytical determinations were not performed in the study, as not required by the test guideline.

B. CYTOTOXICITY
In the preliminary toxicity test, cytotoxicity was observed in tester strain TA100 at concentrations of 3160

µg/plate and above in the absence of metabolic activation. Hence, 3160 µg/plate was chosen as top concentration for the main mutagenicity study.

In the main mutation assay, cytotoxicity, indicated by scarce background lawn and a reduction in the number of revertants, was noted at the top concentration of 3160 µg/plate for all tester strains in both experiments (plate-incorporation and pre-incubation test), both in the presence and absence of metabolic activation.

C. SOLUBILITY

Precipitation of the tests item was observed in the preliminary toxicity test in tester strain TA100 at the top concentration of 5000 µg/plate without metabolic activation. There was no precipitation in the main mutagenicity study.

D. MUTATION ASSAY

There was no increase in the number of revertant colonies when compared to control counts observed in any of the five tester strains following treatment with glyphosate technical tested up to 3160 µg/plate, neither in the presence nor in the absence of metabolic activation.

In both experiments, the number of revertants induced by the vehicle control was within the range of the historical control data for each strain. A distinct increase in the number of revertant colonies was observed for the positive controls, demonstrating the functionality of the S9 mix and the sensitivity of the test system.

Table 5.4.1-6: Mutagenicity study of Glyphosate TC in the *Salmonella typhimurium* Reverse Mutation Assay (*in vitro*) ([redacted], 2010), first experiment

Experiment 1: Standard plate test (SPT)										
Strain	**TA 98**		**TA 100**		**TA 102**		**TA 1535**		**TA 1537**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Vehicle control										
Aqua ad iniectabilia mean	30.7	31.0	160.0	141.7	270.3	283.3	21.7	19.7	5.0	7.0
± *SD*	*± 3.2*	*± 8.7*	*± 19.3*	*± 15.9*	*± 3.2*	*± 20.0*	*± 2.5*	*± 2.1*	*± 1.0*	*± 1.0*
HCD[#] range	*20 - 60*		*100 - 200*		*240 - 320*		*10 - 35*		*3 - 20*	
Test item [µg/plate]										
31.6 mean	26.3	29.3	151.3	166.0	261.3	267.7	15.7	15.7	4.7	7.0
± *SD*	*± 5.5*	*± 4.2*	*± 25.7*	*± 17.5*	*± 8.1*	*± 12.5*	*± 1.5*	*± 2.1*	*± 1.5*	*± 1.0*
100 mean	27.7	29.3	148.0	148.3	275.7	274.3	17.3	15.7	4.3	5.3
± *SD*	*± 6.4*	*± 5.8*	*± 7.5*	*± 7.1*	*± 9.3*	*± 14.5*	*± 2.1*	*± 3.5*	*± 1.2*	*± 0.6*
316 mean	25.0	24.3	142.7	148.7	271.3	281.0	17.0	18.3	4.0	4.0
± *SD*	*± 3.6*	*± 5.1*	*± 14.2*	*± 14.4*	*± 8.0*	*± 7.0*	*± 1.7*	*± 3.5*	*± 1.0*	*± 1.0*
1000 mean	31.0	21.3	136.3	136.0	257.0	271.0	19.0	16.0	2.7	5.0
± *SD*	*± 4.6*	*± 3.2*	*± 21.2*	*± 18.5*	*± 17.3*	*± 11.8*	*± 3.6*	*± 1.0*	*± 0.6*	*± 1.7*
3160 mean	13.3[$]	8.3[$]	65.3[$]	106.0[$]	226.3[$]	265.7[$]	9.7[$]	13.0[$]	2.3[$]	2.0[$]
± *SD*	*± 1.5*	*± 2.5*	*± 11.8*	*± 4.0*	*± 6.0*	*± 1.5*	*± 2.1*	*± 1.7*	*± 1.5*	*± 1.0*
Positive control										
[§] mean	352.0	333.3	894.7	909.3	1038.3	1034.0	247.7	243.7	210.3	212.0

Table 5.4.1-6: Mutagenicity study of Glyphosate TC in the *Salmonella typhimurium* Reverse Mutation Assay (*in vitro*) ([redacted], 2010), first experiment

Experiment 1: Standard plate test (SPT)										
Strain	**TA 98**		**TA 100**		**TA 102**		**TA 1535**		**TA 1537**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
± *SD*	± *52.0*	± *8.3*	± *87.9*	± *69.3*	± *7.8*	± *11.1*	± *64.3*	± *23.2*	± *9.5*	± *5.6*

[§] Information on respective positive control is reported in Material and Method section I.A.2

[#] Historical control data generated in the laboratory of the testing facility

[$] Cytotoxicity indicated by scarce background lawn

Table 5.4.1-7: Mutagenicity study of Glyphosate TC in the *Salmonella typhimurium* Reverse Mutation Assay (*in vitro*) ([redacted], 2010), second experiment

Experiment 2: Pre-incubation test (PIT)										
Strain	**TA 98**		**TA 100**		**TA 102**		**TA 1535**		**TA 1537**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Vehicle control										
Aqua ad iniectabilia mean	35.0	36.0	171.0	136.7	282.0	294.7	24.0	19.3	8.0	9.0
± *SD*	± *4.0*	± *3.6*	± *24.0*	± *8.0*	± *24.2*	± *26.1*	± *3.6*	± *3.1*	± *1.7*	± *1.0*
HCD[#] range	*20 - 60*		*100 - 200*		*240 - 320*		*10 - 35*		*3 - 20*	
Test item										
31.6 mean	34.0	35.0	139.7	132.0	275.0	283.7	16.7	20.7	5.3	8.3
± *SD*	± *3.5*	± *3.0*	*4.6*	± *14.0*	± *8.9*	± *9.0*	± *1.5*	± *1.5*	± *1.5*	± *1.5*
100 mean	30.7	35.7	151.7	129.3	276.0	293.7	20.3	17.0	5.7	7.3
± *SD*	± *7.6*	± *2.9*	± *24.6*	± *21.4*	± *10.1*	± *8.4*	± *2.1*	± *2.6*	± *1.5*	± *1.5*
316 mean	30.0	32.7	169.7	120.3	275.0	285.0	19.7	19.0	6.0	7.7
± *SD*	± *5.3*	± *4.0*	± *17.2*	± *8.3*	± *12.5*	± *7.0*	± *2.1*	± *2.6*	± *1.0*	± *0.6*
1000 mean	30.7	29.3	148.3	109.3	248.3	250.3	20.3	18.0	5.0	6.3
± *SD*	± *4.5*	± *3.5*	± *11.2*	± *2.3*	± *3.1*	± *2.1*	± *3.2*	± *2.6*	± *1.7*	± *1.5*
3160 mean	5.3[$]	6.3[$]	50.7[$]	38.0[$]	75.3[$]	74.7[$]	8.3[$]	8.0[$]	1.3[$]	1.7[$]
± *SD*	± *2.5*	± *1.5*	± *5.1*	± *3.0*	± *14.5*	± *10.0*	± *1.5*	± *2.0*	± *0.6*	± *0.6*
Positive control										
[§] mean	397.7	427.0	934.0	943.3	1040.7	1078.3	303.3	362.3	243.7	235.3
± *SD*	± *24.5*	± *28.5*	± *72.0*	± *51.2*	± *28.9*	± *28.6*	± *29.7*	± *40.1*	± *21.2*	± *12.0*

[§] Information on respective positive control is reported in Material and Method section I.A.2

[#] Historical control data generated in the laboratory of the testing facility

[$] Cytotoxicity indicated by scarce background lawn

III. CONCLUSION

According to the results and under the conditions of the present study, glyphosate technical is not mutagenic in the Ames standard plate and pre-incubation test with and without metabolic activation.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Negative for gene mutation in bacteria (*S. typhimurium* TA 98, TA 100, TA 102, TA 1535 and TA 1537) with and without metabolic activation.
The study was performed according to OECD guideline 471 (1997) and under GLP conditions. Deviations from the guideline were considered to be of minor degree that do not hinder data evaluation. The study is therefore considered valid and acceptable.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.4.1/004
Report author	[redacted]
Report year	2010
Report title	*Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay with Solution of Glyphosate TC spiked with Glyphosine
Report No	1332300
Document No	Not reported
Guidelines followed in study	OECD 471 (1997), Commission Regulation (EC) No. 440/2008 B13/14 (2008)
Deviations from current test guideline OECD 471 (1997)	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Glyphosate technical (batch: 2009051501, purity 97.16 %) spiked with glyphosine was investigated with regard to its potential to induce gene mutation in bacteria in an Ames test. *S. typhimurium* strains TA 98, TA 100, TA 1535 and TA 1537, and *E. coli* strain WP2 uvrA were exposed to the test item in the presence and absence of metabolic activation (phenobarbital and β-naphthoflavone-induced rat liver S9 fraction) for at least 48 hours at 37 °C. Vehicle (deionized water), untreated and positive controls were included in each experiment.

A pre-experiment was conducted to identify cytotoxic concentrations of the test item. In a standard plate test (plate incorporation method), test item concentrations in the range of 3 to 5000 µg/plate did not show

any cytotoxic effects in the presence and absence of metabolic activation. The pre-experiment was designated experiment I of the main mutation assay and analysed for the number of revertant colonies. A second main mutation assay was performed using the preincubation method and concentrations in the same concentration range. In both experiments, all conditions were tested in triplicates. After at least 48 hours of incubation, the mean number of revertant colonies was counted for each plate.

Precipitation of the test substance was observed for all tester strains in the second experiment only (preincubation method) at ≥ 2500 µg/mL in the presence of metabolic activation. In none of the experiments, cytotoxicity was observed.

There was no substantial increase in the number of his^+ or trp^+ revertant colonies observed in any of the five tester strains at any dose level, neither in the presence nor in the absence of metabolic activation.
The number of revertants induced by the vehicle control was within the range of the historical control data for each strain, thus demonstrating an acceptable experimental performance. Appropriate positive control compounds showed a distinct increase in the number of revertant colonies, confirming the activity of the S9 mix and the validity of the test system.

Based on the results of the present study and under the experimental conditions of the test, glyphosate technical spiked with glyphosine did not cause any genotoxic effect in the Ames standard plate and pre-incubation test with and without metabolic activation.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material: Glyphosate technical (5000 mg/L), containing glyphosine (32 mg/L)

Identification:	S1111311
Description:	An aqueous solution of glyphosate technical grade active ingredient (purity 97.16 % w/w), containing 0.63 % (w/w) glyphosine in the technical grade active ingredient.
Lot/Batch #:	2009051501
Purity:	97.16 %
Stability of test compound:	The stability of the test item under storage conditions (at room temperature, light protected) was guaranteed for two weeks from the date of preparation. The stability of the test item in vehicle was not specified in the study report.
Solvent (vehicle) used:	Deionised water

2. Control materials:

Negative control:	Controls which remained untreated were included in each experiment.
Solvent (vehicle) control:	Deionised water
Solvent (vehicle)/final concentration:	0.1 mL per plate
Positive controls:	Please refer to the table below.

Strain	Metabolic activation	Mutagen	Solvent	Conc. [µg/plate]
S. typhimurium strains				
TA 100	-S9	Sodium azide (NaN_3)	Water	10.0
	+S9	2-Aminoanthracene (2-AA)*	DMSO	2.5
TA 1535	-S9	Sodium azide (NaN_3)	Water	10.0
	+S9	2-Aminoanthracene (2-AA)*	DMSO	2.5
TA 98	-S9	4-nitro-o-phenylene-diamine (4-NOPD)	DMSO	10
	+S9	2-Aminoanthracene (2-AA)*	DMSO	2.5
TA 1537	-S9	4-nitro-o-phenylene-diamine (4-NOPD)	DMSO	50.0
	+S9	2-Aminoanthracene (2-AA)*	DMSO	2.5
E. coli strain				
WP2 uvrA	-S9	Methylmethane sulfonate (MMS)	Water	3.0
	+S9	2-Aminoanthracene (2-AA)*	DMSO	10.0

* The functionality of the S9 mix batch used was additionally checked with benzo(a)pyrene and showed the expected results.

3. Metabolic activation:

S9 mix was obtained from the livers of 8 – 12 weeks old male Wistar rats, weighing approx. 220 - 320 g. The animals received intraperitoneal injections of phenobarbital (80 mg/kg bw) and oral administration of β-naphthoflavone (80 mg/kg bw) each on three consecutive days. The livers were prepared 24 h after the last treatment. Aliquots of the S9 mix were thawed prior to each experiment and co-factor was immediately added to prepare S9 mix containing the following components:

S9 mix component	Concentration	Unit
Sodium phosphate buffer (pH 7.4)	100	mM
KCl	33	mM
NADPH-generating system		
Glucose 6-phosphate	5	mM
NADP	4	mM
$MgCl_2$	8	mM
S9	10	% (v/v)

4. Test organisms:

Tester strains				Bacteria batch checked for	
S. typhimurium		***E. coli***			
TA 98	✓	WP2 *uvr*A	✓	deep rough character (rfa)	✓
TA 100	✓	WP2 *uvr*A (pKM101)		ampicillin resistance (R factor plasmid)	✓
TA 1535	✓	WP2 pKM101		UV-light sensitivity (absence of uvrB and uvrA genes in *S. typhimurium* and *E. coli* strains, respectively)	✓
TA 1537	✓				
TA 102					
TA 1538				Histidine and tryptophan auxotrophy (automatically via the spontaneous rate)	✓

5. Test concentrations:

(a) Preliminary cytotoxicity assay/Experiment 1 of the main mutation assay

Plate incorporation test ± S9 mix:	
Concentrations:	*3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate*
Tester strains:	TA 1535, TA 1537, TA 98, TA 100 and WP2 uvrA

Replicates:		*Triplicates*	

(b) Experiment 2 of the main mutation assay

Pre-incubation test ± S9 mix:		
Concentrations:	*3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate*	
Tester strains:	TA 1535, TA 1537, TA 98, TA 100 and WP2 uvrA	
Replicates:	*Triplicates*	

B: STUDY DESIGN AND METHODS

1. **Dates of experimental work:** 17 – 22 Mar 2010
   Finalisation date: 07 Apr 2010

2. **Standard plate test (plate-incorporation test, SPT):**
An aliquot of 0.1 mL test solution or vehicle/positive control, an aliquot of 0.1 mL fresh bacterial culture and 0.5 mL S9 mix (in tests with metabolic activation) or 0.5 mL of S9 substitution buffer (in tests without metabolic activation) were added to 1.0 mL of molten overlay agar (supplemented with 10.5 mg/L L-histidine + 12.2 mg/L biotin or 2.5 mg/L tryptophan). The mixture was mixed in a test tube and poured onto selective agar plates. Each concentration and the controls were tested in triplicates. After solidification, the plates were incubated upside down for at least 48 hours at 37 °C in the dark. After incubation, the background bacterial lawn was examined and the number of bacterial colonies (his^+ or trp^+ revertants) was counted.

3. **Pre-incubation test (PIT):**
0.1 mL of test solution or vehicle/positive control, 0.1 mL pre-cultured bacterial suspension and 0.5 mL of S9 mix (in tests with metabolic activation) or 0.5 mL of S9 mix substitution buffer (in tests without metabolic activation) were mixed in a test tube and shaken at 37 °C for 60 minutes. After pre-incubation, 1.0 mL of molten overlay agar was added to each test tube. Each concentration and the controls were tested in triplicates. The mixture was poured on selective agar plates. After solidification the plates were incubated upside down for at least 48 h at 37°C in the dark. Subsequently, the number of bacterial colonies (his^+ or trp^+ revertants) was counted.

4. **Cytotoxicity**
Toxicity was detected by a
- reduction in the number of spontaneous revertants (below the indication factor of 0.5 when compared to solvent controls)
- clearing or diminution of the background lawn (= reduced his^- or trp^- background growth)

and recorded for all test groups both with and without S9 mix in all experiments.

5. **Statistics**
Results were judged without statistical analysis.

6. **Acceptance criteria**
The test was valid if
- There was a regular background growth in the negative and solvent control.

- The spontaneous reversion rates in the negative and solvent control were in the range of the laboratory's historical data.
- The positive control substances produced a significant increase in mutant colony frequencies.

7. Evaluation criteria

A test item was considered as a mutagen if a biologically relevant increase in the number of revertants exceeding the threshold of twice the colony count of the corresponding solvent control was observed.

A dose dependent increase was considered biologically relevant if the threshold was exceeded at more than one concentration.

An increase exceeding the threshold at only one concentration was judged as biologically relevant if reproduced in an independent second experiment. A dose dependent increase in the number of revertant colonies below the threshold was regarded as an indication of a mutagenic potential if reproduced in an independent second experiment. However, whenever the colony counts remained within the historical range of negative and solvent controls such an increase was not considered biologically relevant.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Analytical determinations were not performed in the study as not required by the test guideline.

B. CYTOTOXICITY

In the preliminary toxicity test, there were no cytotoxic effects observed in any tester strain up to the highest tested concentration of 5000 µg/mL, neither in the presence nor the absence of metabolic activation. Since the second experiment was performed before the results of the preliminary experiment/experiment I were available, both experiments were performed with concentrations from 3 to 5000 µg/plate.

C. SOLUBILITY

Precipitation was observed only in the second experiment at 2500 and 5000 µg/mL in all strains in the presence of metabolic activation. There was no precipitation in the first experiment and no precipitation in the absence of metabolic activation.

D. MUTATION ASSAY

There was no substantial increase in the number of his^+ or trp^+ revertant colonies observed in any of the five tester strains following treatment with glyphosate technical spiked with glyphosine at any dose level, neither in the presence nor in the absence of metabolic activation.

The number of revertants induced by the vehicle control was within the range of the historical control data for each strain, thus demonstrating an acceptable experimental performance.

Appropriate positive control compounds showed a distinct increase in the number of revertant colonies, confirming the activity of the S9 mix and the validity of the test system.

Table 5.4.1-8: *Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay with Solution of Glyphosate TC spiked with Glyphosine ([redacted] 2010), pre-experiment/first experiment

Preliminary experiment / Experiment 1: Standard plate test (SPT)										
Strain	**TA 1535**		**TA 1537**		**TA 98**		**TA 100**		**WP2uvrA**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Untreated										

Table 5.4.1-8: *Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay with Solution of Glyphosate TC spiked with Glyphosine ([redacted] 2010), pre-experiment/first experiment

Preliminary experiment / Experiment 1: Standard plate test (SPT)										
Strain	**TA 1535**		**TA 1537**		**TA 98**		**TA 100**		**WP2uvrA**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
mean	14	21	14	15	36	41	135	153	58	70
± *SD*	± *2*	± *1*	± *1*	± *2*	± *7*	± *5*	± *8*	± *7*	± *8*	± *11*
HCD[#] mean	15	18	12	16	31	39	142	154	53	62
± *SD*	± *3.41*	± *4.69*	± *3.23*	± *3.92*	± *5.67*	± *6.91*	± *21.88*	± *25.80*	± *8.10*	± *8.80*
[range]	*7 - 36*	*8 - 55*	*5 - 27*	*7 - 31*	*14 - 59*	*16 - 84*	*85 - 226*	*94 - 239*	*34 - 80*	*32 - 87*
Vehicle control										
Water mean	16	17	10	20	33	44	132	154	61	72
± *SD*	± *3*	± *2*	± *3*	± *6*	± *5*	± *4*	± *9*	± *14*	± *8*	± *8*
HCD[#] mean	16	19	12	15	30	38	132	144	52	61
± *SD*	± *3.37*	± *4.37*	± *2.84*	± *3.59*	± *5.26*	± *6.58*	± *23.21*	± *25.42*	± *8.11*	± *8.66*
[range]	*8 - 38*	*10 - 41*	*6 - 27*	*7 - 33*	*15 - 52*	*16 - 59*	*94 - 218*	*94 - 241*	*33 - 76*	*34 - 82*
Test item [µg/plate]										
3 mean	14	21	10	20	34	47	131	164	57	70
± *SD*	± *4*	± *5*	± *3*	± *4*	± *4.6*	± *3*	± *6*	± *10*	± *10*	± *11*
10 mean	14	15	9	17	31	39	144	158	65	72
± *SD*	± *5*	± *3*	± *3*	± *3*	± *2.9*	± *7*	± *17*	± *9*	± *4*	± *9*
33 mean	15	16	8	21	33	45	149	165	59	70
± *SD*	± *2*	± *5*	± *1*	± *2*	± *4.7*	± *5*	± *6*	± *8*	± *7*	± *3*
100 mean	17	19	9	17	35	43	146	162	69	75
± *SD*	± *5*	± *5*	± *1*	± *2*	± *2.1*	± *2*	± *17*	± *9*	± *10*	± *5*
333 mean	11	17	11	20	31	43	131	166	59	76
± *SD*	± *3*	± *2*	± *4*	± *1*	± *2.6*	± *7*	± *20*	± *4*	± *9*	± *5*
1000 mean	13	14	13	14	32	41	140	169	68	65
± *SD*	± *4*	± *1*	± *1*	± *5*	± *8.5*	± *7*	± *8*	± *5*	± *1*	± *4*
2500 mean	15	14	8	11	34	50	129	155	47	65
± *SD*	± *1*	± *1*	± *1*	± *2*	± *6.1*	± *1*	± *14*	± *5*	± *7*	± *8*
5000 mean	12	15	9	11	26	40	98	92	35	43
± *SD*	± *0*	± *4*	± *4*	± *3*	± *2.0*	± *6*	± *16*	± *18*	± *7*	± *15*
Positive control										
[§] mean	1926.0	341.7	104.7	436.7	369.3	2072.0	1886.3	3249.3	1057.0	350.0
± *SD*	± *45*	± *8*	± *15*	± *7*	± *20*	± *40*	± *60*	± *170*	± *57*	± *13*
HCD[#] mean	1886	304	101	227	407	1586	1954	2032	808	332
± *SD*	± *242.09*	± *154.47*	± *28.30*	± *67.24*	± *98.13*	± *454.52*	± *426.94*	± *569.62*	± *434.27*	± *154.50*
[range]	*663 - 2690*	*134 - 2404*	*58 - 440*	*68 - 498*	*216 - 897*	*198 - 3309*	*563 - 2844*	*594 - 3724*	*168 - 2528*	*175 - 1718*

[§] Information on respective positive control is reported in Material and Method section I.A.2

Table 5.4.1-8: *Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay with Solution of Glyphosate TC spiked with Glyphosine ([redacted] 2010), pre-experiment/first experiment

Preliminary experiment / Experiment 1: Standard plate test (SPT)										
Strain	**TA 1535**		**TA 1537**		**TA 98**		**TA 100**		**WP2uvrA**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**

[#] Historical control data generated from January - December 2009 in approx. 500 experiments

Table 5.4.1-9: *Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay with Solution of Glyphosate TC spiked with Glyphosine ([redacted] 2010), second experiment

Experiment 2: Pre-incubation test (PIT)										
Strain	**TA 1535**		**TA 1537**		**TA 98**		**TA 100**		**WP2uvrA**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Untreated										
mean	16	21	11	16	30	46	155	169	54	67
± *SD*	± *4*	± *3*	± *3*	± *1*	± *9*	± *4*	± *9*	± *12*	± *7*	± *8*
HCD[#] mean	15	18	12	16	31	39	142	154	53	62
± *SD*	± *3.41*	± *4.69*	± *3.23*	± *3.92*	± *5.67*	± *6.91*	± *21.88*	± *25.80*	± *8.10*	± *8.80*
[range]	*7 - 36*	*8 - 55*	*5 - 27*	*7 - 31*	*14 - 59*	*16 - 84*	*85 - 226*	*94 - 239*	*34 - 80*	*32 - 87*
Vehicle control										
Water mean	17	21	12	18	34	47	167	166	58	67
± *SD*	± *3*	± *1*	± *3*	± *5*	± *4*	± *3*	± *14*	± *9*	± *3*	± *8*
HCD[#] mean	16	19	12	15	30	38	132	144	52	61
± *SD*	± *3.37*	± *4.37*	± *2.84*	± *3.59*	± *5.26*	± *6.58*	± *23.21*	± *25.42*	± *8.11*	± *8.66*
[range]	*8 - 38*	*10 - 41*	*6 - 27*	*7 - 33*	*15 - 52*	*16 - 59*	*94 - 218*	*94 - 241*	*33 - 76*	*34 - 82*
Test item										
3 mean	17	20	14	18	35	44	161	171	53	66
± *SD*	± *3*	± *4*	± *2*	± *2*	± *9*	± *7*	± *4*	± *22*	± *4*	± *3*
10 mean	18	18	14	19	34	45	155	157	55	73
± *SD*	± *3*	± *4*	± *4*	± *4*	± *4*	± *8*	± *17*	± *12*	± *1*	± *12*
33 mean	18	20	12	21	36	44	168	168	59	69
± *SD*	± *3*	± *4*	± *3*	± *2*	± *12*	± *3*	± *19*	± *15*	± *12*	± *10*
100 mean	18	23	11	20	35	45	150	175	55	66
± *SD*	± *3*	± *3*	± *3*	± *1*	± *4*	± *4*	± *2*	± *9*	± *2*	± *12*
333 mean	18	18	12	18[UM]	29	43	170	171	59	63[U]
± *SD*	± *4*	± *3*	± *3*	± *5*	± *1*	± *10*	± *11*	± *17*	± *8*	± *13*
1000 mean	15	23	14	18[UM]	32	49	157	165	56	64[U]
± *SD*	± *2*	± *2*	± *2*	± *4*	± *3*	± *6*	± *12*	± *2*	± *9*	± *6*
2500 mean	12	24[P]	15	12[UMP]	29	45[P]	138	149[P]	52	59[UP]
± *SD*	± *4*	± *1*	± *2*	± *2*	± *4*	± *6*	± *11*	± *12*	± *7*	± *7*

Table 5.4.1-9: *Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay with Solution of Glyphosate TC spiked with Glyphosine ([redacted] 2010), second experiment

Experiment 2: Pre-incubation test (PIT)										
Strain	**TA 1535**		**TA 1537**		**TA 98**		**TA 100**		**WP2uvrA**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
5000 mean ± *SD*	12 ± *2*	22[P] ± *2*	10 ± *5*	11[UMP] ± *1*	20 ± *6*	50[P] ± *1*	129 ± *9*	100[P] ± *12*	34 ± *3*	62[UP] ± *5*
Positive control										
[§] mean ± *SD*	1688 ± *150*	431 ± *16*	123 ± *11*	547 ± *31*	453 ± *10*	2686 ± *742*	1985 ± *99*	3992 ± *38*	691 ± *67*	378 ± *42*
HCD[#] mean ± *SD*	1886 ± *242.09*	304 ± *154.47*	101 ± *28.30*	227 ± *67.24*	407 ± *98.13*	1586 ± *454.52*	1954 ± *426.94*	2032 ± *569.62*	808 ± *434.27*	332 ± *154.50*
[range]	*663 - 2690*	*134 - 2404*	*58 - 440*	*68 - 498*	*216 - 897*	*198 - 3309*	*563 - 2844*	*594 - 3724*	*168 - 2528*	*175 - 1718*

[§] Information on respective positive control is reported in Material and Method section I.A.2

[#] Historical control data generated from January - December 2009 in approx 500 experiments

[P] Precipitation observed

[U] Air bubbles observed

[M] Manual count

III. CONCLUSION

According to the results and under the experimental conditions of the present study, glyphosate technical spiked with glyphosine is not mutagenic in the Ames test (standard plate and pre-incubation method) with and without metabolic activation.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Negative for gene mutation in bacteria (*S. typhimurium* TA 100, TA 98, TA 1535 and TA 1537 and *E. coli* WP2 *uvr*A) with and without metabolic activation.
The study was conducted under GLP conditions and according to OECD guideline 471 (1997) with no deviations. The study is therefore considered valid and acceptable.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.4.1/005
Report author	[redacted]
Report year	2010
Report title	Reverse Mutation Assay using bacteria (*Salmonella typhimurium*) with

	Glyphosate TC
Report No	101268
Document No	Not reported
Guidelines followed in study	OECD 471 (1997), Commission Regulation (EC) No. 440/2008 B.13/14 (2008), US EPA OPPTS 870.5100 (1998)
Deviations from current test guideline OECD 471 (1997)	2-Aminoanthracene was used as sole positive control in the presence of metabolic activation.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

S. typhimurium strains TA 98, TA 100, TA 1535, TA 97a, and TA102 were exposed to glyphosate technical (batch: 200903051, purity: 98.2 %) in the presence and absence of metabolic activation (phenobarbital and β-naphthoflavone-induced rat liver S9 fraction). Test item concentrations in the range of 31.6 to 5000 µg/plate were applied in two independent experiments using the plate-incorporation method (first experiment) and the pre-incubation method (second experiment). Sterility controls (untreated), vehicle (deionised water) and positive controls were included in each experiment. All conditions were tested in triplicates. After an exposure period of at least 48 h the bacterial background lawn was examined and the number of revertant colonies was counted.

There was no biologically relevant increase in the number of revertant colonies observed in any experiment at any tested concentration either in the presence or absence of metabolic activation. The spontaneous mutation rates of negative controls remained within the range of historical control data. Appropriate positive controls induced a distinct response in the number of revertant colonies, thus demonstrating the functionality and validity of the test system. Based on the results and under the conditions of the present study, glyphosate is not mutagenic in the Ames test with and without metabolic activation.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material:

	Glyphosate technical
Identification:	/
Description:	Solid
Lot/Batch #:	200903051
Purity:	98.2 %
Stability of test compound:	The stability of the test item under storage conditions (at room temperature) was guaranteed until 26 Mar 2011.
Solvent (vehicle) used:	DMSO. Due to the low solubility of the test item stock solutions at 50 and 25 mg/mL were prepared and processed by ultrasound for 30 minutes at 37 °C.

2. Control materials:

Negative control:	Sterility controls were performed in accordance with the experimental design, but without addition of bacterial suspension.
Solvent (vehicle) control:	Deionised water and DMSO
Solvent (vehicle)/final concentration:	0.1 mL per plate.
Positive controls:	Please refer to the table below.

Strain	Metabolic activation	Mutagen	Solvent	Conc. [µg/plate]
S. typhimurium strains				
TA 100	-S9	Sodium azide (NaN_3)	Water	10.0
	+S9	2-Aminoanthracene (2-AA)	DMSO	2.5
TA 1535	-S9	Sodium azide (NaN_3)	Water	10.0
	+S9	2-Aminoanthracene (2-AA)	DMSO	2.5
TA 98	-S9	4-Nitro-o-phenylene-diamine (4-NOPD)	DMSO	10
	+S9	2-Aminoanthracene (2-AA)	DMSO	2.5
TA 1537	-S9	4-Nitro-o-phenylene-diamine (4-NOPD)	DMSO	40.0
	+S9	2-Aminoanthracene (2-AA)	DMSO	2.5
TA 102	-S9	Methyl methane sulfonate (MMS)	Water	1.0
	+S9	2-Aminoanthracene (2-AA)	DMSO	10.0

3. Metabolic activation:

S9 mix was obtained from the livers of male Wistar rats that were induced (oral treatment) with 80 mg/kg bw phenobarbital and 100 mg/kg bw β-naphthoflavone for three consecutive days. Aliquots of the S9 mix were thawed prior to each experiment and co-factor was immediately added to prepare S9 mix containing the following components:

S9 mix component	Concentration	Unit
Sodium ortho phosphate buffer (pH 7.4)	100	mM
KCl	33	mM
NADPH-generating system		
Glucose 6-phosphate	5	mM
NADP	4	mM
$MgCl_2$	8	mM
S9	5	% (v/v)

4. Test organisms:

Tester strains				Bacteria batch checked for	
S. typhimurium		***E.coli***			
TA 98	✓	WP2 uvrA		deep rough character (rfa)	✓
TA 100	✓	WP2 uvrA (pKM101)		ampicillin resistance (R factor plasmid)	✓
TA 1535	✓			UV-light sensitivity (absence of uvrB and uvrA genes)	✓
TA 1537	✓				
TA 102	✓				
TA 1538				Histidine auxotrophy (automatically via the spontaneous rate)	✓

5. Test concentrations:

Plate-incorporation test ± S9 mix:	
Concentrations:	31.6, 100, 316, 1000, 2500 and 5000 µg/plate
Tester strains:	TA 1535, TA 1537, TA 98, TA 100 TA 102
Replicates:	Triplicates

Pre-incubation test ± S9 mix:	
Concentrations:	31.6, 100, 316, 1000, 2500 and 5000 µg/plate
Tester strains:	TA 1535, TA 1537, TA 98, TA 100 TA 102
Replicates:	*Triplicates*

B: STUDY DESIGN AND METHODS

1. **Dates of experimental work:** 25 Mar – 06 Apr 2010
   Finalisation date: 08 Apr 2010

2. **Standard plate test (plate-incorporation test, SPT):**
An aliquot of 0.1 mL test solution or vehicle/positive control, an aliquot of 0.1 mL fresh bacterial culture and 0.5 mL S9 mix (in tests with metabolic activation) or 0.5 mL of S9 substitution buffer (in tests without metabolic activation) were added to 2 mL of molten overlay agar (supplemented with 10.5 mg/L L-histidine + 12.2 mg/L biotin). All components were mixed and poured onto minimal agar plates. Each concentration and the controls were tested in triplicates. After incubation at 37 °C for at least 48 h in the dark, the background bacterial lawn was examined and the number of bacterial colonies (his^+ revertants) was counted.

3. **Pre-incubation test (PIT):**
0.1 mL of test solution or vehicle/positive control, 0.1 mL pre-cultured bacterial suspension and 0.5 mL of S9 mix (in tests with metabolic activation) or 0.5 mL of S9 mix substitution buffer (in tests without metabolic activation) were pre-incubated at 37 °C for 60 minutes. Afterwards, 2 mL of overlay agar (supplemented with 10.5 mg/L L-histidine + 12.2 mg/L biotin) were added and poured onto minimal agar plates. Each concentration and the controls were tested in triplicates. After solidification the plates were inverted and incubated for at least 48 h at 37°C in the dark. Subsequently, the background bacterial lawn was examined and the number of bacterial colonies (his^+ revertants) was counted.

4. **Cytotoxicity**
Toxicity was detected by a
- reduction in the number of spontaneous revertants down to a mutation factor of approximately ≤ 0.5 in relation to the solvent control
- clearing or diminution of the background lawn (= reduced his^- background growth)

and recorded for all test groups both with and without S9 mix in all experiments.

5. **Statistics**
Statistical evaluation of the results was not regarded as necessary.

6. **Acceptance criteria**
The test was considered valid for each strain if
- The bacteria demonstrated their typical responses to ampicillin (TA 98, TA 100 and TA 102)
- The control plates with and without S9 mix (mean values of spontaneous reversion rates) were in the range of the laboratory's historical data
- Corresponding background growth on negative control, solvent control and test plates was observed
- The positive controls showed a distinct enhancement of revertant rates over the control plate.

7. **Evaluation criteria**
A test item was considered as a mutagen if the following criteria were met:
- There was a clear and dose-related increase in the number of revertants observed and/or
- There was a biologically relevant positive response for at least one of the dose groups observed in at least one

tester strain with or without metabolic activation.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Analytical determinations were not performed in the study, as not required by the test guideline.

B. CYTOTOXICITY

Cytotoxicity was observed in the first experiment in strain TA100 at 5000 µg/plate and in strain TA1535 at ≥ 2500 µg/plate both in the presence and absence of metabolic activation. In the second experiment cytotoxicity was evident at 5000 µg/plate in tester strain TA100 without metabolic activation and in strain TA1535 with and without metabolic activation.

C. SOLUBILITY

There was no precipitation of the test substance in any tester strain observed up to the highest tested concentration, neither in the presence, not in the absence of metabolic activation.

D. MUTATION ASSAY

There was no biologically relevant increase in the number of revertant colonies observed in any experiment at any tested concentration either in the presence or absence of metabolic activation. The spontaneous mutation rates of negative controls remained within the range of historical control data and appropriate positive controls induced a distinct increase in the number of revertant colonies, thus demonstrating the functionality and validity of the test system.

Table 5.4.1-10: Reverse Mutation Assay using bacteria (*Salmonella typhimurium*) with Glyphosate TC ([redacted], 2010), first experiment (plate-incorporation test)

Experiment 1: Standard plate test (SPT)										
Strain	**TA 98**		**TA 100**		**TA 1535**		**TA 1537**		**TA 102**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Vehicle control										
Water mean	21.0	22.0	104.0	109.0	9.0	9.0	12.0	12.0	290.0	186.0
± *SD*	± *2.3*	± *5.9*	± *12.7*	± *9.0*	± *2.1*	± *1.0*	± *2.5*	± *4.2*	± *8.5*	± *5.1*
DMSO mean	22.0	21.0	72.0	102.0	7.0	6.0	9.0	7.0	244.0	127.0
± *SD*	± *4.2*	± *1.0*	± *14.1*	± *6.6*	± *2.3*	± *1.5*	± *5.1*	± *2.1*	± *10.8*	± *15.0*
Negative control[N]										
HCD[#] mean	24.0	32.1	113.9	114.5	13.3	10.4	11.0	12.1	234.4	283.2
± *SD*	± *4.3*	± *6.1*	± *16.2*	± *16.7*	± *4.7*	± *3.0*	± *3.9*	± *4.2*	± *50.4*	± *61.6*
[range]	*18 - 46*	*18 - 57*	*77 - 163*	*78 - 165*	*5 - 29*	*5 - 27*	*5 - 30*	*5 - 36*	*164 - 309*	*163 - 472*
Test item [µg/plate]										
31.6 mean	23.0	30.0	87.0	123.0	7.0	6.0	11.0	11.0	235.0	141.0
± *SD*	± *1.2*	± *8.1*	± *11.1*	± *10.3*	± *3.2*	± *2.1*	± *3.8*	± *2.1*	± *10.1*	± *9.5*
100 mean	23.0	29.0	81.0	102.0	8.0	7.0	7.0	12.0	238.0	152.0
± *SD*	± *3.2*	± *3.5*	± *10.2*	± *14.2*	± *1.0*	± *4.7*	± *3.2*	± *2.9*	± *6.8*	± *8.7*
316 mean	19.0	30.0	81.0	100.0	6.0	5.0	10.0	9.0	218.0	135.0
± *SD*	± *3.5*	± *2.1*	± *10.6*	± *7.5*	± *1.2*	± *3.1*	± *1.0*	± *2.1*	± *7.1*	± *7.0*

Table 5.4.1-10: Reverse Mutation Assay using bacteria (*Salmonella typhimurium*) with Glyphosate TC ([redacted], 2010), first experiment (plate-incorporation test)

Experiment 1: Standard plate test (SPT)										
Strain	**TA 98**		**TA 100**		**TA 1535**		**TA 1537**		**TA 102**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
1000 mean	18.0	28.0	75.0	119.0	5.0	6.0	12.0	10.0	229.0	167.0
± *SD*	*± 2.3*	*± 3.1*	*± 15.7*	*± 17.2*	*± 0.6*	*± 2.5*	*± 2.9*	*± 2.0*	*± 13.0*	*± 10.8*
2500 mean	22.0	31.0	72.0	98.0	1.0	3.0	7.0	9.0	183.0	130.0
± *SD*	*± 0.6*	*± 3.6*	*± 12.3*	*± 8.7*	*± 1.0*	*± 2.1*	*± 3.0*	*± 2.6*	*± 17.8*	*± 16.4*
5000 mean	15.0	21.0	12.0	51.0	0.0 [B]	1.0 [B]	5.0	8.0	141.0	74.0
± *SD*	*± 4.0*	*± 4.5*	*± 3.5*	*± 13.6*	*± 0.0*	*± 1.2*	*± 3.8*	*± 1.0*	*± 7.8*	*± 12.7*
Positive control										
[§] mean	361.0	1904.0	634.0	2373.0	543.0	1320	110.0	347.0	1593.0	1160.0
± *SD*	*± 12.1*	*± 384.8*	*± 256.6*	*± 99.6*	*± 326.6*	*± 27.9*	*± 14.6*	*± 12.7*	*± 172.1*	*± 119.3*
HCD[#] mean	522.5	2378.0	1002.8	2083.8	1099.2	148.3	140.4	278.4	1601.4	1154.1
± *SD*	*± 145.1*	*± 536.1*	*± 240.2*	*± 528.0*	*± 246.7*	*± 63.0*	*± 34.3*	*± 81.8*	*± 308*	*± 316.4*
[range]	*250 - 1508*	*260 - 3599*	*240 - 2307*	*500 3341*	*389 - 1827*	*31 - 687*	*43 - 453*	*58 - 502*	*550 - 2407*	*419 - 2102*

[§] Information on respective positive control is reported in Material and Method section I.A.2

[#] Historical control data generated in 2007 - 2009

[N] Negative control historical control data combine solvent or vehicle as well as untreated controls

[B] Background lawn reduced

Table 5.4.1-11: Reverse Mutation Assay using bacteria (*Salmonella typhimurium*) with Glyphosate TC ([redacted], 2010), second experiment (pre-incubation test)

Experiment 2: Pre-incubation test (PIT)										
Strain	**TA 98**		**TA 100**		**TA 1535**		**TA 1537**		**TA 102**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Vehicle control										
Water mean	22.0	29.0	118.0	125.0	8.0	11.0	11.0	9.0	351.0	271.0
± *SD*	*± 5.9*	*± 4.7*	*± 9.3*	*± 9.5*	*± 3.0*	*± 2.3*	*± 3.0*	*± 2.9*	*± 7.1*	*± 17.0*
DMSO mean	23.0	26.0	94.0	101.0	8.0	9.0	10.0	9.0	292.0	210.0
± *SD*	*± 6.8*	*± 2.1*	*± 3.5*	*± 20.0*	*± 3.0*	*± 2.3*	*± 5.3*	*± 8.1*	*± 5.6*	*± 13.6*
Negative control[N]										
HCD[#] mean	24.0	32.1	113.9	114.5	13.3	10.4	11.0	12.1	234.4	283.2
± *SD*	*± 4.3*	*± 6.1*	*± 16.2*	*± 16.7*	*± 4.7*	*± 3.0*	*± 3.9*	*± 4.2*	*± 50.4*	*± 61.6*
[range]	*18 - 46*	*18 - 57*	*77 - 163*	*78 - 165*	*5 - 29*	*5 - 27*	*5 - 30*	*5 - 36*	*164 - 309*	*163 - 472*
Test item [µg/plate]										
31.6 mean	20.0	28.0	110.0	112.0	14.0	7.0	8.0	9.0	233.0	195.0

Table 5.4.1-11: Reverse Mutation Assay using bacteria (*Salmonella typhimurium*) with Glyphosate TC ([redacted], 2010), second experiment (pre-incubation test)

Experiment 2: Pre-incubation test (PIT)										
Strain	**TA 98**		**TA 100**		**TA 1535**		**TA 1537**		**TA 102**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
± SD	*± 2.9*	*± 6.7*	*± 7.6*	*± 19.4*	*± 3.8*	*± 3.6*	*± 2.1*	*± 0.6*	*± 15.8*	*± 12.2*
100 mean	18.0	26.0	107.0	103.0	10.0	9.0	7.0	7.0	240.0	184.0
± SD	*± 2.1*	*± 4.2*	*± 6.1*	*± 10.4*	*± 4.4*	*± 3.2*	*± 2.1*	*± 2.1*	*± 8.1*	*± 12.4*
316 mean	23.0	29.0	101.0	109.0	7.0	8.0	11.0	12.0	278.0	205.0
± SD	*± 5.8*	*± 4.0*	*± 10.0*	*± 11.2*	*± 1.2*	*± 4.4*	*± 2.9*	*± 2.3*	*± 5.9*	*± 16.5*
1000 mean	16.0	34.0	84.0	96.0	9.0	10.0	8.0	8.0	252.0	151.0
± SD	*± 0.6*	*± 6.2*	*± 9.6*	*± 6.6*	*± 2.1*	*± 5.5*	*± 1.2*	*± 3.1*	*± 1.2*	*± 21.1*
2500 mean	18.0	32.0	88.0	96.0	5.0	9.0	6.0	8.0	182.0	151.0
± SD	*± 8.5*	*± 3.8*	*± 12.6*	*± 14.1*	*± 2.0*	*± 2.3*	*± 4.2*	*± 2.0*	*± 19.6*	*± 20.1*
5000 mean	18.0	23.0	43.0	82.0	3.0	4.0	9.0	12.0	184.0	116.0
± SD	*± 6.8*	*± 9.1*	*± 6.2*	*± 3.1*	*± 2.9*	*± 1.2*	*± 1.5*	*± 2.0*	*± 32.2*	*± 3.5*
Positive control										
§ mean	595.0	2172.0	722.0	2025.0	812.0	99.0	144.0	240.0	1747.0	1211.0
± SD	*± 66.8*	*± 164.6*	*± 47.8*	*± 124.4*	*± 95.7*	*± 14.0*	*± 16.9*	*± 34.0*	*± 47.2*	*± 210.4*
HCD# mean	522.5	2378.0	1002.8	2083.8	1099.2	148.3	140.4	278.4	1601.4	1154.1
± SD	*± 145.1*	*± 536.1*	*± 240.2*	*± 528.0*	*± 246.7*	*± 63.0*	*± 34.3*	*± 81.8*	*± 308*	*± 316.4*
[range]	*250 - 1508*	*260 - 3599*	*240 - 2307*	*500 - 3341*	*389 - 1827*	*31 - 687*	*43 - 453*	*58 - 502*	*550 - 2407*	*419 - 2102*

§ Information on respective positive control is reported in Material and Method section I.A.2

Historical control data generated in 2007 - 2009

N Negative control historical control data combine solvent or vehicle as well as untreated controls

III. CONCLUSION

Based on the results of the present study and under the experimental conditions of the test, the test item is not mutagenic in the Ames test (standard plate and pre-incubation method) with and without metabolic activation.

3. Assessment and conclusion

<u>Assessment and conclusion by applicant</u>:

Negative for gene mutation in bacteria (*S. typhimurium* TA 100, TA 98, TA 1535, TA 1537 and TA102) with and without metabolic activation.

The study was performed according to OECD guideline 471 (1997) and under GLP conditions. There were only minor deviations, which were considered to not compromise the validity of the study. The study is therefore considered valid and acceptable.

<u>Assessment and conclusion by RMS</u>:

1. Information on the study

Data point	CA 5.4.1/006
Report author	[redacted]
Report year	2009
Report title	Mutagenicity study of Glyphosate TC in the *Salmonella typhimurium* Reverse Mutation Assay (*in vitro*)
Report No	23916
Document No	Not reported
Guidelines followed in study	OECD 471 (1997), Commission Regulation (EC) No. 440/2008 B.13/14 (2008), US EPA OPPTS 870.5100 (1998), ICH S2A (CPMP/ICH/141/95) and ICH S2B (CPMP/ICH/174/95)
Deviations from current test guideline OECD 471 (1997)	Historical control data for the positive controls were not included in the study report.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid[#]
Category study in AIR 5 dossier (L docs)	Category 2a

Following the feedback from the Assessment Group Glyphosate in March 2020, the Notifier is aware of concerns regarding work conducted at this specific contract laboratory (LPT). For the sake of completeness, this study is included in the dossier submission since it was part of the data package of previous EU evaluation processes.

2. Full summary

Glyphosate technical (batch: 20080801, purity 98.8 %) was investigated for its potential to induce gene mutations in bacteria in an Ames test. *S. typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 were exposed to the test item in the presence and absence of metabolic activation (Aroclor 1254-induced rat liver S9 fraction) for 48 to 72 hours at 37 °C. Vehicle (aqua ad iniectabilia) and positive controls were included in each experiment.

Based on the results of a preliminary cytotoxicity test conducted in strain TA100 in the absence of metabolic activation, test item concentrations in the main mutagenicity test ranged from 31.6 to 3160 µg/plate. Two independent experiments were performed, using the standard plate test (plate-incorporation method) and the pre-incubation test. After 48 to 72 hours of incubation, the number of revertant colonies was counted and the bacterial background lawn was examined.

Precipitation of the test item was not reported. Cytotoxicity was observed for all tester strains at 3160 µg/plate in the presence and absence of metabolic activation.

There was no increase in the number of revertant his^+ colonies as compared to vehicle controls observed in any of the five tester strains following treatment with glyphosate technical tested up to concentrations of 3160 µg/plate, neither in the presence, nor in the absence of metabolic activation.

The number of revertants induced by the vehicle control was within the range of the laboratory's historical control data. The positive controls markedly induced the number of revertant colonies in each strain, demonstrating the functionality of the S9 mix and the sensitivity of the test system.

Based on the experimental results, glyphosate technical does not cause gene mutations in bacteria in the Ames standard plate and pre-incubation test with and without metabolic activation.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material: Glyphosate TC

Identification:	37/064/08
Description:	Solid, white
Lot/Batch #:	20080801
Purity:	98.8 %
Stability of test compound:	The stability of the test item under storage conditions (at room temperature) was guaranteed until the mentioned expiry date 01 Aug 2010. The stability of the test item in vehicle was not specified in the study report.
Solvent (vehicle) used:	Aqua ad iniectabilia

2. Control materials:

Negative control:	Sterility controls were not included in the present study
Solvent (vehicle) control:	Aqua ad iniectabilia
Solvent (vehicle)/final concentration:	0.1 mL per plate
Positive controls:	Please refer to the table below.

Strain	Metabolic activation	Mutagen	Solvent	Conc. [µg/plate]
S. typhimurium strains				
TA 100	-S9	Sodium azide	Water	10.0
	+S9	Cyclophosphamide	Water	1500.0
TA 1535	-S9	Sodium azide	Water	10.0
	+S9	Cyclophosphamide	Water	1500.0
TA 98	-S9	2-Nitrofluorene	DMSO	10
	+S9	2-Aminoanthracene	DMSO	2.0
TA 1537	-S9	9-Aminoacridine	Ethanol	100.0
	+S9	2-Aminoanthracene	DMSO	2.0
TA 102	-S9	Methylmethane sulfonate	DMSO	1300.0
	+S9	2-Aminoanthracene	DMSO	2.0

3. Metabolic activation:

S9 mix was obtained from the livers of 20 – 30 rats treated with Aroclor 1254. The pooled fraction exhibited a protein content of 31.55 mg/mL and a cytochrome P450 level of 0.41 nmol/protein. Aliquots of the S9 mix were thawed prior to each experiment and co-factor was immediately added to prepare S9 mix containing the following components:

S9 mix component	Concentration	Unit
Sodium phosphate buffer (pH 7.4)	100.0	mM
KCl	33.0	mM
NADPH-generating system		
Glucose 6-phosphate	5.4	mM
NADP	4.1	mM
$MgCl_2$	8.0	mM
S9	5.0	% (v/v)

4. Test organisms:

Tester strains				Bacteria batch checked for	
S. typhimurium		***E.coli***			
TA 98	✓	WP2 uvrA		deep rough character (rfa)	✓
TA 100	✓	WP2 uvrA (pKM101)		ampicillin resistance (R factor plasmid)	✓
TA 1535	✓			UV-light sensitivity (absence of uvrB genes)	✓
TA 1537	✓				
TA 102	✓				
TA 1538				Histidine auxotrophy (automatically via the spontaneous rate)	✓

5. Test concentrations:

(a) Preliminary cytotoxicity assay

Plate incorporation test - S9 mix:	
Concentrations:	*0.316, 1.0, 3.16, 10.0, 31.6, 100, 316, 1000, 3160 and 5000 µg/plate*
Tester strain:	TA100
Replicates:	*Duplicates*

(b) Main mutation assay

Plate incorporation test ± S9 mix:	
Concentrations:	*31.6, 100, 316, 1000 and 3160 µg/plate*
Tester strains:	TA 98, TA 100, TA 102, TA 1535 and TA 1537
Replicates:	*Triplicates*
Pre-incubation test ± S9 mix:	
Concentrations:	*31.6, 100, 316, 1000 and 3160 µg/plate*
Tester strains:	TA 98, TA 100, TA 102, TA 1535 and TA 1537
Replicates:	*Triplicates*

B: STUDY DESIGN AND METHODS

1. **Dates of experimental work:** 04 – 27 Feb 2009
   Finalisation date: 30 Apr 2009

2. **Standard plate test (plate-incorporation test, SPT):**

An aliquot of 0.1 mL test solution or vehicle/positive control, an aliquot of 0.1 mL fresh bacterial culture and 0.5 mL S9 mix (in tests with metabolic activation) or 0.5 mL of S9 phosphate buffer (in tests without metabolic activation) were added to 2.0 mL of molten overlay agar (supplemented with 0.5 mM L-histidine + 0.5 mM biotin). The test components were mixed by vortexing at low speed, poured onto coded minimal glucose agar plates and the plates were quickly tilted and rotated for homogenous distribution. Each concentration and the controls were tested in triplicates. After solidification, the plates were incubated upside down for 48 to 72 hours at 37 °C in the dark. After incubation, the number of revertant colonies was counted and the presence of bacterial background lawn was confirmed.

3. Pre-incubation test (PIT):
0.1 mL of test solution or vehicle/positive control, 0.1 mL pre-cultured bacterial suspension and 0.5 mL of S9 mix (in tests with metabolic activation) or 0.5 mL of S9 mix phosphate buffer (in tests without metabolic activation) were pre-incubated for 20 minutes at 37 °C using a shaker.
Afterwards, 2.0 mL of molten overlay agar was added to each test tube and the mixture was poured on coded minimal glucose agar plates. Each concentration and the controls were tested in triplicates. After solidification, the plates were incubated upside down for 48 to 72 hours at 37 °C in the dark. After incubation, the number of revertant colonies was counted and the presence of bacterial background lawn was examined.

4. Cytotoxicity
Toxicity was detected by

- a reduction in the number of spontaneous revertants (below 50 % when compared to solvent controls)
- a clearing or diminution of the background lawn (= reduced his^- background growth)
- the degree of survival of the treated cultures

and recorded for all test groups both with and without S9 mix in all experiments.

5. Statistics
Statistical significance for an increased number of revertants compared to the solvent control was determined using the Mann-Whitney U-test. A concentration-related effect was identified using the Spearman's rank correlation coefficient.

6. Acceptance criteria
Acceptance criteria were not defined in the study report.

7. Evaluation criteria
A test item was considered to show a positive response if the following criteria were met:

- The number of revertant colonies was statistically significantly increased compared to the solvent control to at least 2-fold of the solvent control for TA 98, TA 100 and TA 102 and 3-fold of the solvent control for TA 1535 and TA 1537 in both experiments.
- There was a statistically significant, concentration-related effect.
- Positive results were reproducible and the histidine independence of the revertants was confirmed by streaking random samples on histidine-free agar plates

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS
Analytical determinations were not performed in the study as not required by the test guideline.

B. CYTOTOXICITY
In the preliminary toxicity test, cytotoxicity was noted in tester strain TA 100 at concentrations of 3160 and 5000 µg/plate in the absence of metabolic activation. Hence, 3160 µg/plate was chosen as top concentration for the main mutagenicity study.

In the main mutation assay, cytotoxicity (scarce background lawn) was noted for all tester strains at 3160 µg/plate in both experiments (plate-incorporation and pre-incubation test), both in the presence and absence of metabolic activation.

C. SOLUBILITY

Precipitation of the tests item was not reported.

D. MUTATION ASSAY

There was no increase in the number of revertant colonies as compared to control counts observed in any of the five tester strains following treatment with glyphosate technical tested up to 3160 µg/plate, neither in the presence nor in the absence of metabolic activation.
The number of revertants induced by the vehicle control was within the range of the historical control data for each strain. A marked increase in the number of revertant colonies was observed for the positive controls, demonstrating the functionality of the S9 mix and the sensitivity of the test system.

Table 5.4.1-12: Mutagenicity study of Glyphosate TC in the *Salmonella typhimurium* Reverse Mutation Assay (*in vitro*) ([redacted], 2009), first experiment

Experiment 1: Standard plate test (SPT)										
Strain	**TA 98**		**TA 100**		**TA 102**		**TA 1535**		**TA 1537**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Vehicle control										
Aqua ad iniectabilia mean	42.7	42.0	138.7	158.3	269.7	274.0	30.7	30.0	6.7	6.3
± *SD*	*± 11.4*	*± 2.0*	*± 11.7*	*± 16.0*	*± 4.7*	*± 5.6*	*± 4.9*	*± 3.0*	*± 0.6*	*± 0.6*
HCD[#] range	*20 - 60*		*100 - 200*		*240 - 320*		*10 - 35*		*3 - 20*	
Test item [µg/plate]										
31.6 mean	39.7	41.7	126.0	150.7	268.3	279.3	25.7	28.7	6.7	5.0
± *SD*	*± 7.6*	*± 8.3*	*± 7.0*	*± 32.7*	*± 2.5*	*± 5.7*	*± 2.5*	*± 1.5*	*± 1.2*	*± 1.0*
100 mean	33.3	32.0	143.0	134.3	274.0	269.7	25.3	29.0	6.7	7.3
± *SD*	*± 0.6*	*± 1.0*	*± 12.5*	*± 7.2*	*± 4.0*	*± 4.5*	*± 4.5*	*± 2.6*	*± 1.2*	*± 0.6*
316 mean	30.7	42.0	143.0	132.7	259.0	274.7	28.7	28.3	4.7	6.0
± *SD*	*± 1.5*	*± 7.0*	*± 4.6*	*± 2.1*	*± 3.6*	*± 5.5*	*± 3.2*	*± 3.8*	*± 0.6*	*± 1.0*
1000 mean	30.0	32.3	124.0	128.7	266.0	272.0	30.3	32.7	4.7	5.7
± *SD*	*± 1.7*	*± 11.0*	*± 13.1*	*± 9.5*	*± 7.0*	*± 5.3*	*± 0.6*	*± 3.2*	*± 1.5*	*± 1.5*
3160 mean	36.6[$]	26.3[$]	109.0[$]	107.3[$]	259.0[$]	256.0[$]	28.7[$]	28.7[$]	4.3[$]	4.0[$]
± *SD*	*± 2.1*	*± 4.0*	*± 3.6*	*± 2.1*	*± 3.6*	*± 4.4*	*± 2.5*	*± 3.2*	*± 0.6*	*± 1.0*
Positive control										
[§] mean	393.7	387.0	720.3	730.7	1045.0	1159.3	366.7	375.0	364.0	375.3
± *SD*	*28.6*	*± 10.5*	*± 31.2*	*± 9.3*	*± 19.2*	*± 31.4*	*± 8.1*	*± 12.5*	*± 21.0*	*± 24.6*

[§] Information on respective positive control is reported in Material and Method section I.A.2

[#] Historical control data generated in the laboratory of the testing facility

[$] Cytotoxicity indicated by scarce background lawn

Table 5.4.1-13: Mutagenicity study of Glyphosate TC in the *Salmonella typhimurium* Reverse Mutation Assay (*in vitro*) [redacted] 2009), second experiment

Experiment 2: Pre-incubation test (PIT)										
Strain	**TA 98**		**TA 100**		**TA 102**		**TA 1535**		**TA 1537**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Vehicle control										
Aqua ad iniectabilia mean	40.3	52.0	150.0	152.3	267.0	274.3	18.7	19.7	4.3	6.7
± *SD*	*± 6.8*	*± 12.2*	*± 6.2*	*± 9.7*	*± 12.5*	*± 16.9*	*± 6.1*	*± 3.5*	*± 0.6*	*± 0.6*
HCD[#] range	*20 - 60*		*100 - 200*		*240 - 320*		*10 - 35*		*3 - 20*	
Test item										
31.6 mean	43.3	47.7	150.0	166.0	277.7	281.3	22.0	20.0	5.7	6.7
± *SD*	*± 6.7*	*± 3.1*	*± 6.6*	*± 13.7*	*± 7.0*	*± 8.5*	*± 3.6*	*± 2.0*	*± 2.5*	*± 2.3*
100 mean	44.3	50.7	145.3	164.0	275.0	268.7	16.3	19.7	6.3	6.3
± *SD*	*± 5.7*	*± 1.5*	*± 24.6*	*± 1.0*	*± 9.0*	*± 2.5*	*± 2.3*	*± 5.5*	*± 1.2*	*± 0.6*
316 mean	39.0	51.0	161.3	139.7	278.3	271.0	17.0	21.0	6.3	6.7
± *SD*	*± 7.0*	*± 11.5*	*± 10.1*	*± 4.0*	*± 5.0*	*± 6.1*	*± 3.6*	*± 6.1*	*± 1.5*	*± 1.2*
1000 mean	43.0	41.3	146.0	157.0	260.3	264.7	24.0	21.3	6.0	7.0
± *SD*	*± 6.1*	*± 0.6*	*± 10.6*	*± 20.1*	*± 3.2*	*± 8.6*	*± 5.6*	*± 8.1*	*± 1.0*	*± 1.0*
3160 mean	36.3[$]	44.7[$]	140.0[$]	161.0[$]	264.3[$]	262.0[$]	19.0[$]	18.0[$]	5.0[$]	5.7[$]
± *SD*	*± 0.6*	*± 0.6*	*± 8.7*	*± 5.3*	*± 5.7*	*± 3.6*	*± 5.6*	*± 2.6*	*± 1.0*	*± 0.6*
Positive control										
[§] mean	468.7	482.3	691.7	700.7	1178.3	1160.0	366.7	384.0	463.7	439.7
± *SD*	*± 12.6*	*± 5.0*	*± 26.6*	*± 11.7*	*± 15.3*	*± 29.5*	*± 24.9*	*± 12.1*	*± 18.0*	*± 15.7*

[§] Information on respective positive control is reported in Material and Method section I.A.2

[#] Historical control data generated in the laboratory of the testing facility

[$] Cytotoxicity indicated by scarce background lawn

III. CONCLUSION:

According to the results and under the conditions of the present study, glyphosate technical is not mutagenic in the Ames standard plate and pre-incubation test with and without metabolic activation.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Negative for gene mutation in bacteria (*S. typhimurium* TA 98, TA 100, TA 102, TA 1535 and TA 1537) with and without metabolic activation.

The study was conducted according to OECD guideline 471 (1997) and in compliance with GLP. There were only minor deviations when compared to OECD 471 (1997) that do not hinder data evaluation. The study is therefore considered valid and acceptable.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.4.1/007
Report author	[redacted]
Report year	2009
Report title	Glyphosate technical - *Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay
Report No	1264500
Document No	Not reported
Guidelines followed in study	OECD 471 (1997), US EPA OPPTS 870.5100 (1998), Commission Regulation (EC) 2008/440 B13/B14 (2008)
Deviations from current test guideline OECD 471 (1997)	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

S. typhimurium strains TA 98, TA 100, TA 1535 and TA 1537, and *E. coli* strains WP2 uvrA (pKM101) and WP2 pKM101 were exposed to glyphosate technical (batch: 569753, purity: 96.3 %) in the presence and absence of metabolic activation (phenobarbital and β-naphthoflavone-induced rat liver S9 fraction) for 48 hours at 37 °C. Vehicle (deionized water), sterility (untreated) and positive controls were included in each experiment.

A pre-experiment was conducted to identify cytotoxic concentrations of the test item. In a standard plate test (plate incorporation method), test item concentrations in the range of 3 to 5000 µg/plate did not show any cytotoxic effects in the presence and absence of metabolic activation. The pre-experiment was designated experiment I of the main mutation assay and analysed for the number of revertant colonies. A second main mutation assay was performed using the preincubation method and concentrations in the range of 33 to 5000 µg/plate. Both experiments were performed with triplicates. Following 48 hours of incubation, the mean number of revertant colonies was counted for each plate.

Precipitation of the test substance was not reported and there were no indications of cytotoxicity in any of the six tester strains up to the highest tested concentration of 5000 µg/plate.

There was no substantial increase in the number of his^+ or trp^+ revertants observed in any experiment at any dose level, neither in the presence nor absence of metabolic activation. In addition, there was no dose-dependent increase in mutation rates in the range below the generally acknowledged border of biological relevance.

The laboratory's historical control range was exceeded for tester strain WP2 uvrA pKM101 in the untreated and solvent control plates with and without S9 mix in the preliminary toxicity experiment/experiment I, and for tester strain WP2 uvrA pKM101 in the untreated control with S9 mix and the solvent control with

and without S9 mix in experiment II. In strain WP2 pKM101 with and without metabolic activation in experiment I, the number of spontaneous revertants was below the lower limit of the laboratory historical control range. As discussed in the study report, the observations were considered to be the result of biologically irrelevant fluctuations in the number of colonies and were judged to have no detrimental impact on the outcome of the study. Since the number of revertants is consistent in all concentrations for these strains without major variability, and no increase of the number of revertant colonies was observed at all, the overall conclusion of an absence of a mutagenic effect is considered to be valid also for these tester strains.

Appropriate positive controls induced a marked increase in the number of revertants, confirming the activity of the S9 mix and the validity of the test system.

Based on the results of the present study and under the experimental conditions chosen, glyphosate technical is considered not mutagenic in the Ames standard plate and pre-incubation test with and without metabolic activation.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material: Glyphosate technical

Identification:	S1008322
Description:	Not specified
Lot/Batch #:	569753
Purity:	96.3 %
Stability of test compound:	The stability of the test item under storage conditions (at room temperature) and in vehicle were not specified in the study report.
Solvent (vehicle) used:	Deionised water

2. Control materials:

Negative control:	Controls which remained untreated were included in each experiment.
Solvent (vehicle) control:	Deionised water
Solvent (vehicle) final concentration:	0.1 mL per plate
Positive controls:	Please refer to the table below.

Strain	Metabolic activation	Mutagen	Solvent	Conc. [µg/plate]
S. typhimurium strains				
TA 100	-S9	Sodium azide (NaN_3)	Water	10.0
	+S9	2-Aminoanthracene (2-AA)*	DMSO	2.5
TA 1535	-S9	Sodium azide (NaN_3)	Water	10.0
	+S9	2-Aminoanthracene (2-AA)*	DMSO	2.5
TA 98	-S9	4-Nitro-o-phenylene-diamine (4-NOPD)	DMSO	10
	+S9	2-Aminoanthracene (2-AA)*	DMSO	2.5
TA 1537	-S9	4-Nitro-o-phenylene-diamine (4-NOPD)	DMSO	50.0
	+S9	2-Aminoanthracene (2-AA)*	DMSO	2.5
E. coli strains				
WP2 uvrA (pKM101)	-S9	Methylmethane sulfonate (MMS)	Water	3.0
	+S9	2-Aminoanthracene (2-AA)*	DMSO	10.0
WP2 pKM101	-S9	Methylmethane sulfonate (MMS)	Water	3.0
	+S9	2-Aminoanthracene (2-AA)*	DMSO	10.0

* The functionality of the S9 mix batch used was checked with benzo(a)pyrene and showed the expected results.

3. Metabolic activation:

S9 mix was obtained from the livers of 8 – 12 weeks old male Wistar rats, weighing approx. 220 - 320 g. The animals received intraperitoneal injections of phenobarbital (80 mg/kg bw) and oral administration of β-naphthoflavone (80 mg/kg bw) on three consecutive days. The livers were prepared 24 h after the last treatment. Aliquots of the S9 mix were thawed prior to each experiment and co-factor was immediately added to prepare S9 mix containing the following components:

S9 mix component	Concentration	Unit
Sodium phosphate buffer (pH 7.4)	100	mM
KCl	33	mM
NADPH-generating system		
Glucose 6-phosphate	5	mM
NADP	4	mM
$MgCl_2$	8	mM
S9	10	% (v/v)

4. Test organisms:

Tester strains				Bacteria batch checked for	
S. typhimurium		***E.coli***			
TA 98	✓	WP2 uvrA		deep rough character (rfa)	✓
TA 100	✓	WP2 uvrA (pKM101)	✓	ampicillin resistance (R factor plasmid)	✓
TA 1535	✓	WP2 pKM101	✓	UV-light sensitivity (absence of uvrB and uvrA genes in *S. typhimurium* and *E. coli* strains, respectively)	✓
TA 1537	✓				
TA 102					
TA 1538				Histidine and tryptophan auxotrophy (automatically via the spontaneous rate)	✓

5. Test concentrations:

(a) Preliminary cytotoxicity assay/Experiment 1 of the main mutation assay

Plate incorporation test ± S9 mix:	
Concentrations:	*3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate*

Tester strains:	TA 1535, TA 1537, TA 98, TA 100, WP2 uvrA (pKM101) and WP2 pKM101
Replicates:	*Triplicates*

(b) Experiment 2 of the main mutation assay

Pre-incubation test ± S9 mix:	
Concentrations:	*33, 100, 333, 1000, 2500 and 5000 µg/plate*
Tester strains:	TA 1535, TA 1537, TA 98, TA 100, WP2 uvrA (pKM101) and WP2 pKM101
Replicates:	*Triplicates*

B: STUDY DESIGN AND METHODS

1. Dates of experimental work: 23 Sep – 13 Oct 2009
Finalisation date: 18 Dec 2009

2. Standard plate test (plate-incorporation test, SPT):
An aliquot of 0.1 mL test solution or vehicle/positive control, an aliquot of 0.1 mL fresh bacterial culture and 0.5 mL S9 mix (in tests with metabolic activation) or 0.5 mL of S9 substitution buffer (in tests without metabolic activation) were added to 2 mL of molten overlay agar (supplemented with 10.5 mg/L L-histidine + 12.2 mg/L biotin or 2.5 mg/L tryptophan). The mixture was mixed in a test tube and poured onto selective agar plates. Each concentration and the controls were tested in triplicates. After solidification, the plates were incubated upside down for at least 48 hours at 37 °C in the dark. After incubation, the background bacterial lawn was examined and the number of the bacterial colonies (his^+ or trp^+ revertants) was counted.

3. Pre-incubation test (PIT):
0.1 mL of test solution or vehicle/positive control, 0.1 mL pre-cultured bacterial suspension and 0.5 mL of S9 mix (in tests with metabolic activation) or 0.5 mL of S9 mix substitution buffer (in tests without metabolic activation) were mixed in a test tube and shaken at 37 °C for 60 minutes. After pre-incubation, 2 mL of molten overlay agar was added to each test tube. Each concentration and the controls were tested in triplicates. The mixture was poured on selective agar plates. After solidification the plates were incubated upside down for at least 48 h at 37°C in the dark. Subsequently, the number of bacterial colonies (his^+ or trp^+ revertants) was counted.

4. Cytotoxicity
Toxicity was detected by a

- reduction in the number of spontaneous revertants
- clearing or diminution of the background lawn (= reduced his^- or trp^- background growth)

and recorded for all test groups both with and without S9 mix in all experiments.

5. Statistics
Results were judged without statistical analysis.

6. Acceptance criteria
The test was valid if

- There was a regular background growth in the negative and solvent control.

- The spontaneous reversion rates in the negative and solvent control were in the range of the laboratory's historical data.
- The positive control substances produced a significant increase in mutant colony frequencies.

7. Evaluation criteria

A test item was considered as a mutagen if a biologically relevant increased in the number of revertants exceeding the threshold of twice the colony count of the corresponding solvent control is observed.

A dose dependent increase was considered biologically relevant if the threshold is exceeded at more than one concentration.

An increase exceeding the threshold at only one concentration was judged as biologically relevant if reproduced in an independent second experiment. A dose dependent increase in the number of revertant colonies below the threshold was regarded as an indication of a mutagenic potential if reproduced in an independent second experiment. However, whenever the colony counts remained within the historical range of negative and solvent controls such an increase was not considered biologically relevant.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Analytical determinations were not performed in the study, as not required by the test guideline.

B. CYTOTOXICITY

In the preliminary toxicity experiment, there were no indications of cytotoxicity up to the highest tested concentration of 5000 µg/plate. The preliminary experiment was designated as experiment I. Since no cytotoxic effects were observed, 5000 µg/plate were chosen as top concentration for experiment II. The plates incubated with the test item showed normal background growth up to 5000 µg/plate with and without metabolic activation in both independent experiments. No toxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5), occurred in the test groups with and without metabolic activation

C. SOLUBILITY

There was no precipitation of the test substance observed up to the highest concentration tested.

D. MUTATION ASSAY

There was no substantial increase in the number of his^+ or trp^+ revertants observed in any of the six tester strains at any dose level, neither in the presence nor absence of metabolic activation. In addition, there was no dose-dependent increase in mutation rates in the range below the generally acknowledged border of biological relevance.

For tester strain WP2 uvrA pKM101 the laboratory's historical control range was exceeded in the untreated and solvent control plates with and without S9 mix in the preliminary toxicity experiment/experiment I, and in the untreated control with S9 mix and the solvent control with and without S9 mix in experiment II. In strain WP2 pKM101 the lower limit of the laboratory historical control range was not quite reached in the untreated control with and without metabolic activation in experiment I. As discussed in the study reports, these elevated colony counts were considered to be the result of biologically irrelevant fluctuations in the number of colonies and were judged to have no detrimental impact on the outcome of the study.

Appropriate positive controls induced a marked increase in the number of revertants, confirming the activity of the S9 mix and the validity of the test system.

Table 5.4.1-14: *Salmonella Typhimurium* and *Escherichia Coli* Reverse Mutation Assay ([redacted] 2009), pre-experiment/first experiment

Preliminary experiment / Experiment 1: Standard plate test (SPT)												
Strain	**TA 1535**		**TA 1537**		**TA 98**		**TA 100**		**WP2 pKM101**		**WP2 uvrA (pKM101)**	
Metabol. activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Untreated												
mean ± *SD*	14 ± *1*	17 ± *4*	14 ± *1*	15 ± *5*	33 ± *9*	39 ± *6*	138 ± *5*	142 ± *24*	185 ± *6*	212 ± *22*	476 ± *23*	560 ± *10*
HCD[#] mean ± *SD*	17 ± *5.33*	20 ± *6.23*	13 ± *3.38*	18 ± *4.05*	31 ± *5.45*	39 ± *6.53*	139 ± *17.30*	147 ± *21.78*	380 ± *42.63*	441 ± *50.20*	273 ± *39.53*	322 ± *54.45*
[range]	*9 - 38*	*10 - 46*	*5 - 26*	*8 - 31*	*16 - 55*	*19 - 59*	*93 - 205*	*92 - 234*	*255 - 446*	*285 - 512*	*188 - 339*	*216 - 440*
Vehicle control												
DMSO mean ± *SD*	16 ± *5*	18 ± *3*	15 ± *2*	15 ± *2*	35 ± *5*	38 ± *2*	135 ± *13*	148 ± *18*	182 ± *12*	193 ± *21*	480 ± *34*	531 ± *37*
HCD[#] mean ± *SD*	17 ± *5.17*	21 ± *5.82*	13 ± *3.12*	17 ± *3.90*	30 ± *5.59*	39 ± *6.34*	130 ± *18.79*	155 ± *22.54*	374 ± *47.29*	406 ± *44.26*	251 ± *37.68*	281 ± *44.48*
[range]	*9 - 39*	*8 - 41*	*6 - 25*	*9 - 35*	*13 - 59*	*20 - 60*	*89 - 224*	*92 - 218*	*240 - 454*	*268 - 506*	*157 - 312*	*174 - 358*
Test item [µg/plate]												
3 mean ± *SD*	14 ± *5*	16 ± *4*	14 ± *1*	16 ± *3*	28 ± *4*	37 ± *6*	138 ± *18*	157 ± *14*	193 ± *17*	197 ± *26*	486 ± *27*	536 ± *22*
10 mean ± *SD*	18 ± *5*	17 ± *3*	15 ± *2*	16 ± *4*	33 ± *11*	37 ± *6*	143 ± *10*	145 ± *3*	202 ± *3*	210 ± *22*	477 ± *34*	493 ± *29*
33 mean ± *SD*	13 ± *2*	16 ± *3*	16 ± *2*	16 ± *1*	29 ± *2*	40 ± *3*	135 ± *2*	157 ± *14*	188 ± *5*	212 ± *42*	499 ± *38*	545 ± *22*
100 mean ± *SD*	14 ± *0*	16 ± *2*	14 ± *2*	16 ± *4*	29 ± *5*	37 ± *7*	120 ± *10*	149 ± *1*	189 ± *23*	192 ± *12*	494 ± *27*	489 ± *31*
333 mean ± *SD*	15 ± *2*	19 ± *3*	16 ± *2*	18 ± *3*	31 ± *3*	35 ± *6*	128 ± *7*	159 ± *14*	207 ± *11*	204 ± *25*	484 ± *20*	196 ± *15*
1000 mean ± *SD*	14 ± *3*	17 ± *6*	12 ± *4*	16 ± *1*	30 ± *6*	33 ± *5*	138 ± *10*	140 ± *12*	170 ± *15*	179 ± *14*	451 ± *22*	517 ± *26*
2500 mean ± *SD*	11 ± *3*	15 ± *3*	13 ± *3*	14 ± *1*	24 ± *5*	32 ± *3*	122 ± *8*	132 ± *7*	167 ± *31*	189 ± *29*	428 ± *9*	456 ± *23*
5000 mean ± *SD*	10 ± *2*	20 ± *4*	14 ± *1*	18 ± *3*	27 ± *4*	30 ± *2*	89 ± *12*	108 ± *7*	110 ± *16*	136 ± *6*	427 ± *19*	442 ± *4*
Positive control												
[§] mean	1508	302	68	378	302	1188	1574	2215	2705	2333	2997	1930

Table 5.4.1-14: *Salmonella Typhimurium* and *Escherichia Coli* Reverse Mutation Assay ([redacted] 2009), pre-experiment/first experiment

Preliminary experiment / Experiment 1: Standard plate test (SPT)												
Strain	**TA 1535**		**TA 1537**		**TA 98**		**TA 100**		**WP2 pKM101**		**WP2 uvrA (pKM101)**	
Metabol. activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
± SD	*± 52*	*± 50*	*± 4*	*± 105*	*± 4*	*± 9*	*± 118*	*± 144*	*± 106*	*± 145*	*± 332*	*± 138*
HCD[#] mean	2024	294	116	204	489	1455	2160	1839	3058	1920	3020	1972
± SD	*± 315.78*	*± 140.02*	*± 30.52*	*± 69.54*	*± 169.76*	*± 463.01*	*± 342.67*	*± 621.27*	*± 168.37*	*± 468.32*	*± 830.78*	*± 652.16*
[range]	*1041 - 3138*	*102 - 945*	*68 - 407*	*72 - 454*	*211 - 1694*	*200 - 3553*	*588 - 3379*	*404 - 3868*	*1369 - 3567*	*1163 - 3597*	*1522 - 4451*	*1043 - 3848*

§ Information on respective positive control is reported in Material and Method section 1.A.2

Historical control data generated from January - October 2008, representing approx. 600 experiments (approx. 150 experiments for WP2 uvrA pKM101; approx. 80 experiments for WP2 pKM101)

Table 5.4.1-15: *Salmonella Typhimurium* and *Escherichia Coli* Reverse Mutation Assay ([redacted] 2009), second experiment

Experiment 2: Pre-incubation test (PIT)												
Strain	**TA 1535**		**TA 1537**		**TA 98**		**TA 100**		**WP2 pKM101**		**WP2 uvrA (pKM101)**	
Metabol. activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Untreated												
mean	16	18	12	17	30	34	137	142	226	290	433	527
± SD	*± 4*	*± 5*	*± 3*	*± 2*	*± 4*	*± 4*	*± 32*	*± 13*	*± 10*	*± 16*	*± 33*	*± 20*
HCD[#] mean	17	20	13	18	31	39	139	147	380	441	273	322
± SD	*± 5.33*	*± 6.23*	*± 3.38*	*± 4.05*	*± 5.45*	*± 6.53*	*± 17.30*	*± 21.78*	*± 42.63*	*± 50.20*	*± 39.53*	*± 54.45*
[range]	*9 - 38*	*10 - 46*	*5 - 26*	*8 - 31*	*16 - 55*	*19 - 59*	*93 - 205*	*92 - 234*	*255 - 446*	*285 - 512*	*188 - 339*	*216 - 440*
Vehicle control												
DMSO mean	16	17	12	18	27	37	140	152	222	266	474	533
± SD	*± 4*	*± 5*	*± 3*	*± 1*	*± 2*	*± 3*	*± 20*	*± 16*	*± 8*	*± 13*	*± 7*	*± 33*
HCD[#] mean	17	21	13	17	30	39	130	155	374	406	251	281
± SD	*± 5.17*	*± 5.82*	*± 3.12*	*± 3.90*	*± 5.59*	*± 6.34*	*± 18.79*	*± 22.54*	*± 47.29*	*± 44.26*	*± 37.68*	*± 44.48*
[range]	*9 - 39*	*8 - 41*	*6 - 25*	*9 - 35*	*13 - 59*	*20 - 60*	*89 - 224*	*92 - 218*	*240 - 454*	*268 - 506*	*157 - 312*	*174 - 358*
Test item												
33 mean	16	17	15	19	26	35	145	144	256	274	456	555
± SD	*± 4*	*± 3*	*± 2*	*± 2*	*± 3*	*± 1*	*± 9*	*± 12*	*± 28*	*± 31*	*± 31*	*± 14*
100 mean	17	18	11	18	29	33	151	152	239	274	456	603

Table 5.4.1-15: *Salmonella Typhimurium* and *Escherichia Coli* Reverse Mutation Assay ([redacted] 2009), second experiment

Experiment 2: Pre-incubation test (PIT)												
Strain	**TA 1535**		**TA 1537**		**TA 98**		**TA 100**		**WP2 pKM101**		**WP2 uvrA (pKM101)**	
Metabol. activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
± SD	*± 4*	*± 2*	*± 3*	*± 2*	*± 4*	*± 4*	*± 7*	*± 6*	*± 7*	*± 21*	*± 6*	*± 29*
333 mean	16	19	12	18	28	36	143	147	241	249	456	577
± SD	*± 5*	*± 4*	*± 2*	*± 2*	*± 4*	*± 2*	*± 15*	*± 12*	*± 7*	*± 33*	*± 26*	*± 32*
1000 mean	16	12	13	16	29	35	130	139	235	270	437	546
± SD	*± 4*	*± 3*	*± 1*	*± 5*	*± 1*	*± 3*	*± 7*	*± 10*	*± 8*	*± 12*	*± 23*	*± 37*
2500 mean	12	13	11	17	25	36	116	142	231	255	419	483
± SD	*± 2*	*± 1*	*± 3*	*± 3*	*± 4*	*± 1*	*± 18*	*± 11*	*± 19*	*± 16*	*± 38*	*± 27*
5000 mean	9	10	10	15	21	22	102	97	140	228	358	466
± SD	*± 1*	*± 2*	*± 2*	*± 3*	*± 2*	*± 1*	*± 15*	*± 20*	*± 10*	*± 5*	*± 8*	*± 43*
Positive control												
§ mean	1521	297	80	237	366	1651	1673	1840	1657	1259	1777	2095
± SD	*± 275*	*± 16*	*± 3*	*± 11*	*± 31*	*± 162*	*± 255*	*± 169*	*± 34*	*± 7*	*± 67*	*± 20*
HCD# mean	2024	294	116	204	489	1455	2160	1839	3058	1920	3020	1972
± SD	*± 315.78*	*± 140.02*	*± 30.52*	*± 69.54*	*± 169.76*	*± 463.01*	*± 342.67*	*± 621.27*	*± 168.37*	*± 468.32*	*± 830.78*	*± 652.16*
[range]	*1041 - 3138*	*102 - 945*	*68 - 407*	*72 - 454*	*211 - 1694*	*200 - 3553*	*588 - 3379*	*404 - 3868*	*1369 - 5367*	*1163 - 3597*	*1522 - 4451*	*1043 - 3848*

§ Information on respective positive control is reported in Material and Method section I.A.2

Historical control data generated from January - October 2008, representing approx. 600 experiments (approx. 150 experiments for WP2 uvrA pKM101; approx. 80 experiments for WP2 pKM101)

III. CONCLUSION:

Based on the results of the present study and under the experimental conditions of the test, the test item is not mutagenic in the Ames test (standard plate and pre-incubation method) with and without metabolic activation.

3. Assessment and conclusion

<u>Assessment and conclusion by applicant</u>:

Negative for gene mutation in bacteria (*S. typhimurium* TA 100, TA 98, TA 1535 and TA 1537 and *E. coli* WP2 uvrA (pKM101) and WP2 pKM101) with and without metabolic activation.

The study was performed under GLP conditions and compliant with OECD guideline 471 (1997), without any deviations. The study is therefore considered valid and acceptable.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.4.1/008
Report author	[redacted]
Report year	2008
Report title	Evaluation of the mutagenic potential of the test substance Glyphosate Technical by reverse mutation assay in *Salmonella typhimurium* (Ames Test)
Report No	RF-3996.401.392.07
Document No	Not reported
Guidelines followed in study	OECD 471 (1997)
GLP	Yes
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	*S. typhimurium* strains TA 98, TA 100, TA 1535, TA 97a, and TA102 were exposed to glyphosate (batch: 20070606, purity: 98.05 %) in the presence and absence of metabolic activation (Aroclor 1254-induced rat liver S9 fraction). Based on the results of a preliminary cytotoxicity test in tester strain TA100, in which cytotoxicity was observed at 2500 µg/plate in the absence of metabolic activation, concentrations in the range of 1 - 1000 µg/plate were selected for the main mutation assay. Triplicates of all tester strains were exposed via the plate-incorporation method to the test item for 72 hours. Sterility controls (untreated), vehicle (sterile water) and positive controls were included. Following incubation, the number of revertant colonies was counted for each strain.
Short description of results:	There was no clear evidence for cytotoxicity in any tester strain up to the highest concentration (1000 µg/plate) in the presence or absence of S9 mix, based on the number of revertant colonies. In addition, the test substance did not promote an increase in the number of revertant colonies in any strain either in the presence or absence of S9 mix. A statistically significant increase in the number of revertant colonies was observed with TA98 at 1 and 10 µg/plate and with TA100 at 500 µg/plate, both with S9 mix. However, the values were not increased by a factor of at least 2 compared to the values of the solvent controls and there was no dose-response relationship evident, therefore the findings were considered to be without relevance. The spontaneous mutation rates of negative controls remained within the range of historical control data and appropriate positive controls induced a marked increase in the number of revertants, thus demonstrating the functionality and validity of the test system. Based on the results of the present study and under the conditions of the test, glyphosate is not mutagenic in the Ames pre-incubation test with and without metabolic activation.
Reasons for why the study is not considered relevant/reliable or not	No evidence of mutagenicity was obtained in the plate-incorporation assay up to 1000 µg/plate (highest concentration tested). As much lower concentrations were tested than in most

considered as key study:	other studies, the study was not considered as key study (and not accepted in RAR 2015).
Category study in AIR5 dossier (L-docs)	Category 3b

1. Information on the study

Data point	CA 5.4.1/009
Report author	[redacted]
Report year	2007
Report title	*Salmonella typhimurium* and *Escherichia coli* Reverse mutation assay with Glyphosate technical (NUP-05068)
Report No	1061401
Document No	Not reported
Guidelines followed in study	OECD 471 (1997) referenced as EEC Directive 92/69 Method B13/B14; Japanese MAFF (2005)
Deviations from current test guideline OECD 471 (1997)	No reporting of bacterial cell density.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

S. typhimurium strains TA 98, TA 100, TA 1535 and TA 1537, and *E. coli* strain WP2 uvrA were exposed to glyphosate technical (NUP-05068, batch: 200609062, purity: 95.1 %) in the presence and absence of metabolic activation (phenobarbital and β-naphthoflavone-induced rat liver S9 fraction) for 48 h. Untreated, vehicle (deionised water) and positive controls were included in each experiment. A pre-experiment was conducted to identify cytotoxic concentrations of the test item. Test item concentrations in the range of 3 to 5000 µg/plate did not show any cytotoxic effects in the presence and absence of metabolic activation. In the main mutation assay two independent experiments were performed, using the plate incorporation method (first experiment) with concentrations in the range of 3 to 5000 µg/plate and the preincubation method (second experiment) with concentrations in the range of 33 to 5000 µg/plate.

There was no substantial increase in the number of his^+ and trp^+ revertant colony numbers in any of the experiments in any of the five tester strains at any dose level, neither in the presence nor absence of metabolic activation. The spontaneous reversion rate with and without metabolic activation was demonstrated with vehicle controls. Appropriate positive controls validated the sensitivity of the test system and the functionality of the metabolic activity of the S9 mix. Based on the results of the present study, glyphosate is not mutagenic in the Ames test with and without metabolic activation under the experimental conditions of the test.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material: Glyphosate technical

Identification:	NUP-05068
Description:	Crystalline powder
Lot/Batch #:	200609062
Purity:	95.1 %
Stability of test compound:	The stability of the test item under storage conditions (at room temperature) was guaranteed until 14 Sep 2008. The stability of the test item in solvent (vehicle) was not indicated by the sponsor.
Solvent (vehicle) used:	Deionised water

2. Control materials:

Negative control:	Controls which remained untreated were included in each experiment.
Solvent (vehicle) control:	Deionised water
Solvent (vehicle)/final concentration:	0.1 mL per plate.
Positive controls:	Please refer to the table below.

Strain	Metabolic activation	Mutagen	Solvent	Conc. [µg/plate]
S. typhimurium strains				
TA 100	-S9	Sodium azide (NaN_3)	Water	10.0
	+S9	2-Aminoanthracene (2-AA)*	DMSO	2.5
TA 1535	-S9	Sodium azide (NaN_3)	Water	10.0
	+S9	2-Aminoanthracene (2-AA)*	DMSO	2.5
TA 98	-S9	4-Nitro-o-phenylene-diamine (4-NOPD)	DMSO	10
	+S9	2-Aminoanthracene (2-AA)*	DMSO	2.5
TA 1537	-S9	4-Nitro-o-phenylene-diamine (4-NOPD)	DMSO	50.0
	+S9	2-Aminoanthracene (2-AA)*	DMSO	2.5
E. coli strain				
WP2 uvrA	-S9	Methylmethane sulfonate (MMS)	Water	3.0
	+S9	2-Aminoanthracene (2-AA)*	DMSO	10.0

* The functionality of the S9 mix batch used was additionally checked with benzo(a)pyrene and showed the expected results

3. Metabolic activation:

S9 mix was obtained from livers of 8 – 12 weeks old male Wistar HanIbm rats, weighing approx. 220 - 320 g. The animals received intraperitoneal injections of phenobarbital (80 mg/kg bw) and oral administration of β-naphthoflavone (80 mg/kg bw) on three consecutive days. The livers were prepared 24 h after the last treatment. The S9 mix was thawed prior to each experiment and co-factor was immediately added to prepare S9 mix containing the following components:

S9 mix component	Concentration	Unit
Sodium phosphate buffer (pH 7.4)	100	mM
KCl	33	mM
NADPH-generating system		
Glucose 6-phosphate	5	mM
NADP	5	mM
$MgCl_2$	8	mM
S9	10	% (v/v)

4. Test organisms:

Tester strains				Bacteria batch checked for	
S. typhimurium		***E.coli***			
TA 98	✓	WP2 uvrA	✓	deep rough character (rfa)	✓
TA 100	✓	WP2 uvrA (pKM101)		ampicillin resistance (R factor plasmid)	✓
TA 1535	✓			UV-light sensitivity (absence of uvrB and uvrA genes in *S. typhimurium* and *E. coli* strains, respectively)	✓
TA 1537	✓				
TA 102					
TA 1538				Histidine and tryptophan auxotrophy (automatically via the spontaneous rate)	✓

5. Test concentrations:

(a) Preliminary cytotoxicity assay:

Plate incorporation test ± S9 mix:	
Concentrations:	*3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate*
Tester strains:	TA 1535, TA 1537, TA 98, TA 100 and WP2 uvrA
Replicates:	*Triplicates*

(b) Mutation assays:

Plate incorporation test ± S9 mix:	
Concentrations:	*3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate*
Tester strains:	TA 1535, TA 1537, TA 98, TA 100 and WP2 uvrA
Replicates:	*Triplicates*
Pre-incubation test ± S9 mix:	
Concentrations:	*33, 100, 333, 1000, 2500 and 5000 µg/plate*
Tester strains:	TA 1535, TA 1537, TA 98, TA 100 and WP2 uvrA
Replicates:	*Triplicates*

B: STUDY DESIGN AND METHODS

1. Dates of experimental work: 15 – 25 Jan 2007
Finalisation date: 16 Mar 2007

2. Standard plate test (plate-incorporation test, SPT):
An aliquot of 0.1 mL test solution or vehicle/positive control, an aliquot of 0.1 mL fresh bacterial culture and 0.5 mL S9 mix (in tests with metabolic activation) or 0.5 mL of S9 substitution buffer (in tests without metabolic activation) were added to 2 mL of molten agar (supplemented with 10.5 mg/L L-histidine + 12.2

mg/L biotin or 2.5 mg/L tryptophan). The mixture was thoroughly shaken and poured onto minimal agar plates. Each concentration and the controls were tested in triplicates. After incubation at 37 °C for at least 48 h in the dark, the background bacterial lawn was examined and the bacterial colonies (his^+ or trp^+ revertants) were counted.

3. Pre-incubation test (PIT):
0.1 mL of test solution or vehicle/positive control, 0.1 mL pre-cultured bacterial suspension and 0.5 mL of S9 mix (in tests with metabolic activation) or 0.5 mL of S9 mix substitution buffer (in tests without metabolic activation) were mixed in a test tube and shaken at 37 °C for 60 minutes. After pre-incubation 2 mL of molten overlay agar was added to each test tube. Each concentration and the controls were tested in triplicates. The mixture was poured on selective agar plates. After solidification the plates were incubated upside down for at least 48 h at 37°C in the dark. Subsequently, the number of bacterial colonies (his^+ or trp^+ revertants) were counted.

4. Cytotoxicity
Toxicity was detected by a

- reduction in the number of spontaneous revertants
- clearing or diminution of the background lawn (= reduced his^- or trp^- background growth)

and recorded for all test groups both with and without S9 mix in all experiments.

5. Statistics
Results were judged without statistical analysis.

6. Acceptance criteria
The test was valid if

- There was a regular background growth in the negative and solvent control.
- The spontaneous reversion rates in the negative and solvent control were in the range of the laboratory's historical data.
- The positive control substances produced a significant increase in mutant colony frequencies.

7. Evaluation criteria
A test item was considered as a mutagen if a biologically relevant increase in the number of revertants exceeding the threshold of twice (strains TA 98, TA 100, and WP2 uvrA) or thrice (strains TA 1535 and TA 1537) the colony count of the corresponding solvent control was observed.
A dose dependent increase was considered biologically relevant if the threshold was exceeded at more than one concentration.
An increase exceeding the threshold at only one concentration was judged as biologically relevant if reproduced in an independent second experiment. A dose dependent increase in the number of revertant colonies below the threshold was regarded as an indication of a mutagenic potential if reproduced in an independent second experiment. However, whenever the colony counts remained within the historical range of negative and solvent controls such an increase was not considered biologically relevant.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS
Analytical determinations were not performed in the study, as not required by the test guideline.

B. CYTOTOXICITY
In the preliminary toxicity test, the test substance did not show any cytotoxicity to any strain up to the highest dose of 5000 µg/plate with and without metabolic activation.
In the second experiment of the main mutation assay toxicity, a reduction in the number of revertant colonies, was observed in tester strain TA 1537 at 5000 µg/plate in the absence of metabolic activation.

C. SOLUBILITY

There was no precipitation of the test substance observed up to the highest dose tested.

D. MUTATION ASSAY

There was no relevant increase in the number of his^+ or trp^+ revertants observed in any experiment at any tested concentration, neither in the presence nor in the absence of metabolic activation. In addition, there was no dose-dependent increase in mutation rates in the range below the generally acknowledged border of biological relevance.

The number of revertants induced by the vehicle control was slightly above the range of historical control data (except for strain TA 98). The finding was judged to be based on biologically irrelevant fluctuations and was considered to have no impact on the outcome of the study. The absence of impact was confirmed by the negative results obtained with the test solution at all tested concentrations in all strains.

Appropriate positive control compounds induced a marked increase in the number of revertants, demonstrating the validity of the test system and the functionality of the S9 mix.

Table 5.4.1-16: *Salmonella typhimurium* and *Escherichia coli* Reverse mutation assay with Glyphosate technical ([redacted] 2007), first experiment

Experiment 1: Standard plate test (SPT)										
Strain	**TA 1535**		**TA 1537**		**TA 98**		**TA 100**		**WP2 uvrA**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Untreated										
mean ± *SD*	18 ± *8*	24 ± *4*	13 ± *4*	19 ± *6*	20 ± *8*	31 ± *3*	147 ± *18*	162 ± *7*	68 ± *9*	81 ± *14*
HCD# mean ± *SD*	20.4 ± *4.4*	24.2 ± *5.5*	11.6 ± *4.0*	17.1 ± *5.4*	30.2 ± *6.6*	39 ± *7.5*	138.2 ± *21.6*	150.1 ± *24.2*	55.9 ± *8.6*	65.6 ± *10.4*
[range]	*11 - 31*	*10 - 38*	*4 - 28*	*7 - 34*	*16 - 60*	*18 - 64*	*86 - 216*	*96 - 214*	*36 - 76*	*33 - 91*
Vehicle control										
Water mean ± *SD*	23 ± *4*	22 ± *5*	10 ± *2*	20 ± *7*	21 ± *2*	30 ± *7*	134 ± *8*	163 ± *8*	73 ± *7*	90 ± *11*
HCD# mean ± *SD*	20.8 ± *4.7*	24.7 ± *5.9*	11.2 ± *3.7*	16.2 ± *5.0*	28.1 ± *6.1*	37.9 ± *7.4*	130.7 ± *20.8*	147.0 ± *25.5*	55.8 ± *7.2*	63.9 ± *9.1*
[range]	*9 - 35*	*7 - 43*	*5 - 28*	*6 - 36*	*15 - 49*	*20 - 57*	*87 - 197*	*84 - 255*	*31 - 74*	*34 - 84*
Test item [µg/plate]										
3 mean ± *SD*	25.3 ± *3*	27 ± *3*	11 ± *1*	21 ± *3*	24 ± *4*	35 ± *2*	127 ± *9*	157 ± *6*	65 ± *9*	90 ± *18*
10 mean ± *SD*	16 ± *5*	22 ± *6*	13 ± *5*	20 ± *5*	24 ± *5*	44 ± *4*	132 ± *14*	153 ± *9*	79 ± *10*	97 ± *5*
33 mean ± *SD*	16 ± *6*	19 ± *5*	11 ± *4*	20 ± *3*	27 ± *2*	30 ± *2*	127 ± *7*	157 ± *3*	86 ± *5*	93 ±*17*
100 mean ± *SD*	15 ± *2*	22 ± *1*	11 ± *4*	20 ± *4*	26 ± *8*	33 ± *9*	126 ± *14*	142 ± *15*	70 ± *9*	83 ± *9*
333 mean	23	22	9	15	25	38	145	147	63	88

Table 5.4.1-16: *Salmonella typhimurium* and *Escherichia coli* Reverse mutation assay with Glyphosate technical ([redacted] 2007), first experiment

Experiment 1: Standard plate test (SPT)										
Strain	**TA 1535**		**TA 1537**		**TA 98**		**TA 100**		**WP2 uvrA**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
± SD	*± 3*	*± 11*[#]	*± 2*	*± 6*	*± 5*	*± 5*	*± 9*	*± 10*	*± 3*	*± 19*
1000 mean	17	27	12	20	24	31	140	151	69	84
± SD	*± 4*	*± 2*	*± 3*	*± 4*	*± 2*	*± 7*	*± 8*	*± 4*	*± 10*	*± 9*
2500 mean	19	23	11	15	20	31	110	149	69	81
± SD	*± 4*	*± 3*	*± 5*	*± 8*	*± 9*	*± 2*	*± 13*	*± 24*	*± 5*	*± 10*
5000 mean	18	24	14	15	22	22	106	129	57	69
± SD	*± 1*	*± 2*	*± 9*	*± 2*	*± 6*	*± 8*	*± 11*	*± 1*	*± 2*	*± 2*
Positive control										
[§] mean	1885	398	100	301	378	1472	2060	2778	1558	269
± SD	*± 55*	*± 21*	*± 4*	*± 7*	*± 10*	*± 116*	*± 80*	*± 91*	*± 86*	*± 15*
HCD[#] mean	1422	332.0	99.8	276.8	439.0	1839.4	2083.1	2372.9	991.0	319.4
± SD	*± 464.7*	*± 95.3*	*± 32.5*	*± 132.6*	*± 155.2*	*± 898.6*	*± 281.3*	*± 958.4*	*± 522.9*	*± 84.8*
[range]	*781 - 4900*	*107 - 695*	*53 - 425*	*59 - 746*	*176 - 1818*	*407 - 4891*	*616 - 2872*	*417 - 5230*	*249 - 1810*	*211 - 9.30*

[§] Information on respective positive control is reported in Material and Method section I.A.2
[#] Historical control data from May 2005 – June 2006 representing approx. 200 experiments
[#:] Contamination, analysis not possible in plate no. 2, mean of two plates

Table 5.4.1-17: *Salmonella typhimurium* and *Escherichia coli* Reverse mutation assay with Glyphosate technical ([redacted] 2007), second experiment

Experiment 2: Pre-incubation test (PIT)										
Strain	**TA 1535**		**TA 1537**		**TA 98**		**TA 100**		**WP2 uvrA**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Untreated										
mean	17	20	13	17	29	30	143	185	54	68
± SD	*± 4*	*± 1*	*± 8*	*± 6*	*± 9*	*± 7*	*± 1*	*± 7*	*± 11*	*± 5*
HCD[#] mean	20.4	24.2	11.6	17.1	30.2	39	138.2	150.1	55.9	65.6
± SD	*± 4.4*	*± 5.5*	*± 4.0*	*± 5.4*	*± 6.6*	*± 7.5*	*± 21.6*	*± 24.2*	*± 8.6*	*± 10.4*
[range]	*11 - 31*	*10 - 38*	*4 - 28*	*7 - 34*	*16 - 60*	*18 - 64*	*86 - 216*	*96 - 214*	*36 - 76*	*33 - 91*
Vehicle control										
Water mean	21	19	11	11	24	28	123	186	52	73
± SD	*± 4*	*± 4*	*± 4*	*± 2*	*± 4*	*± 9*	*± 3*	*± 9*	*± 5*	*± 7*
HCD[#] mean	20.8	24.7	11.2	16.2	28.1	37.9	130.7	147.0	55.8	63.9
± SD	*± 4.7*	*± 5.9*	*± 3.7*	*± 5.0*	*± 6.1*	*± 7.4*	*± 20.8*	*± 25.5*	*± 7.2*	*± 9.1*
[range]	*9 - 35*	*7 - 43*	*5 - 28*	*6 - 36*	*15 - 49*	*20 - 57*	*87 - 197*	*84 - 255*	*31 - 74*	*34 - 84*

Table 5.4.1-17: *Salmonella typhimurium* and *Escherichia coli* Reverse mutation assay with Glyphosate technical ([redacted] 2007), second experiment

Experiment 2: Pre-incubation test (PIT)										
Strain	**TA 1535**		**TA 1537**		**TA 98**		**TA 100**		**WP2 uvrA**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Test item [µg/plate]										
33 mean	16	15	14	18	26	31	133	180	61	81
± *SD*	± *2*	± *5*	± *2*	± *3*	± *4*	± *6*	± *3*	± *9*	± *4*	± *10*
100 mean	19	23	10	11	28	29	140	169	53	66
± *SD*	± *6*	± *4*	± *3*	± *2*	± *11*	± *2*	± *4*	± *37*	± *1*	± *3*
333 mean	19	22	10	15	26	30	140	191	44	69
± *SD*	± *5*	± *6*	± *4*	± *5*	± *2*	± *2*	± *11*	± *29*	± *4*	± *5*
1000 mean	19	23	9	15	28	29	143	192	50	67
± *SD*	± *6*	± *6*	± *3*	± *6*	± *8*	± *6*	± *13*	± *17*	± *7*	± *9*
2500 mean	17	22	10	11	26	23	112	163	48	53
± *SD*	± *2*	± *6*	± *5*	± *3*	± *2*	± *8*	± *4*	± *5*	± *4*	± *3*
5000 mean	11	23	4	11	20	24	95	126	25	55
± *SD*	± *3*	± *9*	± *3*	± *4*	± *9*	± *2*	± *10*	± *4*	± *12*	± *12*
Positive control										
[§] mean	1934	365	112	179	530	764	1861	2004	568	261
± *SD*	± *82*	± *17*	± *7*	± *18*	± *11*	± *77*	± *100*	± *334*	± *27*	± *20*
HCD[#] mean	1422.0	332.0	99.8	276.8	439.0	1839.4	2083.1	2372.9	991.0	319.4
± *SD*	± *464.7*	± *95.3*	± *32.5*	± *132.6*	± *155.2*	± *898.6*	± *281.3*	± *958.4*	± *522.9*	± *84.8*
[range]	*781 - 4900*	*107 - 695*	*33 - 425*	*59 - 746*	*176 - 1818*	*407 - 4891*	*616 - 2872*	*417 - 5230*	*249 - 1810*	*211 - 9.30*

[§] Information on respective positive control is reported in Material and Method section I.A.2
[#] Historical control data from May 2005 – June 2006 representing approx. 200 experiments

III. CONCLUSION

In conclusion, under the experimental conditions reported, the test item (NUP-05068) did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used. There is no indication for mutagenicity in bacteria (Ames test) with and without metabolic activation.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Negative for gene mutation in bacteria (*S. typhimurium* TA 100, TA 98, TA 1535 and TA 1537 and *E. coli* WP2 uvrA) with and without metabolic activation.

The study was performed according to OECD guideline 471 (1997) and under GLP conditions. There were only minor deviations when compared to the guideline, which were considered to not compromise the validity of the study. The study is therefore considered valid and acceptable.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.4.1/010
Report author	[redacted]
Report year	2007
Report title	*Salmonella typhimurium* and *Escherichia coli* Reverse mutation assay with Glyphosate technical (NUP-05070)
Report No	1061402
Document No	Not reported
Guidelines followed in study	OECD 471 (1997) referenced as EEC Directive 92/69 Method B13/B14; Japanese MAFF (2005)
Deviations from current test guideline OECD 471 (1997)	No reporting of bacterial cell density.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

S. typhimurium strains TA 98, TA 100, TA 1535 and TA 1537, and *E. coli* strain WP2 uvrA were exposed to glyphosate technical (NUP-05070, batch: 20060901, purity: 97.7 %) in the presence and absence of metabolic activation (phenobarbital and β-naphthoflavone-induced rat liver S9 fraction) for 48 h. Untreated, vehicle (deionised water) and positive controls were included in each experiment. A pre-experiment was conducted to identify cytotoxic concentrations of the test item. In a standard plate test (plate incorporation method), cytotoxicity was observed as a reduced bacterial background lawn for strain TA 1537 at 333 – 5000 µg/plate in the presence of S9 mix, for strain TA 100 at ≥ 2500 µg/plate in the presence of S9 mix and for strain WP2 uvrA at 5000 µg/plate in the presence and absence of S9 mix.

The pre-experiment was designated experiment 1 of the main mutation assay. A second experiment was performed in the main mutation assay using the preincubation method and concentrations in the range of 33 to 5000 µg/plate. Both experiments were performed with triplicates. There was no reduced bacterial growth in the second experiment. Toxicity, evident as a reduced number of revertant colonies, was observed for strain WP2 uvrA at 5000 µg/plate without S9 mix in the first experiment and for strains TA 98 and WP2 uvrA at 5000 µg/plate in the presence and absence of S9 mix.

There was no substantial increase in the number of his^+ and trp^+ revertant colony numbers in any of the five tester strains at any dose level, neither in the presence nor absence of metabolic activation.

The spontaneous reversion rate with and without metabolic activation of the solvent (vehicle) control was within the range of historical control data. The sensitivity of the test system and the functionality of the S9 mix was demonstrated with appropriate positive controls. Based on the results of the present study and under the experimental conditions of the test, glyphosate is not mutagenic in the Ames test with and without metabolic activation.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material: Glyphosate technical

Identification:	NUP-05070
Description:	Crystalline powder
Lot/Batch #:	20060901
Purity:	97.7 %
Stability of test compound:	The stability of the test item under storage conditions (at room temperature) was guaranteed until 01 Sep 2008. The stability of the test item in solvent (vehicle) was guaranteed for 30 days at room temperature.
Solvent (vehicle) used:	Deionised water

2. Control materials:

Negative control:	Controls which remained untreated were included in each experiment.
Solvent (vehicle) control:	Deionised water
Solvent (vehicle)/final concentration:	0.1 mL per plate.
Positive controls:	Please refer to the table below.

Strain	Metabolic activation	Mutagen	Solvent	Conc. [µg/plate]
S. typhimurium strains				
TA 100	-S9	Sodium azide (NaN_3)	Water	10.0
	+S9	2-Aminoanthracene (2-AA)*	DMSO	2.5
TA 1535	-S9	Sodium azide (NaN_3)	Water	10.0
	+S9	2-Aminoanthracene (2-AA)*	DMSO	2.5
TA 98	-S9	4-nitro-o-phenylene-diamine (4-NOPD)	DMSO	10
	+S9	2-Aminoanthracene (2-AA)*	DMSO	2.5
TA 1537	-S9	4-nitro-o-phenylene-diamine (4-NOPD)	DMSO	50.0
	+S9	2-Aminoanthracene (2-AA)*	DMSO	2.5
E. coli strain				
WP2 uvrA	-S9	Methylmethane sulfonate (MMS)	Water	3.0
	+S9	2-Aminoanthracene (2-AA)*	DMSO	10.0
* The functionality of the S9 mix batch used was additionally checked with benzo(a)pyrene and showed the expected results				

3. Metabolic activation:

S9 mix was obtained from the livers of 8 – 12 weeks old male Wistar HanIbm rats, weighing approx. 220 - 320 g. The animals received intraperitoneal injections of phenobarbital (80 mg/kg bw) and oral administration of β-naphthoflavone (80 mg/kg bw) on three consecutive days. The livers were prepared 24 h after the last treatment. The S9 mix was thawed prior to each experiment and co-factor was immediately added to prepare S9 mix containing the following components:

S9 mix component	Concentration	Unit
Sodium phosphate buffer (pH 7.4)	100	mM
KCl	33	mM
NADPH-generating system		
Glucose 6-phosphate	5	mM
NADP	5	mM
$MgCl_2$	8	mM
S9	10	% (v/v)

4. Test organisms:

Tester strains				Bacteria batch checked for	
S. typhimurium		***E. coli***			
TA 98	✓	WP2 uvrA	✓	deep rough character (rfa)	✓
TA 100	✓	WP2 uvrA (pKM101)		ampicillin resistance (R factor plasmid)	✓
TA 1535	✓			UV-light sensitivity (absence of uvrB and uvrA genes in *S. typhimurium* and *E. coli* strains, respectively)	✓
TA 1537	✓				
TA 102					
TA 1538				Histidine and tryptophan auxotrophy (automatically via the spontaneous rate)	✓

5. Test concentrations:

(a) Preliminary cytotoxicity assay/Experiment 1 of the main mutation assay

Plate incorporation test ± S9 mix:	
Concentrations:	*3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate*
Tester strains:	TA 1535, TA 1537, TA 98, TA 100 and WP2 uvrA
Replicates:	*Triplicates*

(b) Experiment 2 of the main mutation assay

Pre-incubation test ± S9 mix:	
Concentrations:	*33, 100, 333, 1000, 2500 and 5000 µg/plate*
Tester strains:	TA 1535, TA 1537, TA 98, TA 100 and WP2 uvrA
Replicates:	*Triplicates*

B: STUDY DESIGN AND METHODS

1. Dates of experimental work: 16 – 25 Jan 2007
Finalisation date: 16 Mar 2007

2. Standard plate test (plate-incorporation test, SPT):
An aliquot of 0.1 mL test solution or vehicle/positive control, an aliquot of 0.1 mL fresh bacterial culture

and 0.5 mL S9 mix (in tests with metabolic activation) or 0.5 mL of S9 substitution buffer (in tests without metabolic activation) were added to 2 mL of molten agar (supplemented with 10.5 mg/L L-histidine + 12.2 mg/L biotin or 2.5 mg/L tryptophan). The mixture was thoroughly shaken and poured onto minimal agar plates. Each concentration and the controls were tested in triplicates. After incubation at 37 °C for at least 48 h in the dark, the background bacterial lawn was examined and the bacterial colonies (his^+ or trp^+ revertants) were counted.

3. Pre-incubation test (PIT):
0.1 mL of test solution or vehicle/positive control, 0.1 mL pre-cultured bacterial suspension and 0.5 mL of S9 mix (in tests with metabolic activation) or 0.5 mL of S9 mix substitution buffer (in tests without metabolic activation) were mixed in a test tube and shaken at 37 °C for 60 minutes. After pre-incubation 2 mL of molten overlay agar was added to each test tube. Each concentration and the controls were tested in triplicates. The mixture was poured on selective agar plates. After solidification the plates were incubated upside down for at least 48 h at 37°C in the dark. Subsequently, the number of bacterial colonies (his^+ or trp^+ revertants) were counted.

4. Cytotoxicity
Toxicity was detected by a

- reduction in the number of spontaneous revertants
- clearing or diminution of the background lawn (= reduced his^- or trp^- background growth)

and recorded for all test groups both with and without S9 mix in all experiments.

5. Statistics
Results were judged without statistical analysis.

6. Acceptance criteria
The test was valid if

- There was a regular background growth in the negative and solvent control.
- The spontaneous reversion rates in the negative and solvent control were in the range of the laboratory's historical data.
- The positive control substances produced a significant increase in mutant colony frequencies.

7. Evaluation criteria
A test item was considered as a mutagen if a biologically relevant increase in the number of revertants exceeding the threshold of twice (strains TA 98, TA 100, and WP2 uvrA) or thrice (strains TA 1535 and TA 1537) the colony count of the corresponding solvent control was observed.
A dose dependent increase was considered biologically relevant if the threshold is exceeded at more than one concentration.
An increase exceeding the threshold at only one concentration was judged as biologically relevant if reproduced in an independent second experiment. A dose dependent increase in the number of revertant colonies below the threshold was regarded as an indication of a mutagenic potential if reproduced in an independent second experiment. However, whenever the colony counts remained within the historical range of negative and solvent controls such an increase was not considered biologically relevant.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS
Analytical determinations were not performed in the study, as not required by the test guideline.

B. CYTOTOXICITY
Cytotoxicity, indicated by a reduced background lawn was evident in the pre-test/first experiment in strain

TA 1537 at concentrations in the range of 333 – 5000 µg/plate in the presence of S9 mix, in strain TA 100 at 2500 – 5000 µg/plate in the presence of S9 mix and in strain WP2 uvrA at 5000 µg/plate both in the presence and absence of S9 mix. There was no reduced bacterial background growth in the second experiment.

Toxic effects evident as a reduction in the number of revertant colonies was observed for strain WP2 uvrA at 5000 µg/plate without S9 mix in the first experiment and for strains TA 98 and WP2 uvrA at 5000 µg/plate with and without S9 mix in the second experiment.

C. SOLUBILITY

There was no precipitation of the test substance observed up to the highest dose tested.

D. MUTATION ASSAY

No relevant increase in the number of his^+ or trp^+ revertants was observed in any experiment at any tested concentration either in the presence or absence of metabolic activation. In addition, there was no dose-dependent increase in mutation rates in the range below the generally acknowledged border of biological relevance.

The number of revertants detected in the vehicle control was within expected range for each strain, thus demonstrating an acceptable experimental performance.

Appropriate positive control compounds induced a marked increase in the number of revertants, demonstrating the validity of the test system and the functionality of the S9 mix.

Table 5.4.1-18: *Salmonella typhimurium* and *Escherichia coli* Reverse mutation assay with Glyphosate technical ([redacted] 2007), pre-experiment/first experiment

Pre-experiment/Experiment 1: Standard plate test (SPT)										
Strain	**TA 1535**		**TA 1537**		**TA 98**		**TA 100**		**WP2 uvrA**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Untreated										
mean ± *SD*	20 ± *0*	20 ± *3*	7 ± *2*	9 ± *0*	29 ± *3*	30 ± *5*	131 ± *5*	138 ± *5*	57 ± *5*	68 ± *9*
HCD# mean ± *SD*	20.4 ± *4.4*	24.2 ± *5.5*	11.6 ± *4.0*	17.1 ± *5.4*	30.2 ± *6.6*	39 ± *7.5*	138.2 ± *21.6*	150.1 ± *24.2*	55.9 ± *8.6*	65.6 ± *10.4*
[range]	*11 - 31*	*10 - 38*	*4 - 28*	*7 - 34*	*16 - 60*	*18 - 64*	*86 - 216*	*96 - 214*	*36 - 76*	*33 - 91*
Vehicle control										
Water mean ± *SD*	18 ± *1*	24 ± *3*	14 ± *2*	13 ± *9*	27 ± *4*	33 ± *7*	131 ± *9*	140 ± *11*	61 ± *12*	78 ± *10*
HCD# mean ± *SD*	20.8 ± *4.7*	24.7 ± *5.9*	11.2 ± *3.7*	16.2 ± *5.0*	28.1 ± *6.1*	37.9 ± *7.4*	130.7 ± *20.8*	147.0 ± *25.5*	55.8 ± *7.2*	63.9 ± *9.1*
[range]	*9 - 35*	*7 - 43*	*5 - 28*	*6 - 36*	*15 - 49*	*20 - 57*	*87 - 197*	*84 - 255*	*31 - 74*	*34 - 84*
Test item [µg/plate]										
3 mean ± *SD*	16 ± *1*	20 ± *5*	14 ± *4*	8 ± *2*	27 ± *4*	36 ± *0*	128 ± *19*	138 ± *11*	45 ± *4*	66 ± *5*
10 mean ± *SD*	21 ± *2*	21 ± *1*	12 ± *2*	9 ± *2*	31 ± *2*	37 ± *3*	138 ± *11*	149 ± *7*	51 ± *4*	68 ± *9*

Table 5.4.1-18: *Salmonella typhimurium* and *Escherichia coli* Reverse mutation assay with Glyphosate technical ([redacted] 2007), pre-experiment/first experiment

Pre-experiment/Experiment 1: Standard plate test (SPT)										
Strain	**TA 1535**		**TA 1537**		**TA 98**		**TA 100**		**WP2 uvrA**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
33 mean	21	24	13	22	29	36	138	133	64	69
± *SD*	± *2*	± *4*	± *1*	± *5*	± *3*	± *7*	± *5*	± *6*	± *20*	± *5*
100 mean	17	23	15	21	31	41	127	147	56	65
± *SD*	± *6*	± *8*	± *6*	± *1*	± *4*	± *7*	± *16*	± *6*	± *7*	± *6*
333 mean	14	20	9 R	13	28	37	117	141	58	67
± *SD*	± *5*	± *7*	± *1*	± *3*	± *9*	± *6*	± *4*	± *10*	± *3*	± *5*
1000 mean	13	17	9 R	18	30	37	130	150	45	63
± *SD*	± *3*	± *5*	± *6*	± *5*	± *6*	± *5*	± *17*	± *10*	± *7*	± *6*
2500 mean	18	21	9 R	11	25	31	119 R	150	43	65
± *SD*	± *4*	± *6*	± *3*	± *4*	± *2*	± *3*	± *10*	± *5*	± *3*	± *3*
5000 mean	20	16	9 R	16	23	31	98 R	123	27 R	50 R
± *SD*	± *2*	± *7*	± *2*	± *3*	± *1*	± *6*	± *5*	± *39*	± *9*	± *5*
Positive control										
§ mean	1935	309	82	81	503	1094	2447	1595	1324	241
± *SD*	± *62*	± *19*	± *5*	± *6*	± *3*	± *33*	± *87*	± *107*	± *69*	± *13*
HCD# mean	1422.0	332.0	99.8	276.8	439.0	1839.4	2083.1	2372.9	991.0	319.4
± *SD*	± *464.7*	± *95.3*	± *32.5*	± *132.6*	± *155.2*	± *898.6*	± *281.3*	± *958.4*	± *522.9*	± *84.8*
[range]	*781 - 4900*	*107 - 695*	*53 - 425*	*59 - 746*	*176 - 1818*	*407 - 4891*	*616 - 2872*	*417 - 5230*	*249 - 1810*	*211 - 9.30*

§ Information on respective positive control is reported in Material and Method section I.A.2
# Historical control data from May 2005 – June 2006 representing approx.. 200 experiments
R Reduced background growth

Table 5.4.1-19: *Salmonella typhimurium* and *Escherichia coli* Reverse mutation assay with Glyphosate technical ([redacted] 2007), second experiment

Experiment 2: Pre-incubation test (PIT)										
Strain	**TA 1535**		**TA 1537**		**TA 98**		**TA 100**		**WP2 uvrA**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Untreated										
mean	10 BM	9 BM	14	24	21 BM	43 BM	145	158	62	66
± *SD*	± *3*	± *3*	± *6*	± *5*	± *2*	± *3*	± *15*	± *20*	± *14*	± *11*
HCD #2 mean	20.4	24.2	11.6	17.1	30.2	39.0	138.2	150.1	55.9	65.6
± *SD*	± 4.4	± 5.5	± 4.0	± 5.4	± 6.6	± 7.5	± 21.6	± 24.2	± 8.6	± 10.4
[range]	*11 - 31*	*10 - 38*	*4 - 28*	*7 - 34*	*16 - 60*	*18 - 64*	*86 - 216*	*96 - 214*	*36 - 76*	*33 - 91*
Vehicle control										

Table 5.4.1-19: *Salmonella typhimurium* and *Escherichia coli* Reverse mutation assay with Glyphosate technical ([redacted] 2007), second experiment

Experiment 2: Pre-incubation test (PIT)										
Strain	**TA 1535**		**TA 1537**		**TA 98**		**TA 100**		**WP2 uvrA**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Water mean	9 [BM]	8 [BM]	16	24	21 [BM]	45 [BM]	130	211	53	69
± SD	*± 1*	*± 1*	*± 5*	*± 8*	*± 4*	*± 5*	*± 3*	*± 29*	*± 2*	*± 6*
HCD[#] mean	20.8	24.7	11.2	16.2	28.1	37.9	130.7	147.0	55.8	63.9
± SD	*± 4.7*	*± 5.9*	*± 3.7*	*± 5.0*	*± 6.1*	*± 7.4*	*± 20.8*	*± 25.5*	*± 7.2*	*± 9.1*
[range]	*9 - 35*	*7 - 43*	*5 - 28*	*6 - 36*	*15 - 49*	*20 - 57*	*87 - 197*	*84 - 255*	*31 - 74*	*34 - 84*
Test item [µg/plate]										
33 mean	8 [BM]	6 [BM]	13	24	16 [BM]	37 [BM]	131	180	54	80
± SD	*± 2*	*± 1*	*± 1*	*± 12*	*± 2*	*± 8*	*± 5*	*± 15*	*± 6*	*± 9*
100 mean	9 [BM]	11 [BM]	17	17	20 [BM]	38 [BM]	146	197	64	77
± SD	*± 1*	*± 4*	*± 7*	*± 3*	*± 4*	*± 8*	*± 3*	*± 17*	*± 2*	*± 9*
333 mean	10 [BM]	14 [BM]	18	18	18 [BM]	38 [BM]	152	183	65	67
± SD	*± 3*	*± 2*	*± 3*	*± 3*	*± 2*	*± 1*	*± 5*	*± 20*	*± 7*	*± 7*
1000 mean	7 [BM]	10 [BM]	16	19	18 [BM]	33 [BM]	150	156	49	64
± SD	*± 1*	*± 1*	*± 4*	*± 5*	*± 2*	*± 3*	*± 2*	*± 20*	*± 12*	*± 9*
2500 mean	8 [BM]	12 [BM]	14	22	14 [BM]	23 [BM]	137	163	34	68
± SD	*± 1*	*± 3*	*± 3*	*± 2*	*± 2*	*± 6*	*± 27*	*± 11*	*± 6*	*± 29*
5000 mean	9 [BM]	10 [BM]	10	18	12 [BM]	18 [BM]	127	130	30	49
± SD	*± 3*	*± 2*	*± 6*	*± 2*	*± 2*	*± 4*	*± 12*	*± 6*	*± 5*	*± 7*
Positive control										
[§] mean	2225	301	131	186	1012	1014	2234	1472	371	230
± SD	*± 22*	*± 45*	*± 23*	*± 13*	*± 105*	*± 104*	*± 37*	*± 220*	*± 75*	*± 16*
HCD[#] mean	1422.0	332.0	99.8	276.8	439.0	1839.4	2083.1	2372.9	991.0	319.4
± SD	*± 464.1*	*± 95.3*	*± 32.5*	*± 132.6*	*± 155.2*	*± 898.6*	*± 281.3*	*± 958.4*	*± 522.9*	*± 84.8*
[range]	*781 - 4900*	*107 - 695*	*53 - 425*	*59 - 746*	*176 - 1818*	*407 - 4891*	*616 - 2872*	*417 - 5230*	*249 - 1810*	*211 - 9.30*

[§] Information on respective positive control is reported in Material and Method section I.A.2
[#] Historical control data from May 2005 – June 2006 representing approx. 200 experiments
[B] Extensive bacterial growth
[M] Manual count

III. CONCLUSION:

According to the results of the present study and under the conditions of the test, the test item (NUP-05070) is not mutagenic in the Ames test (standard plate and pre-incubation method) with and without metabolic activation.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Negative for gene mutation in bacteria (*S. typhimurium* TA 100, TA 98, TA 1535 and TA 1537 and *E. coli* WP2 uvrA) with and without metabolic activation.

The study was performed according to OECD guideline 471 (1997) and under GLP conditions. There were only minor deviations when compared to the guideline, which were considered to not compromise the validity of the study. The study is therefore considered valid and acceptable.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.4.1/011
Report author	[redacted]
Report year	2007
Report title	*Salmonella typhimurium* and *Escherichia coli* Reverse mutation assay with Glyphosate technical (NUP-05067)
Report No	1061403
Document No	Not reported
Guidelines followed in study	OECD 471 (1997) referenced as EEC Directive 92/69 Method B13/B14; Japanese MAFF (2005)
Deviations from current test guideline OECD 471 (1997)	No reporting of bacterial cell density.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

This study was performed to investigate the potential of glyphosate technical (NUP-05070, batch: 0609-1, purity: 95.0 %) to induce gene mutations in *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98, and TA 100, and the *Escherichia coli* strain WP2 uvrA. All strains were exposed, to the test item, vehicle (deionised water), untreated and appropriate positive controls in the presence and absence of metabolic activation (phenobarbital and β-naphthoflavone-induced rat liver S9 fraction) for at least 48 h.
A pre-experiment was conducted to identify cytotoxic concentrations of the test item. In a standard plate test (plate incorporation method), the highest test item concentration (5000 µg/plate) induced cytotoxicity in strain WP2 uvrA in the absence of metabolic activation. The pre-experiment was designated experiment 1 of the main mutation assay and analysed for the number of revertant colonies. A second main mutation assay was performed using the pre-incubation method and concentrations in the range of 33 to 5000 µg/plate. Both experiments were performed with triplicates.

In the second experiment, cytotoxicity was observed at 5000 µg/plate for strain WP2 uvrA in the presence and absence of S9 mix.

No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with the test item at any dose level, in any of the experiments, neither in the presence nor absence

of metabolic activation. The spontaneous reversion rate with and without metabolic activation of the solvent control was within the range of historical control data. A marked increase in the number of revertant colonies was obtained with appropriate positive controls, demonstrating the sensitivity of the test system and the functionality of the S9 mix. Based on the results of the present study and under the experimental conditions of the test, glyphosate is not mutagenic in the Ames test with and without metabolic activation.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material: Glyphosate technical

Identification:	NUP-05070
Description:	Crystalline powder
Lot/Batch #:	0609-1
Purity:	95.0 %
Stability of test compound:	The stability of the test item under storage conditions (at room temperature) was guaranteed until 15 Aug 2008. The stability of the test item in solvent (vehicle) was guaranteed for 30 days at room temperature.
Solvent (vehicle)used:	Deionised water

2. Control materials:

Negative control:	Controls which remained untreated were included in each experiment.
Solvent (vehicle) control:	Deionised water
Solvent (vehicle)/final concentration:	0.1 mL per plate.
Positive controls:	Please refer to the table below.

Strain	Metabolic activation	Mutagen	Solvent	Conc. [µg/plate]
S. typhimurium strains				
TA 100	-S9	Sodium azide (NaN_3)	Water	10.0
	+S9	2-Aminoanthracene (2-AA)*	DMSO	2.5
TA 1535	-S9	Sodium azide (NaN_3)	Water	10.0
	+S9	2-Aminoanthracene (2-AA)*	DMSO	2.5
TA 98	-S9	4-nitro-o-phenylene-diamine (4-NOPD)	DMSO	10
	+S9	2-Aminoanthracene (2-AA)*	DMSO	2.5
TA 1537	-S9	4-nitro-o-phenylene-diamine (4-NOPD)	DMSO	50.0
	+S9	2-Aminoanthracene (2-AA)*	DMSO	2.5
E. coli strain				
WP2 uvrA	-S9	Methylmethane sulfonate (MMS)	Water	3.0
	+S9	2-Aminoanthracene (2-AA)*	DMSO	10.0
* The functionality of the S9 mix batch used was checked with benzo(a)pyrene and showed the expected results.				

3. Metabolic activation:

S9 mix was obtained from the livers of 8 – 12 weeks old male Wistar HanIbm rats, weighing approx. 220 - 320 g. The animals received intraperitoneal injections of phenobarbital (80 mg/kg bw) and oral administration of β-naphthoflavone (80 mg/kg bw) on three consecutive days. The livers were prepared 24 h after the last treatment. The S9 mix was thawed prior to each experiment and co-factor was immediately added to prepare S9 mix containing the following components:

S9 mix component	Concentration	Unit
Sodium phosphate buffer (pH 7.4)	100	mM
KCl	33	mM
NADPH-generating system		
Glucose 6-phosphate	5	mM
NADP	5	mM
$MgCl_2$	8	mM
S9	10	% (v/v)

4. Test organisms:

Tester strains				Bacteria batch checked for	
S. typhimurium		*E.coli*			
TA 98	✓	WP2 uvrA	✓	deep rough character (rfa)	✓
TA 100	✓	WP2 uvrA (pKM101)		ampicillin resistance (R factor plasmid)	✓
TA 1535	✓			UV-light sensitivity (absence of uvrB and uvrA genes in *S. typhimurium* and *E. coli* strains, respectively)	✓
TA 1537	✓				
TA 102					
TA 1538				Histidine and tryptophan auxotrophy (automatically via the spontaneous rate)	✓

5. Test concentrations:

(a) Preliminary cytotoxicity assay/Experiment 1 of the main mutation assay

Plate incorporation test ± S9 mix:	
Concentrations:	*3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate*
Tester strains:	TA 1535, TA 1537, TA 98, TA 100 and WP2 uvrA
Replicates:	*Triplicates*

(b) Experiment 2 of the main mutation assay

Pre-incubation test ± S9 mix:	
Concentrations:	*33, 100, 333, 1000, 2500 and 5000 µg/plate*
Tester strains:	TA 1535, TA 1537, TA 98, TA 100 and WP2 uvrA
Replicates:	*Triplicates*

B: STUDY DESIGN AND METHODS

1. Dates of experimental work: 16 – 25 Jan 2007
Finalisation date: 16 Mar 2007

2. Standard plate test (plate-incorporation test, SPT):
An aliquot of 0.1 mL test solution or vehicle/positive control, an aliquot of 0.1 mL fresh bacterial culture and 0.5 mL S9 mix (in tests with metabolic activation) or 0.5 mL of S9 substitution buffer (in tests without metabolic activation) were added to 2 mL of molten agar (supplemented with 10.5 mg/L L-histidine + 12.2 mg/L biotin or 2.5 mg/L tryptophan). The mixture was thoroughly shaken and poured onto selective agar plates. Each concentration and the controls were tested in triplicates. After incubation at 37 °C for at least 48 h in the dark, the background bacterial lawn was examined and the bacterial colonies (his^+ or trp^+ revertants) were counted.

3. Pre-incubation test (PIT):
0.1 mL of test solution or vehicle/positive control, 0.1 mL pre-cultured bacterial suspension and 0.5 mL of S9 mix (in tests with metabolic activation) or 0.5 mL of S9 mix substitution buffer (in tests without metabolic activation) were mixed in a test tube and shaken at 37 °C for 60 minutes. After pre-incubation 2 mL of molten overlay agar was added to each test tube. Each concentration and the controls were tested in triplicates. The mixture was poured on selective agar plates. After solidification the plates were incubated upside down for at least 48 h at 37°C in the dark. Subsequently, the number of bacterial colonies (his^+ or trp^+ revertants) were counted.

4. Cytotoxicity
Toxicity was detected by a

- reduction in the number of spontaneous revertants
- clearing or diminution of the background lawn (= reduced his^- or trp^- background growth)

and recorded for all test groups both with and without S9 mix in all experiments.

5. Statistics
Results were judged without statistical analysis.

6. Acceptance criteria
The test was valid if

- There was a regular background growth in the negative and solvent control.
- The spontaneous reversion rates in the negative and solvent control were in the range of the laboratory's historical data.
- The positive control substances produced a significant increase in mutant colony frequencies.

7. Evaluation criteria
A test item was considered as a mutagen if a biologically relevant increase in the number of revertants exceeding the threshold of twice (strains TA 98, TA 100, and WP2 uvrA) or thrice (strains TA 1535 and TA 1537) the colony count of the corresponding solvent control was observed.
A dose dependent increase was considered biologically relevant if the threshold was exceeded at more than one concentration.
An increase exceeding the threshold at only one concentration was judged as biologically relevant if reproduced in an independent second experiment. A dose dependent increase in the number of revertant colonies below the threshold was regarded as an indication of a mutagenic potential if reproduced in an independent second experiment. However, whenever the colony counts remained within the historical range of negative and solvent controls such an increase was not considered biologically relevant.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Analytical determinations were not performed in the study, as not required by the test guideline.

B. CYTOTOXICITY

Cytotoxicity indicated by a reduced background lawn was observed in the pre-test/first experiment in *E. coli* strain WP2 uvrA at 5000 µg/plate in the absence of metabolic activation. There was no reduced bacterial growth noted in the second experiment. Toxic effects evident as a reduction in the number of revertant colonies was observed at minor degree in both experiments at 5000 µg/plate in strain WP2 uvrA in the absence of metabolic activation.

C. SOLUBILITY

There was no precipitation of the test substance observed up to the highest dose tested.

D. MUTATION ASSAY

There was no substantial increase in the number of his^+ or trp^+ revertants observed in any experiment at any tested concentration either in the presence or absence of metabolic activation. In addition, there was no dose-dependent increase in mutation rates in the range below the generally acknowledged border of biological relevance.

The spontaneous mutation rates of negative controls remained within the range of historical control data and appropriate positive controls induced a marked increase in the number of revertants, thus demonstrating the functionality and validity of the test system.

Table 5.4.1-20: *Salmonella typhimurium* and *Escherichia coli Reverse* mutation assay with Glycophosate technical ([redacted] 2007), pre-experiment/first experiment

Pre-experiment/Experiment 1: Standard plate test (SPT)										
Strain	**TA 1535**		**TA 1537**		**TA 98**		**TA 100**		**WP2 uvrA**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Untreated										
mean ± *SD*	19 ± *8*	21 ± *5*	11 ± *4*	20 ± *4*	33 ± *6*	41 ± *7*	139 ± *16*	135 ± *2*	53 ± *10*	72 ± *10*
HCD# mean ± *SD*	20.4 ± *4.4*	24.2 ± *5.5*	11.6 ± *4.0*	17.1 ± *5.4*	30.2 ± *6.6*	39.0 ± *7.5*	138.2 ± *21.6*	150.1 ± *24.2*	55.9 ± *8.6*	65.6 ± *10.4*
[range]	*11 - 31*	*10 - 38*	*4 - 28*	*7 - 34*	*16 - 60*	*18 - 64*	*86 - 216*	*96 - 214*	*36 - 76*	*33 - 91*
Vehicle control										
Water mean ± *SD*	20 ± *6*	21 ± *2*	10 ± *4*	15 ± *2*	24 ± *5*	34 ± *4*	127 ± *8*	138 ± *3*	53 ± *3*	73 ± *4*
HCD# mean ± *SD*	20.8 ± *4.7*	24.7 ± *5.9*	11.2 ± *3.7*	16.2 ± *5.0*	28.1 ± *6.1*	37.9 ± *7.4*	130.7 ± *20.8*	147.0 ± *25.5*	55.8 ± *7.2*	63.9 ± *9.1*
[range]	*9 - 35*	*7 - 43*	*5 - 28*	*6 - 36*	*15 - 49*	*20 - 57*	*87 - 197*	*84 - 255*	*31 - 74*	*34 - 84*
Test item [µg/plate]										
3 mean ± *SD*	19 ± *4*	23 ± *4*	9 ± *3*	15 ± *5*	28 ± *6*	33 ± *3*	126 ± *7*	127 ± *7*	44 ± *7*	69 ± *14*
10 mean ± *SD*	*20* ± *6*	*22* ± *3*	*10* ± *2*	*16* ± *0*	*28* ± *4*	*41* ± *9*	*136* ± *11*	*139* ± *4*	*54* ± *6*	*77* ± *2*
33 mean	21	20	11	14	26	34	128	148	51	74

Table 5.4.1-20: *Salmonella typhimurium* and *Escherichia coli Reverse* mutation assay with Glyphosate technical ([redacted] 2007), pre-experiment/first experiment

Pre-experiment/Experiment 1: Standard plate test (SPT)										
Strain	**TA 1535**		**TA 1537**		**TA 98**		**TA 100**		**WP2 uvrA**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
± *SD*	± *2*	± *2*	± *3*	± *3*	± *10*	± *8*	± *16*	± *9*	± *10*	± *10*
100 mean	20	16	12	12	25	32	131	148	51	71
± *SD*	± *7*	± *7*	± *4*	± *4*	± *2*	± *7*	± *8*	± *1*	± *6*	± *14*
333 mean	12	24	14	16	25	35	118	121	54	76
± *SD*	± *3*	± *4*	± *2*	± *1*	± *5*	± *7*	± *10*	± *6*	± *4*	± *11*
1000 mean	*20*	*21*	*8*	*19*	*28*	*38*	*119*	*144*	*52*	*67*
± *SD*	± *8*	± *9*	± *2*	± *3*	± *7*	± *2*	± *15*	± *6*	± *5*	± *4*
2500 mean	18	19	9	15	24	34	135	137	43	60
± *SD*	± *5*	± *2*	± *2*	± *4*	± *3*	± *2*	± *16*	± *9*	± *4*	± *9*
5000 mean	17	22	9	18	21	28	116	112	22 [R]	55
± *SD*	± *6*	± *3*	± *1*	± *3*	± *7*	± *6*	± *3*	± *8*	± *6*	± *7*
Positive control										
§ mean	1991	345	102	208	436	1459	2196	2023	1304	322
± *SD*	± *41*	± *11*	± *9*	± *9*	± *43*	± *133*	± *97*	± *40*	± *54*	± *16*
HCD# mean	1422.0	332.0	99.8	276.8	439.0	1839.4	2083.1	2372.9	991.0	319.4
± *SD*	± *464.7*	± *95.3*	± *32.5*	± *132.6*	± *155.2*	± *898.6*	± *281.3*	± *958.4*	± *522.9*	± *84.8*
[range]	*781 - 4900*	*107 - 695*	*53 - 425*	*59 - 746*	*176 - 1818*	*407 - 4891*	*616 - 2872*	*417 - 5230*	*249 - 1810*	*211 - 9.30*

§ Information on respective positive control is reported in Material and Method section I.A.2

\# Historical control data from May 2005 – June 2006 representing approx. 200 experiments

R Reduced background growth

Table 5.4.1-21: *Salmonella typhimurium* and *Escherichia coli Reverse* mutation assay with Glyphosate technical ([redacted] 2007), second experiment

Experiment 2: Pre-incubation test (PIT)										
Strain	**TA 1535**		**TA 1537**		**TA 98**		**TA 100**		**WP2 uvrA**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Untreated										
mean	12	21	11	24	28	40	124	153	52	68
± *SD*	± *2*	± *2*	± *4*	± *2*	± *7*	± *9*	± *27*	± *16*	± *3*	± *17*
HCD# mean	20.4	24.2	11.6	17.1	30.2	39.0	138.2	150.1	55.9	65.6
± *SD*	± *4.4*	± *5.5*	± *4.0*	± *5.4*	± *6.6*	± *7.5*	± *21.6*	± *24.2*	± *8.6*	± *10.4*
[range]	*11 - 31*	*10 - 38*	*4 - 28*	*7 - 34*	*16 - 60*	*18 - 64*	*86 - 216*	*96 - 214*	*36 - 76*	*33 - 91*
Vehicle control										
Water mean	16	28	8	18	32	41	150	164	58	66
± *SD*	± *7*	± *2*	± *2*	± *3*	± *3*	± *4*	± *2*	± *2*	± *5*	± *10*

Table 5.4.1-21: *Salmonella typhimurium* and *Escherichia coli Reverse* mutation assay with Glyphosate technical ([redacted] 2007), second experiment

Experiment 2: Pre-incubation test (PIT)										
Strain	**TA 1535**		**TA 1537**		**TA 98**		**TA 100**		**WP2 uvrA**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
HCD[#] mean ± *SD*	20.8 ± *4.7*	24.7 ± *5.9*	11.2 ± *3.7*	16.2 ± *5.0*	28.1 ± *6.1*	37.9 ± *7.4*	130.7 ± *20.8*	147.0 ± *25.5*	55.8 ± *7.2*	63.9 ± *9.1*
[range]	*9 - 35*	*7 - 43*	*5 - 28*	*6 - 36*	*15 - 49*	*20 - 57*	*87 - 197*	*84 - 255*	*31 - 74*	*34 - 84*
Test item [µg/plate]										
33 mean ± *SD*	19 ± *4*	24 ± *4*	13 ± *4*	21 ± *4*	28 ± *6*	44 ± *10*	131 ± *8*	167 ± *14*	54 ± *2*	68 ± *18*
100 mean ± *SD*	16 ± *6*	22 ± *8*	14 ± *1*	22 ± *8*	26 ± *3*	36 ± *14*	144 ± *7*	161 ± *11*	54 ± *11*	62 ± *4*
333 mean ± *SD*	15 ± *5*	23 ± *9*	11 ± *4*	23 ± *9*	30 ± *4*	33 ± *3*	147 ± *3*	166 ± *19*	49 ± *12*	73 ± *9*
1000 mean ± *SD*	13 ± *4*	21 ± *2*	10 ± *4*	21 ± *2*	30 ± *4*	39 ± *2*	148 ± *8*	154 ± *32*	41 ± *7*	67 ± *7*
2500 mean ± *SD*	19 ± *3*	19 ± *8*	10 ± *1*	19 ± *8*	24 ± *3*	39 ± *13*	135 ± *3*	153 ± *11*	30 ± *4*	49 ± *9*
5000 mean ± *SD*	10 ± *3*	21 ± *2*	9 ± *2*	21 ± *2*	25 ± *9*	42 ± *8*	105 ± *13*	146 ± *34*	24 ± *11*	49 ± *5*
Positive control										
[§] mean ± *SD*	2045 ± *99*	335 ± *12*	105 ± *5*	229 ± *45*	356 ± *28*	1008 ± *128*	2246 ± *39*	2331 ± *280*	705 ± *40*	287 ± *18*
HCD[#] mean ± *SD*	1422.0 ± *464.7*	332.0 ± *95.3*	99.8 ± *32.5*	276.8 ± *132.6*	439.0 ± *155.2*	1839.4 ± *898.6*	2083.1 ± *281.3*	2372.9 ± *958.4*	991.0 ± *522.9*	319.4 ± *84.8*
[range]	*781 - 4900*	*107 - 695*	*53 - 425*	*59 - 746*	*176 - 1818*	*407 - 4891*	*616 - 2872*	*417 - 5230*	*249 - 1810*	*211 - 9.30*

[§] Information on respective positive control is reported in Material and Method section I.A.2
[#] Historical control data from May 2005 – June 2006 representing approx. 200 experiments

III. CONCLUSION:

Based on the results of the present study and under the experimental conditions of the test, the test item is not mutagenic in the Ames test (standard plate and pre-incubation method) with and without metabolic activation.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Negative for gene mutation in bacteria (*S. typhimurium* TA 100, TA 98, TA 1535 and TA 1537 and *E. coli* WP2 uvrA) with and without metabolic activation.

The study was performed according to OECD guideline 471 (1997) and under GLP conditions. There were only minor deviations when compared to the guideline, which were considered to not compromise

the validity of the study. The study is therefore considered valid and acceptable.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.4.1/012
Report author	[redacted]
Report year	2007
Report title	Bacterial reverse mutation test (Ames Test) for Glifosato Téchnico
Report No	RL3393/2007-2.0AM-B
Document No	Not reported
Guidelines followed in study	OECD 471 (1997)
Deviations from current test guideline OECD 471 (1997)	2-Aminoanthracene was used as sole positive control in the presence of metabolic activation. Only a single experiment was performed, without giving a justification for not conducting a confirmatory experiment. Historical control data were restricted to solvent controls, but not differentiated for exposures in the presence or absence of metabolic activation.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

S. typhimurium strains TA 98, TA 100, TA 1535, TA 1537 and TA 102 were exposed to glyphosate technical (batch: 2007091801, purity: 98.01 %) in the presence and absence of metabolic activation (Aroclor 1254-induced rat liver S9 fraction). Based on the results of a preliminary cytotoxicity test, in which no toxicity was observed up to 5000 µg/plate, concentrations for the main mutation assay were selected. In the main experiment the tester strains were exposed via the plate-incorporation method to test item concentrations in the range of 648 to 5000 µg/plate for 72 hours. Vehicle (sterile water) and positive controls were included. Following incubation, the bacterial background lawn was examined and the number of revertant colonies was counted for each strain.

Precipitation of the test substance was not reported. Cytotoxicity as indicated by a statistically significant reduction in the number of revertant colonies was observed at 5000 µg/plate in strain TA 1537 in the absence of S9 mix.

There was no substantial increase in the number of his^+ and trp^+ revertants (exceeding a mutation rate by a factor of at least 2 when compared to solvent controls) observed in any experiment either in the presence or absence of metabolic activation and no dose-response relationship in the range below the generally acknowledged border of biological significance. Vehicle controls were within the range of historical control data and positive controls demonstrated the sensitivity and the functionality of the test substance.
Based on the results of the present study, glyphosate is not mutagenic in the Ames pre-incubation test with and without metabolic activation under the experimental conditions of the test.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material: Glyphosate technical

Identification:	Glifosato Téchnico Helm
Description:	Solid
Lot/Batch #:	2007091801
Purity:	98.01 %
Stability of test compound:	The stability of the test item under storage conditions was guaranteed until 17 Sep 2009. In addition, it was confirmed by CIPAC MT 46 at 54 °C for 14 days
Solvent (vehicle) used:	Dimethylsulfoxide (DMSO)

2. Control materials:

Negative control:	Not examined
Solvent (vehicle) control:	Dimethylsulfoxide (DMSO)
Solvent (vehicle)/final concentration:	0.1 mL per plate.
Positive controls:	Please refer to the table below.

Strain	Metabolic activation	Mutagen	Conc. [µg/plate]
TA 100	-S9	Sodium azide	5.0
	+S9	2-Aminoanthracene	2.5
TA 1535	-S9	Sodium azide	5.0
	+S9	2-Aminoanthracene	2.5
TA 98	-S9	2-Nitrofluorene	0.5
	+S9	2-Aminoanthracene	2.5
TA 1537	-S9	ICR 191-Acridine	10.0
	+S9	2-Aminoanthracene	2.5
TA 102	-S9	Mitomycin C	0.5
	+S9	2-Aminoanthracene	2.5

3. Metabolic activation:

S9 mix was purchased from [redacted] ([redacted]). The homogenate was produced from the livers of rats which were induced with Aroclor 1254. The concentration of protein in S9 fraction employed in the assay was 34.9 mg/mL.

4. Test organisms:

Tester strains				Bacteria batch checked for	
S. typhimurium		***E.coli***			
TA 98	✓	WP2 uvrA		deep rough character (rfa)	✓
TA 100	✓	WP2 uvrA (pKM101)		ampicillin resistance (R factor plasmid)	✓
TA 1535	✓			UV-light sensitivity (absence of uvrB and uvrA genes)	✓
TA 1537	✓				
TA 102	✓				
TA 1538				Histidine auxotrophy (automatically via the spontaneous rate)	✓

5. Test concentrations:

(a) Preliminary cytotoxicity assay:

Plate incorporation test without S9 mix:	
Concentrations:	*8, 40, 200, 1000 and 5000 µg/plate*
Tester strains:	TA 100
Replicates:	*Triplicates*

(b) Mutation assay:

Plate incorporation test ± S9 mix:	
Concentrations:	*648, 1080, 1800, 3000 and 5000 µg/plate*
Tester strains:	TA 1535, TA 1537, TA 98, TA 100 and TA 102
Replicates:	*Triplicates in a single experiment.*

B: STUDY DESIGN AND METHODS

1. Dates of experimental work: 06 Nov – 03 Dec 2007
Finalisation date: 13 Dec 2007

2. Standard plate test (plate-incorporation test, SPT):
An aliquot of 0.1 mL test solution or vehicle/positive control, an aliquot of 0.1 mL fresh bacterial culture and 0.5 mL S9 mix (in tests with metabolic activation) or 0.5 mL of S9 substitution buffer (in tests without metabolic activation) were added to 3 mL of top agar. Each suspension was incubated on selective agar plates for 72 h. After incubation the background bacterial lawn was examined and the bacterial colonies (his^+ or trp^+ revertants) were counted.

3. Cytotoxicity
Cytotoxicity was investigated in a preliminary dose-range finding study, which was performed in tester strain TA100 in the absence of metabolic activation. Toxicity was detected by a

- decrease in the number of revertants
- clearing or diminution of the background lawn (= reduced his^- background growth)

and recorded for all test groups both with and without S9 mix.

4. Statistics
Analysis of variance (ANOVA) was performed on the data to identify statistical significance (p ANOVA < 0.05)

5. Acceptance criteria
The following acceptance criteria were defined:

- Presence of background lawn in the test plates.
- Spontaneous revertant colonies of the negative control were in the range reported in literature and established in the laboratory by historical control values.
- Positive controls showed mutagenicity in all tested strains.

6. Evaluation criteria

Results were judged positive when the following criteria were met:

- The mutation rates after 72 hours of incubation of strains exposed to the test chemical were higher than 2 for strains TA 98, TA 100 and TA 102 or higher than 3 for strains TA 1535 and TA1537.
- The positive result was statistically significant (pANOVA < 0.05) and a clear dose-related increase in the number of revertants was observed.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Analytical determinations were not performed in the study, as not required by the test guideline.

B. CYTOTOXICITY

In the preliminary cytotoxicity experiment, the test substance did not show any toxicity to any strain up to the highest dose tested, neither in the presence, nor in the absence of metabolic activation.

According to the result tables of the study report, in the main experiment, cytotoxicity as indicated by a statistically significant reduction in the mean number of revertant colonies, was observed at 5000 µg/plate in strain TA 1537 in the absence of S9 mix. There was no cytotoxicity observed in any other strain, neither in the presence, nor absence of S9 mix. These observations on cytotoxicity listed in the result tables of the study report are inconsistent with the observations described in the text of the study report, where a reduction in the mean number of revertant colonies was observed at 5000 µg/plate for strain TA 1537 in the presence of S9 mix and for strain TA 102 in the absence of S9 mix.

C. SOLUBILITY

Precipitation of the test substance was not reported.

D. MUTATION ASSAY

No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment at any concentration level, neither in the presence nor absence of metabolic activation. There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological significance.

The number of revertants induced by the vehicle control was within expected range of historical control data and data published in literature for each strain, thus demonstrating an acceptable experimental performance.

Appropriate positive control compounds induced a marked increase in the number of revertants, demonstrating the functionality of the S9 mix and the validity of the test system.

Table 5.4.1-22: Bacterial reverse mutation test (Ames Test) ([redacted], 2007)

Standard plate test (SPT)										
Strain	**TA 98**		**TA 100**		**TA 102**		**TA 1535**		**TA1537**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Vehicle control										
DMSO mean	34	32	204	203	312	307	33	30	12	9
± SD	*± 3*	*± 3*	*± 4*	*± 2*	*± 4*	*± 6*	*± 3*	*± 2*	*± 1*	*± 2*
HCD[#] *[range]*	*17-75*		*60-220*		*240-320*		*5-50*		*3-25*	

Table 5.4.1-22: Bacterial reverse mutation test (Ames Test) ([redacted], 2007)

Standard plate test (SPT)										
Strain	**TA 98**		**TA 100**		**TA 102**		**TA 1535**		**TA1537**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Test item [µg/plate]										
648 mean	29	30	209	204	311	312	32	28	10	14
± *SD*	± *2*	± *2*	± *8*	± *4*	± *6*	± *4*	± *2*	± *4*	± *2*	± *2*
1080 mean	32	34	203	204	309	307	33	31	10	14
± *SD*	± *4*	± *4*	± *5*	± *4*	± *6*	± *5*	± *2*	± *4*	± *3*	± *5*
1800 mean	33	37	203	209	308	305	29	28	9	8
± *SD*	± *3*	± *7*	± *2*	± *8*	± *8*	± *6*	± *1*	± *2*	± *2*	± *1*
3000 mean	31	35	208	213	308	304	34	33	13	7
± *SD*	± *6*	± *2*	± *8*	± *6*	± *4*	± *4*	± *3*	± *2*	± *4*	± *2*
5000 mean	34	36	209	208	287	304	33	28	7	9
± *SD*	± *2*	± *5*	± *8*	± *11*	± *9*	± *4*	± *5*	± *5*	± *2*	± *4*
Positive control[$]										
[§] mean	1076	3290	2926	2828	3136	1890	2576	272	3501	258
± *SD*	± *62*	± *337*	± *99*	± *79*	± *317*	± *218*	± *119*	± *28*	± *120*	± *8*

[§] Information on respective positive control is reported in Material and Method section I.A.2
[#] Historical control data, time frame of generation not specified
[$] The positive control was tested in duplicates only.

III. CONCLUSION:

According to the results of the present study and under the experimental conditions of the test, the test item is not mutagenic in the Ames plate-incorporation test with and without metabolic activation.

3. Assessment and conclusion

<u>Assessment and conclusion by applicant</u>:

Negative for gene mutation in bacteria (*S. typhimurium* TA 98, TA 100, TA 1535, TA 1537 and TA 102) with and without metabolic activation.

The study was performed under GLP conditions and according to OECD guideline 471 (1997), including some deviations from the guideline. No justification was provided for conducting only a single experiment according to the plate incorporation method, however, given the clearly negative result, this is considered acceptable. Further deviations to OECD guideline 471 (1997) were considered to be of minor degree and to not compromise the validity of the study. The study was considered to provide supporting information.

<u>Assessment and conclusion by RMS</u>:

1. Information on the study

Data point	CA 5.4.1/013
Report author	[redacted]
Report year	1996
Report title	Glyphosate Acid: An Evaluation of Mutagenic Potential Using *S. typhimurium* and *E. coli*.
Report No	CTL/P/4874 (Syngenta)
Document No	YV3611
Guidelines followed in study	OECD 471 (1983); OECD 472 (1983); U.S. EPA FIFRA Guidelines, Subdivision F (1991); EEC Directive 92/69 Method B.13/B14 (1992)
Deviations from current test guideline OECD 471 (1997)	No historical control data provided. No information on cytotoxicity reported. Evaluation of cytotoxicity and precipitation were not reported, but concentrations were tested up to limit doses. 2-Aminoanthracene was used as sole indicator of the efficacy of the S9 mix in all strains. No confirmation of bacterial cell density.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

S. typhimurium strains TA 98, TA 100, TA 1535 and TA 1537, and *E. coli* strains WP2P and WP2 uvrA were exposed to glyphosate acid (batch: P24, purity: 95.6 %) in the presence and absence of metabolic activation (phenobarbital and β-naphthoflavone-induced rat liver S9 fraction). The test was performed using the plate-incorporation method (first experiment) and the pre-incubation method (second experiment) and concentrations in the range of 100 – 5000 µg/plate. Solvent (DMSO) and positive controls were included in each experiment. After 72 hours of incubation at 37 °C, the bacterial background lawn was inspected and the number of revertant colonies was examined.

Evaluation of precipitation and cytotoxicity was not provided in the study report. Cytotoxicity was evaluated retrospectively by comparing the presented mean numbers of revertant colonies induced by the test item to the mean number of spontaneous revertants observed in the corresponding solvent control. Indications for cytotoxicity were observed in the first experiment in tester strains TA1535, TA 98 and TA 100 at ≥ 2500 µg/plate and in tester strains WP2P and WP2 uvrA at 5000 µg/plate in the absence of S9 mix. In the second experiment, cytotoxicity was observed at ≥ 2500 µg/plate for strain TA 98 in the absence of S9 mix, at 5000 µg/plate for strains TA 1537 and WP2uvrA in the absence of S9 mix and for strains TA 1535, TA 98, TA 100 and WP2P in the presence and absence of S9 mix.

There was no statistically singificant, reproducible increase in in the number of his^+ and trp^+ revertant colony numbers in any of the six tester strains at any dose level, neither in the presence nor absence of metabolic activation. Statistical significance in the mean number of revertant colonies was observed for tester strains TA 100 and WP2P at single concentrations in the presence or absence of S9 mix, however, the results were not reproducible in independent experiments and showed no dose-response relationship and were thus considered to be incidental. The spontaneous reversion rate with and without metabolic activation was demonstrated with vehicle controls. Appropriate positive controls validated the sensitivity of the test system and the functionality of the metabolic activity of the S9 mix.

Based on the results and under the experimental conditions of the present study, there is no indication for mutagenicity in the Ames standard plate and pre-incubation test with and without metabolic activation.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material: Glyphosate acid

Identification:	Y04707/034
Description:	White solid
Lot/Batch #:	P24
Purity:	95.6 %
Stability of test compound:	The stability of the test item under storage conditions (at ambient temperature in the dark) was guaranteed until the stated expiry date (no date provided). The stability of the test item in solvent (vehicle) was not indicated by the sponsor.
Solvent (vehicle) used:	Dimethylsulfoxide (DMSO)

2. Control materials:

Negative control:	Not specified
Solvent (vehicle) control:	DMSO
Solvent (vehicle)/final concentration:	10 µL per plate.
Positive controls:	Please refer to the table below.

Strain	Metabolic activation	Mutagen	Solvent	Conc. [µg/plate]
S. typhimurium strains				
TA 100	-S9	Sodium azide (NaN_3)	DMSO	0.5, 1.0 and 2.0
	+S9	2-Aminoanthracene (2AA)	DMSO	0.2, 0.5 and 1.0
TA 1535	-S9	Sodium azide (NaN_3)	DMSO	0.5, 1.0 and 2.0
	+S9	2-Aminoanthracene (2AA)	DMSO	0.5, 1.0 and 2.0
TA 98	-S9	Daunorubicin Hydrochloride (DR)	DMSO	0.2, 0.5 and 1.0
	+S9	2-Aminoanthracene (2AA)	DMSO	0.2, 0.5 and 1.0
TA 1537	-S9	Acridine Mutagen ICR191 Dihydrochloride (ACM)	DMSO	0.5, 1.0 and 2.0
	+S9	2-Aminoanthracene (2AA)	DMSO	0.5, 1.0 and 2.0
E. coli strains				
WP2 uvrA	-S9	N-Ethyl-N-nitro-N-nitrosoguanidine (ENNG)	DMSO	0.2, 0.5 and 1.0
	+S9	2-Aminoanthracene (2AA)	DMSO	1, 2 and 5
WP2P	-S9	Mitomycin C (MMC)	DMSO	0.2, 0.5 and 1.0
	+S9	2-Aminoanthracene (2AA)	DMSO	5, 10 and 20

3. Metabolic activation:

S9 mix was obtained from the livers of male Sprague-Dawley rats, dosed once daily by oral gavage for three days with a combined phenobarbital (80 mg/kg bw) and β-naphthoflavone (100 mg/kg bw) corn oil solution. The livers were prepared one day after the third dose. A 25 % (w/v) homogenate fraction was prepared using sucrose-Tris-EDTA buffer (250:50:1 mM), adding 3 mL S9 fraction to 7 mL buffer. Afterwards 20 mL co-factor solution were immediately added containing the following components:

S9 mix component	Concentration	Unit
Sodium phosphate buffer (pH 7.4)	150	mM
KCl	49.5	mM
NADPH-generating system		
Glucose 6-phosphate	7.55	mM
NADP, Na salt	6	mM
$MgCl_2$	12	mM
S9	25	% (v/v)

4. Test organisms:

Tester strains				Bacteria batch checked for	
S. typhimurium		*E.coli*			
TA 98	✓	WP2 uvrA	✓	deep rough character (rfa)	✓
TA 100	✓	WP2 P	✓	ampicillin resistance (R factor plasmid)	✓
TA 1535	✓			UV-light sensitivity (absence of uvrB and uvrA genes in *S. typhimurium* and *E. coli* strains, respectively)	✓
TA 1537	✓				
TA 102					
TA 1538				Histidine and tryptophan auxotrophy (automatically via the spontaneous rate)	✓

5. Test concentrations:

Plate incorporation test ± S9 mix:		
Concentrations:	*100, 200, 500, 1000, 2500 and 5000 µg/plate*	
Tester strains:	TA 1535, TA 1537, TA 98, TA 100, WP2P and WP2 uvrA	
Replicates:	*Triplicates*	
Pre-incubation test ± S9 mix:		
Concentrations:	*100, 200, 500, 1000, 2500 and 5000 µg/plate*	
Tester strains:	TA 1535, TA 1537, TA 98, TA 100, WP2P and WP2 uvrA	
Replicates:	*Triplicates*	

B: STUDY DESIGN AND METHODS

1. Dates of experimental work: 28 Nov – 11 Dec 1995
Finalisation date: 16 Feb 1996

2. Standard plate test (plate-incorporation test, SPT):
An aliquot of 0.1 mL test solution or vehicle/positive control, an aliquot of 0.1 mL fresh bacterial overnight culture and 0.5 mL S9 mix (in tests with metabolic activation) or 0.5 mL of S9 substitution buffer (in tests without metabolic activation) were added to 2 mL of molten agar (supplemented with 0.5 mg histidine/0.5 mM biotin stock solution (10 mL solution : 100 mL agar) or 10 mL 0.5 mM stock tryptophan per 100 mL agar). The mixture was thoroughly shaken and poured rapidly onto Vogel Bonner agar plates. Each

concentration and the controls were tested in triplicates. After incubation at 37 °C for 72 h in the dark, the plates were checked for microbial contamination, the background bacterial lawn was examined and the number of the bacterial colonies (his$^+$ or trp$^+$ revertants) was counted.

3. Pre-incubation test (PIT):

0.1 mL of test solution or vehicle/positive control were prepared by adding 0.02 mL volumes of stock solution in DMSO to phosphate buffered saline. Afterwards, the formulations, 0.1 mL pre-cultured bacterial suspension and 0.5 mL of S9 mix (in tests with metabolic activation) or 0.5 mL of S9 mix substitution buffer (in tests without metabolic activation) were mixed in a test tube and shaken at 37 °C for 60 minutes. After pre-incubation 2 mL of molten overlay agar (supplemented with 0.5 mg histidine/0.5 mM biotin stock solution (10 mL solution : 100 mL agar) or 10 mL 0.5 mM stock tryptophan per 100 mL agar) was added to each test tube. Each concentration and the controls were tested in triplicates. The mixture was poured onto Vogel Bonner agar plates. After solidification the plates were incubated for 72 h at 37°C in the dark. Subsequently, the plates were checked for microbial contamination, the bacterial background lawn was examined and the number of bacterial colonies (his$^+$ or trp$^+$ revertants) was counted.

4. Cytotoxicity

Evaluation of cytotoxicity was not further specified in the study report. Thus, retrospectively, cytotoxicity was evaluated by comparing the presented mean numbers of revertant colonies induced by the test item to the mean number of spontaneous revertants observed in the corresponding solvent control. Cytotoxicity was considered evident when the mean number of revertant colonies induced by the test item concentration was ≤ 0.5 fold of the mean number of spontaneous revertant colonies induced by the solvent control.

5. Statistics

An assessment of statistical significance was carried out using a one-tailed Student's t-test. Values of $p < 0.01$ are treated as significant with values of $0.01 \leq p < 0.05$ being indicative of a possible effect.

6. Acceptance criteria

The test was valid if

- The concurrent solvent control data were acceptable
- The positive control data showed unequivocal positive responses.

Failure of one or more tester strain/S9 combinations does not invalidate the data for the remainder of a concurrent experiment.

7. Evaluation criteria

A positive response in an individual experiment was achieved when one or both of the following criteria were met:

- A statistically significant dose-related increase in the mean number of revertant colonies was obtained.
- A two-fold or greater increase of statistical significance in the mean number of revertant colonies was observed at one or more concentrations.

For a positive response in an individual experiment to be considered indicative of an unequivocal positive, i.e. mutagenic, result for that strain/S9 combination, then the observed effects must be consistently reproducible.

A negative response in an individual experiment was achieved when

- There was no statistically significant dose-related increase in the mean number of revertant colonies per plate observed for the test substance.
- In the absence of any such dose response, no increase in colony numbers was observed at any test concentration, which exceeded 2x the concurrent solvent control.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Analytical determinations were not performed in the study, as not required by the test guideline.

B. CYTOTOXICITY

Indications for cytotoxicity were observed in the first experiment in tester strains TA 1535, TA 98 and TA 100 at ≥ 2500 µg/plate and in tester strains WP2P and WP2 uvrA at 5000 µg/plate in the absence of S9 mix and in the second experiment for tester strains TA 1535, TA 1537, TA 98, TA 100 and WP2P with and without S9 mix and in tester strain WP2 uvrA in the absence of S9 mix.

C. SOLUBILITY

There was no precipitation of the test substance reported.

D. MUTATION ASSAY

Although a statistically significant increase in the mean number of revertant colonies was obtained in tester strains TA 100 and WP2P at 500 µg/plate in the presence and absence of S9 mix, respectively, there was no dose response relationship and the observations were not consistent in individual experiments.

The number of revertants induced by the test item were comparable to those of the corresponding solvent controls. The positive control compounds induced the expected results, indicating the functionality of the S9 mix and demonstrating the validity of the test system.

Table 5.4.1-23: Glyphosate Acid: An Evaluation of Mutagenic Potential Using *S. Typhimurium* and *E. Coli*. ([redacted], 1996), first experiment, plate-incorporation test

Experiment 1: Standard plate test (SPT)												
Strain	**TA 1535**		**TA 1537**		**TA 98**		**TA 100**		**WP2P**		**WP2 uvrA**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Vehicle control												
DMSO mean	10.2	10.2	2.8	3.2	23.8	23.4	69.8	80	36.7	45.8	121.6	154
± *SD*	*± 3.2*	*± 4.3*	*± 0.8*	*± 1.6*	*± 5.2*	*± 4.8*	*± 4.3*	*± 5.5*	*± 7.1*	*± 3.5*	*± 5.2*	*± 8.2*
Test item [µg/plate]												
100 mean	9.7	12.3	2.3	2.0	23.0	25.3	69.7	79.0	34.0	44.0	127.3	150.0
± *SD*	*± 1.2*	*± 6.5*	*± 1.5*	*± 1.0*	*± 6.2*	*± 6.1*	*± 3.8*	*± 9.0*	*± 5.3*	*± 6.6*	*± 10.6*	*± 11.8*
200 mean	8.3	14.0	2.0	3.3	23.0	23.7	68.0	80.3	39.3	51.0	111.0	162.3
± *SD*	*± 4.0*	*± 3.6*	*± 0.0*	*± 1.5*	*± 1.7*	*± 2.3*	*± 3.5*	*± 8.5*	*± 2.1*	*± 7.0*	*± 9.2*	*± 14.6*
500 mean	11.0	11.7	2.3	2.3	20.7	25.0	67.3	94.7**	32.7	54.0*	124.3	162.0
± *SD*	*1.7*	*± 3.8*	*± 1.2*	*± 1.5*	*± 4.0*	*± 5.3*	*± 2.1*	*± 1.5*	*± 3.5*	*± 5.2*	*± 21.5*	*± 5.6*
1000 mean	7.0	13.3	2.3	3.3	24.0	25.0	60.0	89.3	36.7	43.7	133.7	139.7
± *SD*	*± 1.7*	*± 2.3*	*± 2.3*	*± 0.6*	*± 2.0*	*± 3.5*	*± 1.7*	*± 13.3*	*± 4.6*	*± 4.0*	*± 17.0*	*± 26.4*
2500 mean	4.7	8.7	1.7	3.0	10.7	20.3	19.3	72.0	26.3	44.0	102.7	151.3
± *SD*	*± 2.9*	*± 2.5*	*± 1.2*	*± 2.6*	*± 6.0*	*± 6.7*	*± 7.4*	*± 3.6*	*± 1.2*	*± 2.6*	*± 16.2*	*6.7*
5000 mean	1.0	7.0	1.7	1.0	2.7	7.0	1.7	51.7	13.7	23.7	64.0	134.0
± *SD*	*± 1.0*	*± 3.5*	*± 0.6*	*± 1.0*	*± 2.5*	*± 5.3*	*± 2.1*	*± 13.7*	*± 4.5*	*± 3.5*	*± 4.0*	*± 18.3*

Table 5.4.1-23: Glyphosate Acid: An Evaluation of Mutagenic Potential Using *S. Typhimurium* and *E. Coli*. ([redacted], 1996), first experiment, plate-incorporation test

Experiment 1: Standard plate test (SPT)												
Strain	**TA 1535**		**TA 1537**		**TA 98**		**TA 100**		**WP2P**		**WP2 uvrA**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Positive control												
[§] 1. mean	114.0**	80.0**	14.0**	20.5**	160.5**	114.5**	245.5**	207.0**	103.5**	84.5**	405.0**	296.5**
± SD	*± 18.4*	*± 11.3*	*± 5.7*	*± 2.1*	*± 14.8*	*± 0.7*	*± 49*	*± 12.7*	*± 0.7*	*± 10.6*	*± 9.9*	*± 13.4*
[§] 2. mean	234.0**	99.0**	27.0**	36.0**	384.5**	287.0**	727.0**	552.5**	149.0**	127.5**	2004.0**	1050.0**
± SD	*± 1.4*	*± 21.2*	*± 9.9*	*± 8.5*	*± 0.7*	*± 2.8*	*± 0.0*	*± 60.1*	*± 8.5*	*± 10.6*	*± 96.2*	*± 118.8*
[§] 3. mean	752.5**	191.0**	63.0**	70.0**	734.5**	361.5**	1165.5**	1037.5**	182.5**	163.0**	2737.0**	2349.5**
± SD	*± 111.0*	*± 12.7*	*± 7.1*	*± 0.0*	*± 101.1*	*± 6.4*	*± 40.3*	*± 30.4*	*± 3.5*	*± 12.7*	*± 315.4*	*± 109.6*

[§] Information on respective positive control is reported in Material and Method section I.A.2. 3 Concentrations were used per plate, 1 = low, 2 = middle, 3 high.
* P < 0.05;
** P < 0.01 (One sided t-Test assumes Test > Control)

Table 5.4.1-24: Glyphosate Acid: An Evaluation of Mutagenic Potential Using *S. Typhimurium* and *E. Coli*. ([redacted], 1996), second experiment, pre-incubation test

Experiment 2: Pre-incubation test (PIT)												
Strain	**TA 1535**		**TA 1537**		**TA 98**		**TA 100**		**WP2P**		**WP2 uvrA**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Vehicle control												
DMSO mean	9.2	11.4	3.0	3.8	18.2	24.2	81.8	83.0	37.4	54.2	98.8	134.6
± SD	*± 3.1*	*± 0.9*	*± 1.2*	*± 1.3*	*± 2.6*	*± 7.7*	*± 10.1*	*± 6.6*	*± 2.9*	*± 4.9*	*± 10.4*	*± 15.5*
Test item												
100 mean	10.3	9.7	3.7	5.0	19.0	18.7	80.0	87.7	34.0	56.0	99.0	150.7
± SD	*± 3.2*	*± 0.6*	*± 1.5*	*± 1.0*	*± 2.0*	*± 2.1*	*± 3.6*	*± 2.5*	*± 1.0*	*± 2.0*	*± 9.5*	*± 19.6*
200 mean	8.3	10.7	3.3	4.7	20.3	22.7	92.3	81.0	40.0	52.7	97.0	135.0 [C]
± SD	*± 4.0*	*± 3.1*	*± 1.2*	*± 1.5*	*± 2.1*	*± 2.1*	*± 4.0*	*± 7.5*	*± 3.6*	*± 8.1*	*± 15.1*	*± 15.6*
500 mean	9.0	10.7	3.3	4.7	23.0*	22.3	71.3	82.0	42.3*	54.7	94.7	140.0
± SD	*± 1.0*	*± 0.6*	*± 1.5*	*± 0.6*	*± 2.6*	*± 2.9*	*± 3.2*	*± 6.2*	*± 4.0*	*± 5.1*	*± 1.5*	*± 16.4*
1000 mean	7.7	10.0	3.0	3.3	20.0	19.3	75.0	85.3	38.3	51.7	101.0	134.0
± SD	*± 1.5*	*± 1.7*	*± 1.0*	*± 0.6*	*± 5.3*	*± 2.9*	*± 6.6*	*± 3.2*	*± 2.3*	*± 2.5*	*± 6.0*	*± 6.2*

Table 5.4.1-24: Glyphosate Acid: An Evaluation of Mutagenic Potential Using *S. Typhimurium* and *E. Coli*. ([redacted], 1996), second experiment, pre-incubation test

Experiment 2: Pre-incubation test (PIT)												
Strain	**TA 1535**		**TA 1537**		**TA 98**		**TA 100**		**WP2P**		**WP2 uvrA**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
2500 mean	7.7	8.0	2.7	4.0	13.0	11.3	63.3	56.3	24.0	43.3	88.7	134.3
± SD	*± 3.8*	*± 1.7*	*± 0.6*	*± 1.0*	*± 1.7*	*± 7.4*	*± 5.0*	*± 16.2*	*± 2.6*	*± 7.4*	*± 7.5*	*± 18.8*
5000 mean	3.3	5.0	0.7	2.3	5.7	4.0	22.3	28.0	14.7	23.7	39.7	92.7
± SD	*± 2.5*	*± 2.6*	*± 1.2*	*± 0.6*	*± 5.5*	*± 0.0*	*± 2.3*	*± 10.8*	*± 3.8*	*± 10.6*	*± 11.6*	*± 17.8*
Positive control												
§ 1. mean	148.5**	36.0**	10.5*	13.0**	69.0**	85.0**	232.0**	163.0**	88.0**	104.0**	210.0**	368.5**
± SD	*± 4.9*	*± 12.7*	*± 7.8*	*± 7.1*	*± 7.1*	*± 22.6*	*± 2.8*	*± 56.6*	*± 11.3*	*± 18.4*	*± 11.3*	*± 37.5*
§ 2. mean	275.0**	92.5**	33.0**	25.5**	258.0**	219.5**	418.5**	327.0**	140.5**	128.5**	377.5**	1337.0**
± SD	*± 5.7*	*± 4.9*	*± 11.3*	*± 10.6*	*± 11.3*	*± 27.6*	*± 30.4*	*± 9.9*	*± 17.7*	*± 0.7*	*± 33.2*	*± 328.1*
§ 3. mean	786.0**	143.0**	63.5**	50.0**	927.0**	422.0**	1150.0**	759.5**	180.5**	143.5**	1460.5**	2170.0**
± SD	*± 28.3*	*± 2.8*	*± 2.1*	*± 5.7*	*± 25.5*	*± 108.9*	*± 108.9*	*± 201.5*	*± 3.5*	*± 3.5*	*± 159.1*	*± 48.1*

§ Information on respective positive control is reported in Material and Method section I.A.2. 3 Concentrations were used per plate, 1 = low, 2 = middle, 3 high.

C = 1 of 3 plates was contaminated

* P < 0.05

** P < 0.01 (One sided t-Test assumes Test ≥ Control)

III. CONCLUSION:

In conclusion, under the experimental conditions reported, the test item is not mutagenic in the Ames test (standard plate and pre-incubation method) with and without metabolic activation.

3. Assessment and conclusion

<u>Assessment and conclusion by applicant</u>:

Negative for gene mutation in bacteria (*S. typhimurium* TA 100, TA 98, TA 1535 and TA 1537 and *E. coli* WP2P and WP2 uvrA) with and without metabolic activation.

The study was performed according to OECD guideline 471 (1997) and compliant with GLP. There were only minor deviations from the guideline, which were considered to not compromise the outcome of the study. The study is therefore considered valid and acceptable.

<u>Assessment and conclusion by RMS</u>:

1. Information on the study

Data point	CA 5.4.1/014
Report author	[redacted]
Report year	1996
Report title	Technical glyphosate: Reverse mutation assay "Ames test" using *Salmonella typhimurium* and *Escherichia coli*
Report No	434/014
Document No	Not reported
Guidelines followed in study	OECD 471 (1983), Commission Directive (EC) 92/69/EEC Method B14 (1992), US EPA (TSCA) guidelines
Deviations from current test guideline OECD 471 (1997)	2-Aminoanthracene was used as sole positive control substance demonstrating the functionality of the S9 mix. It was reported that cytotoxicity was observed, but it was not indicated for which strain and for which concentration. Historical control data on vehicle and positive controls were not provided. In the repeat-experiment, no parameter was changed. Both experiments were conducted under the same conditions using the plate incorporation method.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Technical glyphosate (batch: H95D161A, purity 95.3 %) was investigated in the Ames standard plate test (plate-incorporation method) for its potential to induce gene mutations in bacteria. *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* strain WP2uvrA were exposed to the test item in the presence and absence of metabolic activation (Aroclor 1254-induced rat liver S9 fraction) for approximately 48 hours at 37 °C. Vehicle (distilled water) and positive controls were included in each experiment. Based on the results of a preliminary toxicity test in strains TA100 and WP2uvrA, the concentrations for the main mutagenicity study were selected.

In two independent experiments, the cells were exposed to test item concentrations in the range of 50 to 5000 µg/plate. After 48 hours of incubation, the plates were scored for revertant colonies and examined for a thinning of the bacterial background lawn.

There was no precipitation of the test item observed. Cytotoxicity was reported, but detailed observations on cytotoxicity of individual strains and concentrations were not specified in the study report. Cytotoxicity was evaluated retrospectively by comparing the presented mean numbers of revertant colonies induced by the test item to the mean number of spontaneous revertants observed in the corresponding vehicle control. A cytotoxic effect was evident when the number of revertant colonies was 50 % of the value in corresponding vehicle controls. In the main mutagenicity test, cytotoxicity evident as a decrease in the frequency of revertant colonies was observed in the second experiment only at 5000 µg/plate, in tester strains TA98, TA100 and TA1535 in the absence of S9 mix and in tester strain TA1535 in the presence of S9 mix.

There was no significant increase in the frequency of revertant colonies as compared to vehicle controls

recorded for any of the five bacteria strains at any dose level, either with or without metabolic activation.

The number of revertants induced by the vehicle control was within the range of spontaneous revertant rates for each strain. All of the positive control chemicals produced marked increases in the frequency of revertant colonies, demonstrating the activity of the S9 mix.

Based on the experimental results and under the experimental conditions of the present study, technical glyphosate is negative for gene mutation in bacteria in the Ames standard plate test (plate incorporation method) in the presence and absence of metabolic activation.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material: Technical Glyphosate

Identification:	/
Description:	White powder,
Lot/Batch #:	H95D161A
Purity:	95.3 %
Stability of test compound:	The stability of the test item under storage conditions (at room temperature) and the stability of the test item in vehicle was not stated in the study report (at the responsibility of the sponsor).
Solvent (vehicle) used:	Sterile distilled water

2. Control materials:

Negative control:	Controls which remained untreated were included in each experiment.
Solvent (vehicle) control:	Sterile distilled water
Solvent (vehicle) /final concentration:	0.1 mL per plate
Positive controls:	Please refer to the table below.

Strain	Metabolic activation	Mutagen	Conc. [µg/plate]
S. typhimurium strains			
TA 100	-S9	N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG)	3.0
	+S9	2-Aminoanthracene (2AA)	1.0
TA 1535	-S9	N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG)	5.0
	+S9	2-Aminoanthracene (2AA)	2.0
TA 98	-S9	4-Nitroquinoline-1-oxide (4NQO)	0.2
	+S9	2-Aminoanthracene (2AA)	0.5
TA 1537	-S9	9-Aminoacridine (9AA)	80
	+S9	2-Aminoanthracene (2AA)	2.0
E. coli strain			
WP2 uvrA	-S9	N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG)	2.0
	+S9	2-Aminoanthracene (2AA)	10.0

3. Metabolic activation:

S9 mix was obtained from the livers of male Sprague-Dawley rats, weighing approx. 200 g. The animals received a single intraperitoneal injection of Aroclor 1254 at 500 mg/kg bw. The rats were sacrificed 5 days after administration and the liver homogenate was prepared. Prior to the experiment, the S9 mix was prepared immediately by mixing S9 fraction and co-factor.

S9 mix component	Concentration	Unit
Sodium phosphate buffer (pH 7.4)	100	mM
KCl	33	mM
NADPH-generating system		
Glucose 6-phosphate	5	mM
NADP	4	mM
$MgCl_2$	8	mM
S9	10	% (v/v)

4. Test organisms:

Tester strains				Bacteria batch checked for	
S. typhimurium		*E.coli*			
TA 98	✓	WP2 uvrA	✓	deep rough character (rfa)	✓
TA 100	✓	WP2 uvrA (pKM101)		ampicillin resistance (R factor plasmid)	✓
TA 1535	✓			UV-light sensitivity (absence of uvrB and uvrA genes in *S. typhimurium* and *E. coli* strains, respectively)	✓
TA 1537	✓				
TA 102					
TA 1538				Histidine and tryptophan auxotrophy (automatically via the spontaneous rate)	✓

5. Test concentrations:

(a) Preliminary cytotoxicity assay

Plate incorporation test - S9 mix:	
Concentrations:	*50, 150, 500, 1500 and 5000 µg/plate*
Tester strain:	TA 100 and WP2 uvrA
Replicates:	*Duplicate*

(b) Experiment 1 of the main mutation assay

Plate incorporation test ± S9 mix:	
Concentrations:	*50, 150, 500, 1500 and 5000 µg/plate*
Tester strain:	TA 98, TA 100, TA 1535, TA 1537 and WP2 uvrA
Replicates:	*Triplicate*

(c) Experiment 2 of the main mutation assay

Plate incorporation test ± S9 mix:			
Concentrations:		*50, 150, 500, 1500 and 5000 µg/plate*	
Tester strains:	TA 98, TA 100, TA 1535, TA 1537 and WP2 uvrA		
Replicates:		*Triplicate*	

B: STUDY DESIGN AND METHODS

1. Dates of experimental work: 19 Aug – 13 Nov 1995
Finalisation date: 20 Feb 1996

2. Standard plate test (plate-incorporation test, SPT):
An aliquot of 0.1 mL test solution or vehicle/positive control, an aliquot of 0.1 mL fresh bacterial culture and 0.5 mL S9 mix (in tests with metabolic activation) or 0.5 mL of S9 phosphate buffer (in tests without metabolic activation) were added to 2.0 mL of molten, histidine/tryptophan supplemented medium. All compounds were mixed and equally distributed onto the surface of sterile Vogel-Bonner minimal agar plates. After approximately 48 hours of incubation at 37 °C, the plates were scored for revertant colonies and examined for a diminution of the bacterial background lawn.

3. Cytotoxicity
Toxicity was detected by

- a reduction in the number of spontaneous revertant colonies
- a clearing or diminution of the background lawn (= reduced his^- and trp^- background growth)

and recorded for all test groups both with and without S9 mix in all experiments.

4. Statistics
The test results were analysed for statistical significance according to Kirkland (1989)[11].

5. Acceptance criteria
Acceptance criteria were not defined in the study report. Prior to use, the bacteria strains where checked for characteristics, viability and spontaneous reversion rates.

6. Evaluation criteria
A test item was considered to show a positive response if it induced a dose-related, statistically significant increase in the mutation rate of at least twice the spontaneous reversion rate in one or more strains in the presence and/or absence of metabolic activation in both experiments at sub-toxic levels.
A test item was considered to show a negative response if the number of induced revertants compared to spontaneous revertants were less than two-fold at each dose level, the intervals of which should be between 2 and 5 fold and extent to the limits imposed by toxicity, solubility or up to the maximum recommended dose of 5000 µg/plate.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS
Analytical determinations were not performed in the study, as not required by the test guideline.

[11] Kirkland, D.J., UKEMS Sub-committee on Guidelines for Mutagenicity Testing. Report Part III (1989), Cambridge University Press.

B. CYTOTOXICITY

Although cytotoxicity was noted in the preliminary toxicity study and in the main mutagenicity study, detailed observations on cytotoxicity of individual strains and concentrations were not further specified in the study report. Cytotoxicity was evaluated retrospectively by comparing the presented mean numbers of revertant colonies induced by the test item to the mean number of spontaneous revertants observed in the corresponding vehicle control. A cytotoxic effect was evident when the number of revertant colonies was 50 % or less of the value in corresponding vehicle controls.

In the preliminary toxicity test (-S9 mix), cytotoxicity was observed in strain TA 100 at 5000 µg/plate.

In the main mutagenicity test, cytotoxicity evident as a decrease in the frequency of revertant colonies was observed in the second experiment only at 5000 µg/plate, in tester strains TA 98, TA 100 and TA 1535 in the absence of S9 mix and in tester strain TA 1535 in the presence of S9 mix.

C. SOLUBILITY

Precipitation of the tests item was not reported.

D. MUTATION ASSAY

There was no significant increase in the frequency of revertant colonies as compared to vehicle controls recorded for any of the five bacteria strains at any dose level, either with or without metabolic activation.

The number of revertants induced by the vehicle control was within the range of spontaneous revertant rates for each strain. All of the positive control chemicals produced marked increases in the frequency of revertant colonies, demonstrating the activity of the S9 mix.

Table 5.4.1-25: Technical glyphosate: Reverse mutation assay "Ames test" using *Salmonella typhimurium* and *Escherichia coli* ([redacted] 1996), first experiment

Experiment 1: Standard plate test (SPT)										
Strain	**TA100**		**TA1535**		**WP2uvrA**		**TA 98**		**TA 1537**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Untreated										
mean	74	/	8	/	11	/	11	/	6	/
± *SD*	*± 15.3*		*± 1.2*		*± 0.6*		*± 1.2*		*± 1.5*	
Vehicle control										
Water mean	128	116	13	12	18	18	20	36	8	8
± *SD*	*± 11.5*	*± 8.2*	*± 2.5*	*± 3.8*	*± 6.7*	*± 6.7*	*± 5.7*	*± 10.8*	*± 2.3*	*± 4.4*
Test item [µg/plate]										
50 mean	124	105	17	10	13	15	25	32	11	9
± *SD*	*± 16.8*	*± 12.9*	*± 2.6*	*± 2.0*	*± 2.5*	*± 3.1*	*± 8.2*	*± 3.1*	*± 5.0*	*± 3.6*
150 mean	106	101	13	10	20	18	17	29	6	12
± *SD*	*± 19.2*	*± 15.6*	*± 4.6*	*± 4.2*	*± 3.2*	*± 4.0*	*± 3.8*	*± 2.6*	*± 3.1*	*± 2.9*
500 mean	121	118	15	11	16	18	19	29	12	11
± *SD*	*± 3.8*	*± 13.5*	*± 4.0*	*± 1.0*	*± 2.5*	*± 3.1*	*± 3.1*	*± 9.0*	*± 2.1*	*± 4.0*
1500 mean	106	93	13	11	19	20	21	36	10	10
± *SD*	*± 12.9*	*± 10.2*	*± 6.7*	*± 3.1*	*± 6.7*	*± 3.1*	*± 9.9*	*± 8.1*	*± 3.1*	*± 2.6*
5000 mean	109	116	13	10	22	22	17	36	9	11
± *SD*	*± 20.2*	*± 18.7*	*± 2.0*	*± 0.6*	*± 4.0*	*± 5.5*	*± 9.6*	*± 8.6*	*± 2.6*	*± 2.3*

Table 5.4.1-25: Technical glyphosate: Reverse mutation assay "Ames test" using *Salmonella typhimurium* and *Escherichia coli* ([redacted] 1996), first experiment

Experiment 1: Standard plate test (SPT)										
Strain	**TA100**		**TA1535**		**WP2uvrA**		**TA 98**		**TA 1537**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Positive control										
§ mean	307	406	587	110	1926	616	164	981	263	148
± *SD*	*± 6.0*	*± 69.2*	*± 222.4*	*± 2.3*	*± 96.0*	*± 111.2*	*± 18.5*	*± 93.6*	*± 86.5*	*± 21.5*

§ Information on respective positive control is reported in Material and Method section I.A.2

Table 5.4.1-26: Technical glyphosate: Reverse mutation assay "Ames test" using *Salmonella typhimurium* and *Escherichia coli* ([redacted] 1996), second experiment

Experiment 2: Standard plate test (SPT)										
Strain	**TA100**		**TA1535**		**WP2uvrA**		**TA 98**		**TA 1537**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Untreated										
mean	123	/	16	/	20	/	26	/	11	/
± *SD*	*± 16.6*		*± 3.2*		*± 0.6*		*± 3.8*		*± 5.6*	
Vehicle control										
DMSO mean	156	149	38	17	29	27	32	41	16	11
± *SD*	*± 10.7*	*± 17.0*	*± 7.0*	*± 5.3*	*± 8.0*	*± 3.2*	*± 5.0*	*± 11.9*	*± 1.0*	*± 2.5*
Test item										
50 mean	166	142	42	13	25	25	34	31	14	7
± *SD*	*± 1.5*	*± 13.3*	*± 1.2*	*± 4.6*	*± 1.7*	*± 2.6*	*± 5.9*	*± 6.7*	*± 2.6*	*± 1.0*
150 mean	165	141	32	13	18	25	31	33	11	13
± *SD*	*± 2.5*	*± 8.7*	*± 4.0*	*± 4.0*	*± 3.2*	*± 2.3*	*± 6.5*	*± 4.2*	*± 4.5*	*± 5.6*
500 mean	152	121	33	13	43	23	28	35	10	11
± *SD*	*± 1.5*	*± 15.3*	*± 5.8*	*± 3.1*	*± 37.3*	*± 5.0*	*± 7.0*	*± 4.5*	*± 5.1*	*± 2.1*
1500 mean	147	109	27	14	13	22	33	28	12	11
± *SD*	*± 5.9*	*± 5.1*	*± 7.0*	*± 5.1*	*± 1.7*	*± 2.3*	*± 8.1*	*± 2.6*	*± 3.5*	*± 1.7*
5000 mean	78	107	30	8	19	15	13	32	8	7
± *SD*	*± 9.5*	*± 23.7*	*± 0.6*	*± 1.5*	*± 2.9*	*± 1.0*	*± 7.2*	*± 7.0*	*± 1.5*	*± 3.5*
Positive control										
§ mean	725	585	482	110	991	248	183	287	865	222
± *SD*	*± 34.1*	*± 28.0*	*± 14.2*	*± 1.5*	*± 15.0*	*± 23.7*	*± 50.8*	*± 43.5*	*± 137.5*	*± 28.3*

§ Information on respective positive control is reported in Material and Method section I.A.2

III. CONCLUSION:

Based on the results and under the experimental conditions of the present study, technical glyphosate is negative for gene mutation in bacteria in the Ames standard plate test (plate incorporation method) in the presence and absence of metabolic activation.

3. Assessment and conclusion

Assessment and conclusion by applicant:
Negative for gene mutation in bacteria (*S. typhimurium* TA 100, TA 98, TA 1535, TA 1537 and *E. coli* WP2 uvrA) with and without metabolic activation.

The study was conducted according to OECD guideline 471 (1983) and in compliance with GLP. There were only minor deviations when compared to the currently valid OECD guideline 471 (1997), which were considered to not compromise the validity of the study. The study was considered valid and acceptable.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.4.1/015
Report author	[redacted]
Report year	1995
Report title	HR-001: Reverse Mutation Test
Report No	IET 94-0142
Document No	Not reported
Guidelines followed in study	U.S. EPA FIFRA Guidelines, Subdivision F; similar to OECD guideline 471 (1997)
Deviations from current test guideline OECD 471 (1997)	Compared to OECD 471 (1997) information on historical control data were not reported. In the repeat-experiment, no parameter was changed.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

S. typhimurium strains TA 98, TA 100, TA 1535 and TA 1537, and *E. coli* strain WP2 uvrA were exposed to glyphosate technical (HR-001, batch: 940908-1, purity: 95.68 %) in the presence and absence of metabolic activation (phenobarbital and 5,6 benzoflavone-induced rat liver S9 fraction). The test was performed for 48 hours at 37 °C using the pre-incubation method. Vehicle (sterile water) and positive controls were included in each experiment. The concentrations were selected based on a preliminary dose-range finding test in which the test item did not show cytotoxicity to any strain up to the highest dose of 5000 µg/plate tested. In two independent experiments of the main mutagenicity test, the test item was tested according to the pre-incubation method in triplicates of five concentrations ranging from 156 to 5000 µg/plate.

Precipitation of the test substance was not reported, although test item formulations were used at concentrations exceeding the mentioned range of solubility of the solvent. A relevant increase in the number of his[+] and trp[+] revertants (exceeding a factor of 2 when compared to solvent controls) was not observed in any experiment either with or without S9 mix. Vehicle controls showed low spontaneous reversion rates with and without metabolic activation. The positive controls induced the appropriate response in the corresponding strains, thus demonstrating the sensitivity of the test system and the metabolic activity of the S9 mix employed.

Based on the results of the present study, glyphosate is not mutagenic in the Ames pre-incubation test with and without metabolic activation under the experimental conditions chosen.

I. MATERIALS AND METHODS

A: MATERIALS

1. **Test material:** Glyphosate technical

Identification: HR-001

Description: White crystals

Lot/Batch #: 940908-1

Purity: 95.68 %

Stability of test compound: The stability of the test item under storage conditions (at approx. 5 °C in a dark cold room) and the stability of the test item in vehicle were not specified.

Solvent used: Sterile water

2. **Control materials:**

Negative control: Not specified

Solvent control: Sterile water

Solvent/final concentration: The test substance was applied at concentrations > 12 mg/mL using 0.1 mL per plate.

Positive controls: Please refer to the table below.

Strain	Metabolic activation	Mutagen	Solvent	Conc. [µg/plate]
S. typhimurium strains				
TA 100	-S9	2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2)	DMSO	0.01
	+S9	2-Aminoanthracene	DMSO	1.0
TA 1535	-S9	Sodium azide (NaN_3)	Water	0.5
	+S9	2-Aminoanthracene	DMSO	2.0
TA 98	-S9	2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2)	DMSO	0.1
	+S9	2-Aminoanthracene	DMSO	0.5
TA 1537	-S9	9-Aminoacridine (9-AA)	Water	80
	+S9	2-Aminoanthracene	DMSO	2.0
E. coli strain				
WP2 uvrA	-S9	2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2)	DMSO	0.01
	+S9	2-Aminoanthracene	DMSO	10.0

3. **Metabolic activation:**

S9 mix was purchased from [redacted] ([redacted]; Lot no. RAA-316). The homogenate was produced from the livers of male Sprague-Dawley rats, weighing 192 – 229 g, that received intraperitoneal injections of phenobarbital (30 mg/kg bw on Day 1, each 60 m g/kg bw on Days 2, 3 and 4) and 5,6 benzoflavone on Day 3. The S9 mix was prepared immediately before the experiment by mixing S9 fraction and co-factor.

S9 mix component	**Concentration**	**Unit**
Sodium phosphate buffer (pH 7.4)	100	mM
KCl	33	mM
NADPH-generating system		
Glucose 6-phosphate	5	mM
NADH	4	mM
NADP	4	mM
$MgCl_2$	8	mM
S9	10	% (v/v)

4. Test organisms:

Tester strains				**Bacteria batch checked for**	
S. typhimurium		***E.coli***			
TA 98	✓	WP2 uvrA	✓	deep rough character (rfa)	✓
TA 100	✓	WP2 uvrA (pKM101)		ampicillin resistance (R factor plasmid)	✓
TA 1535	✓			UV-light sensitivity (absence of uvrB and uvrA genes in *S. typhimurium* and *E. coli* strains, respectively)	✓
TA 1537	✓				
TA 102					
TA 1538				Histidine and tryptophan auxotrophy (automatically via the spontaneous rate)	✓

5. Test concentrations:

(a) Preliminary cytotoxicity assay:

Pre-incubation assay ± S9 mix:	
Concentrations:	*0, 200, 500, 1000, 2000 and 5000 µg/plate*
Tester strains:	TA 1535, TA 1537, TA 98, TA 100 and WP2 uvrA
Replicates:	*A single plate was used per condition.*

(b) Mutation assays:

Pre-incubation assay ± S9 mix:	
Concentrations:	*0, 156, 313, 625, 1250, 2500 and 5000 µg/plate*
Tester strains:	TA 1535, TA 1537, TA 98, TA 100 and WP2 uvrA
Replicates:	*Triplicates in two independent experiments*

B: STUDY DESIGN AND METHODS

1. **Dates of experimental work:** 21 Feb – 09 Mar 1995
   Finalisation date: 03 Apr 1995

2. **Pre-incubation test (PIT):**
0.1 mL of test solution or vehicle/positive control, 0.1 mL pre-cultured bacterial suspension and 0.5 mL of S9 mix (in tests with metabolic activation) or 0.5 mL of 100 mM sodium phosphate buffer (in tests without metabolic activation) were pre-incubated for 20 minutes at 37 °C using a shaker. Subsequently 2 mL of aminoacid-supplemented molten agar was added to each test tube. The contents were mixed uniformly and overlaid on the minimal glucose agar plate. Each concentration and the controls were tested in triplicates. After an incubation period of 48 h at 37 °C, the number of bacterial colonies (his^+ or trp^+ revertants) were counted.

3. **Cytotoxicity**
Cytotoxicity was investigated in a preliminary dose-range finding study. Toxicity was detected by a
- decrease in the number of revertants
- clearing or diminution of the background lawn (= reduced his^- or trp^- background growth)

and recorded for all test groups both with and without S9 mix in all experiments.

4. **Statistics**
Results were judged without statistical analysis.

5. **Acceptance criteria**
The test was valid if
- The culture of the tester strains, the solution of the test substance and S9 mix were free from contamination or other bacteria.
- A normal number of spontaneous revertant colonies was observed for the solvent control.
- An at least 3-fold increase above the solvent control in the mean number of revertants was observed in the positive control.

6. **Evaluation criteria**
Results were judged positive without statistical analysis when the following criteria were met:
- A two-fold or greater increase above solvent control in the mean number of revertants was observed.
- This increase in the number of revertants was accompanied by a dose-response relationship.
- This increase in the number of revertants was reproducible.

Reproducibility of results was confirmed by two independent experiments.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS
Analytical determinations were not performed in the study, as not required by the test guideline.

B. CYTOTOXICITY
In the preliminary cytotoxicity test, the test substance did not show any cytotoxicity to any strain up to the highest dose of 5000 µg/plate with and without metabolic activation.

In the main mutation study there was also no cytotoxicity observed up to the highest concentration tested.

C. SOLUBILITY

There was no precipitation of the test substance observed.

D. MUTATION ASSAY

A relevant increase in the number of his^+ or trp^+ revertants was not observed in any experiment at any tested concentration either in the presence or absence of metabolic activation.

The number of revertants induced by the vehicle control was within expected range for each strain, thus demonstrating an acceptable experimental performance.

Appropriate positive control compounds induced a marked increase in the number of revertants, demonstrating the validity of the test system and the functionality of the S9 mix.

Table 5.4.1-27: Reverse Mutation Test ([redacted], 1995), first experiment

Experiment 1: Pre-incubation test (PIT)										
Strain	**TA 100**		**TA 1535**		**WP2 uvrA**		**TA 98**		**TA 1537**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Vehicle control										
Water mean	117	78	12	9	21	21	37	35	3	7
± *SD*	± *6*	± *5*	± *3*	± *0*	± *3*	± *1*	± *11*	± *4*	± *2*	± *5*
Test item [µg/plate]										
156 mean	119	83	11	6	12	19	40	36	3	9
± *SD*	± *9*	± *4*	± *2*	± *4*	± *2*	± *3*	± *10*	± *2*	± *2*	± *3*
313 mean	117	79	11	7	16	19	42	31	4	5
± *SD*	± *12*	± *5*	± *1*	± *2*	± *5*	± *4*	± *6*	± *3*	± *1*	± *3*
625 mean	139	99	9	6	15	19	39	30	2	8
± *SD*	± *8*	± *11*	± *3*	± *4*	± *1*	± *1*	± *10*	± *5*	± *2*	± *3*
1250 mean	125	93	9	6	22	22	43	37	5	6
± *SD*	± *3*	± *17*	± *1*	± *2*	± *4*	± *5*	± *6*	± *4*	± *4*	± *1*
2500 mean	106	73	3	7	15	16	38	39	3	7
± *SD*	± *12*	± *12*	± *1*	± *6*	± *3*	± *2*	± *10*	± *2*	± *2*	± *3*
5000 mean	105	56	4	3	20	16	39	25	2	4
± *SD*	± *11*	± *9*	± *2*	± *2*	± *5*	± *6*	± *7*	± *5*	± *1*	± *2*
Positive control										
[§] mean	510	606	524	392	305	522	621	360	786	75
± *SD*	± *13*	± *70*	± *37*	± *58*	± *28*	± *15*	± *7*	± *41*	± *82*	± *6*

[§] Information on respective positive control is reported in Material and Method section I.A.2

Table 5.4.1-28: Reverse Mutation Test ([redacted], 1995), second experiment

Experiment 2: Pre-incubation test (PIT)										
Strain	**TA 100**		**TA 1535**		**WP2 uvrA**		**TA 98**		**TA 1537**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Vehicle control										

Table 5.4.1-28: Reverse Mutation Test [redacted], 1995), second experiment

Experiment 2: Pre-incubation test (PIT)										
Strain	**TA 100**		**TA 1535**		**WP2 uvrA**		**TA 98**		**TA 1537**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Water mean	146	123	9	8	16	17	24	37	5	7
± *SD*	± *11*	± *12*	± *1*	± *2*	± *4*	± *8*	± *4*	± *5*	± *1*	± *2*
Test item [µg/plate]										
156 mean	137	112	10	7	18	15	18	32	7	10
± *SD*	± *7*	± *5*	± *3*	± *3*	± *3*	± *1*	± *4*	± *9*	± *4*	± *4*
313 mean	*140*	*125*	*7*	*7*	*19*	*13*	*20*	*29*	*4*	*9*
± *SD*	± *13*	± *12*	± *2*	± *3*	± *2*	± *4*	± *4*	± *6*	± *1*	± *2*
625 mean	*136*	*113*	*8*	*8*	*17*	*20*	*18*	*35*	*3*	*9*
± *SD*	± *22*	± *4*	± *3*	± *1*	± *3*	± *5*	± *4*	± *6*	± *2*	± *4*
1250 mean	*136*	*107*	*7*	*7*	*15*	*14*	*15*	*28*	*3*	*9*
± *SD*	± *12*	± *9*	± *2*	± *3*	± *5*	± *1*	± *7*	± *4*	± *2*	± *1*
2500 mean	144	89	6	7	18	19	10	20	3	8
± *SD*	± *15*	± *10*	± *2*	± *3*	± *4*	± *7*	± *1*	± *6*	± *1*	± *3*
5000 mean	117	67	10	4	14	17	9	17	4	4
± *SD*	± *14*	± *3*	± *4*	± *2*	± *5*	± *4*	± *2*	± *4*	± *3*	± *2*
Positive control										
§ mean	595	768	527	322	252	605	742	327	909	87
± *SD*	± *23*	± *56*	± *87*	± *32*	± *27*	± *35*	± *36*	± *20*	± *131*	± *9*

§ Information on respective positive control is reported in Material and Method section I.A.2

III. CONCLUSION:

According to the results of the present study, the test item is not mutagenic in the Ames pre-incubation test with and without metabolic activation under the experimental condition of the test.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Negative for gene mutation in bacteria (*S. typhimurium* TA 100, TA 98, TA 1535 and TA 1537 and *E. coli* WP2 uvrA) with and without metabolic activation.

The study was conducted according to OECD guideline 471 (1983) and in compliance with GLP. There were only minor deviations when compared to the currently valid OECD guideline 471 (1997), which were considered to not compromise the validity of the study. The study was considered valid and acceptable.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.4.1/016
Report author	[redacted]
Report year	1995
Report title	Study of the ability of the test article glyphosate to induce gene mutations in strains of *Salmonella typhimurium*
Report No	940724
Document No	Not reported
Guidelines followed in study	The study was conducted according to the main criteria of OECD TG 471 (1983)
GLP	Yes
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	*S. typhimurium* strains TA 98, TA 100, TA 102, TA 1535 and TA 1537 were exposed to glyphosate (purity not reported) in the presence and absence of metabolic activation (liver S9 fraction). Test item concentrations were in the range of 50 – 5000 µg/plate.
Short description of results:	Negative for mutagenicity in bacteria (Ames test) up to limit concentrations of 5000 µg/plate in the presence and absence of metabolic activation.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	Considered invalid based on the information in RAR (2015).
Reasons why the study report is not available for submission	The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL.
Category study in AIR5 dossier (L-docs)	Category 4b

1. Information on the study

Data point:	CA 5.4.1/017
Report author	[redacted]
Report year	1995
Report title	Glyphosate: Reverse mutation assay "Ames test" using *Salmonella typhimurium*
Report No	710/20
Document No	Not reported
Guidelines followed in study	The study was conducted according to the main criteria of OECD TG 471 (1983)
GLP	Yes
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	*S. typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 were exposed to glyphosate (purity: 95 %) in the presence and absence of metabolic activation (liver S9 fraction). Test item concentrations were in the range of 8 – 5000 µg/plate.

Short description of results:	Negative for mutagenicity in bacteria (Ames test) up to limit concentrations of 5000 µg/plate in the presence and absence of metabolic activation.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	Considered invalid based on the information in RAR (2015).
Reasons why the study report is not available for submission	The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL.
Category study in AIR5 dossier (L-docs)	Category 4b

1. Information on the study

Data point	CA 5.4.1/018
Report author	[redacted]
Report year	1993
Report title	Mutagenicity – *Salmonella typhimurium* reverse mutation assay (Ames test)
Report No	887-MUT.AMES
Document No	Not reported
Guidelines followed in study	OECD 471 (1983)
Deviations from current test guideline OECD 471 (1997)	The test was conducted in 4 valid strains only. Strains like *S. typhimurium* TA 102 or *E. coli* WP2 enabling the detection of cross-linking mutagens were not included. In addition, the bacterial cell density was not confirmed and no historical control data were provided. Further, acceptance criteria were not specified in the study report.
Previous evaluation	Not accepted in RAR 2015
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

Glyphosate technical (batch 046, purity: 96.0 %) was assessed for its ability to cause gene mutations in *S. typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 in the presence and absence of metabolic activation (Aroclor 1254-induced rat liver S9 fraction). Based on the results of a preliminary cytotoxicity test, in which no cytotoxicity was noted up to a concentration of 1000 µg/plate, concentrations for the main mutagenicity assay were selected. Two independent plate-incorporation tests (standard plate tests) were performed, using glyphosate concentrations in the range of 1 – 1000 µg/plate. Solvent (distilled water) and appropriate positive controls were included in each experiment. After 48 hours of incubation at 37 °C, the bacterial background lawn was examined and the number of revertant colonies was counted for

each plate.

There was no precipitation up to the highest tested concentration, neither in the presence nor absence of S9 mix. Cytotoxicity, evident as a thinning of the bacterial background lawn, was noted at 1000 µg/plate; however, it was not specified for which strains the observation was made and whether the observation was made in the presence or absence of metabolic activation.

There was no statistically significant, toxicologically relevant increase in the number of revertant colonies observed in any experiment up to the highest concentration, neither in the presence nor in the absence of metabolic activation. Although a statistically significant increase in the number of his$^+$ revertant colonies of about 40 % was observed for strain TA 100 at the top concentration of 1000 µg/plate in the presence of metabolic activation, the increase was less than two-fold compared to the number of revertant colonies induced by the solvent control and therefore considered to be incidental.

The number of revertant colonies induced by the vehicle and appropriate positive controls were in the expected range, demonstrating the functionality of the S9 mix and the sensitivity of the test system.

Under the conditions of the test, glyphosate technical was negative for gene mutation in bacteria in the presence and absence of metabolic activation.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material: Glyphosate technical

Identification:	FSG 03090 h/05, March 1990
Description:	Solid white coloured crystals, odourless
Lot/Batch #:	046
Purity:	96.0 %
Stability of test compound:	The stability of the test item under storage conditions (at room temperature) was guaranteed until the expected expiry date July 1994. The stability of the test item in solvent (vehicle) was not specified.
Solvent (vehicle) used:	Distilled water

2. Control materials:

Negative control:	A negative control was not employed in this study.
Solvent (vehicle) control:	Distilled water
Solvent (vehicle)/final concentration:	0.1 mL per plate.
Positive controls:	Please refer to table below.

Strain	Metabolic activation	Mutagen	Conc. [µg/plate]
S. typhimurium strains			
TA 100	-S9	Sodium azide	0.5
	+S9	2-Aminofluorene	20.0
TA 1535	-S9	Sodium azide	0.5
	+S9	Sodium azide	0.5
TA 98	+S9	2-Nitrofluorene	2.0
	+S9	2-Aminofluorene	20.0
TA 1537	-S9	9-Aminoacridine	50.0
	+S9	9-Aminoacridine	50.0
TA 1538	-S9	2-Nitrofluorene	5.0
	+S9	2-Aminofluorene	20.0

3. Metabolic activation:

S9 mix was produced from the livers of male Wistar rats, that received a single intraperitoneal injection of Aroclor 1254 at a dose level of 500 mg/kg bw. The livers were prepared five days after the injection. The S9 mix was thawed prior to each experiment and co-factor was immediately added to prepare S9 mix containing the following components:

S9 mix component	Concentration	Unit
Phosphate buffer (pH 7.4)		
KCl	33	mM
NADPH-generating system		
Glucose 6-phosphate, K salt	5	mM
NADP, di-Na salt	4	mM
$MgCl_2$	8	mM
S9	10	% (v/v)

4. Test organisms:

Tester strains				Bacteria batch checked for	
S. typhimurium		***E.coli***			
TA 98	✓	WP2 uvrA		deep rough character (rfa)	✓
TA 100	✓	WP2 uvrA (pKM101)		ampicillin resistance (R factor plasmid)	✓
TA 1535	✓			UV-light sensitivity (absence of uvrB and uvrA genes in *S. typhimurium* and *E. coli* strains, respectively)	✓
TA 1537	✓				
TA 1538	✓			Histidine and tryptophan auxotrophy (automatically via the spontaneous rate)	✓

5. Test concentrations:

(a) Preliminary cytotoxicity assay:

Plate incorporation test ± S9 mix:	
Concentrations:	*10, 30, 100, 350 and 1000 µg/plate*
Tester strains:	TA 100

Replicates:	*Duplicates*

(b) Mutation assays:

Plate incorporation test ± S9 mix:	
Concentrations:	*1, 3, 10, 100 and 1000 µg/plate*
Tester strains:	TA 98, TA 100, TA 1535, TA 1537 and TA 1538
Replicates:	*Triplicates*

B: STUDY DESIGN AND METHODS

1. Dates of experimental work: Dec 1992 – Jan 1993
Finalisation date: 30 Apr 1993

2. Standard plate test (plate-incorporation test, SPT):
An aliquot of 0.1 mL test solution, vehicle or positive control, an aliquot of 0.1 mL bacterial culture (2 × 10^9 cells/mL) and 0.5 mL S9 mix (in tests with metabolic activation) or 0.5 mL of 0.05 M phosphate buffer (in tests without metabolic activation) were added to 2 mL of soft agar (supplemented with 0.5 mM L-histidine + 0.5 mM D-biotin). After vortexing, the mixture was overlaid onto the surface of minimal bottom agar plates. Each concentration and the controls were tested in triplicates. The cells were incubated at 37 °C for 48 hours. Afterwards, the background bacterial lawn was examined and the bacterial colonies were counted.

3. Cytotoxicity
Toxicity was detected by a

- reduction in the number of spontaneous revertants
- clearing or diminution of the background lawn (= reduced his^- background growth)

and recorded for all test groups both with and without S9 mix in all experiments.

4. Statistics
For each triplicate plating, an average and standard deviation were calculated. The results were evaluated for statistical analysis; however, it was not specified in the study report which kind of test was used for statistical evaluation.

5. Acceptance criteria
Acceptance criteria were not specified in the study report.

6. Evaluation criteria
A test item was considered as a possible mutagen if the number of revertant colonies was at least two-fold the number of spontaneous revertants observed for the solvent control.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS
Analytical determinations were not performed in the study, as not required by the test guideline.

B. CYTOTOXICITY

In the preliminary toxicity test in strain TA 100, a slight thinning of the bacterial background lawn was observed at 350 and 1000 µg/plate in the presence of metabolic activation. The number of revertant colonies was comparable to those of solvent controls.

In the main mutagenicity assay, cytotoxicity, evident as a slight thinning of the bacterial background lawn, was noted at the highest concentration of 1000 µg/plate. However, it was not specified for which strains the observation was made and whether the observation was made in the presence or absence of metabolic activation. Evaluation of the colony shape and size revealed that upon treatment with glyphosate at ≥ 100 µg/plate rough edged colonies were noticed.

C. SOLUBILITY

Precipitation of the test item in minimal basal agar plates was tested in a preliminary precipitation test. There was no precipitation up to the highest concentration of 1000 µg/plate. In the main mutagenicity assay there was as well no precipitation reported at any of the tested concentration, either in the presence or in absence of S9 mix.

D. MUTATION ASSAY

There was no statistically significant, toxicologically relevant increase in the number of revertant colonies observed in any experiment up to the highest concentration, neither in the presence nor in the absence of metabolic activation. A statistically significant increase in the number of his^+ revertant colonies of about 40 % was observed for strain TA 100 at the top concentration of 1000 µg/plate in the presence of metabolic activation, but as the increase was less than two-fold compared to the number of revertant colonies induced by the solvent control, the observation was considered incidental.

The number of revertant colonies induced by the vehicle and appropriate positive controls were in the expected range, demonstrating the functionality of the S9 mix and the sensitivity of the test system.

Table 5.4.1-29: Study of the ability of the test article glyphosate to induce gene mutations in strains of *Salmonella typhimurium* ([redacted], 1993), first experiment

Experiment 1: Standard plate test (SPT)										
Strain	**TA 98**		**TA 100**		**TA 1535**		**TA 1537**		**TA 1538**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Vehicle control										
Water mean	27	19	120	114	11	16	6	12	31	11
± *SD*	± *2.0*	± *2.0*	± *9.0*	± *5.1*	± *4.5*	± *2.0*	± *1.7*	± *2.6*	± *1.5*	± *1.0*
Test item [µg/plate]										
1 mean	34	21	99	124	12	12	10	14	31	10
± *SD*	± *2.5*	± *1.5*	± *2.5*	± *11.1*	± *1.0*	± *2.5*	± *5.7*	± *4.6*	± *2.8*	± *4.1*
3 mean	31	18	109	134	10	18	6	15	33	9
± *SD*	± *0.6*	± *2.0*	± *30.3*	± *9.0*	± *3.2*	± *2.0*	± *0.6*	± *5.0*	± *1.0*	± *3.2*
10 mean	28	31	117	120	10	18	4	9	29	8
± *SD*	± *1.0*	± *3.6*	± *9.2*	± *4.6*	± *3.4*	± *4.9*	± *0.6*	± *2.5*	± *1.2*	± *1.5*
100 mean	32	25	125	154	7	16	7	17	28	11
± *SD*	± *4.5*	± *4.0*	± *30.2*	± *9.6*	± *0.6*	± *4.0*	± *1.7*	± *4.0*	± *2.3*	± *2.0*
1000	31	22	126	**155***	7	15	7	11	28	9

Table 5.4.1-29: Study of the ability of the test article glyphosate to induce gene mutations in strains of *Salmonella typhimurium* ([redacted], 1993), first experiment

Experiment 1: Standard plate test (SPT)										
Strain	**TA 98**		**TA 100**		**TA 1535**		**TA 1537**		**TA 1538**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
mean ± *SD*	 ± *5.5*	 ± *3.8*	 ± *12.5*	 ± *14.2*	 ± *1.1*	 ± *1.0*	 ± *2.6*	 ± *3.5*	 ± *3.0*	 ± *3.7*
Positive control										
§ mean	78	89	473	356	190	375	89	74	84	84
± *SD*	± *10.4*	± *3.6*	± *41.6*	± *40.4*	± *10.0*	± *25.0*	± *16.3*	± *8.1*	± *20.1*	± *6.0*

§ Information on respective positive control is reported in Material and Method section A.2

* A statistically significant increase in the number of his+ revertant colonies of about 40 % was observed for strain TA100 at the top concentration of 1000 µg/plate in the presence of metabolic activation (trial 1/2 combined), but as the increase was less than two-fold compared to the number of revertant colonies induced by the solvent control, the observation was considered incidental

Table 5.4.1-30: Study of the ability of the test article glyphosate to induce gene mutations in strains of *Salmonella typhimurium* ([redacted] 1993), second experiment

Experiment 2: Standard plate test (SPT)										
Strain	**TA 98**		**TA 100**		**TA 1535**		**TA 1537**		**TA 1538**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Vehicle control										
Water mean	14	17	91	111	12	9	5	4	26	6
± *SD*	± *4.0*	± *3.4*	± *4.3*	± *18.0*	± *1.7*	± *1.5*	± *2.5*	± *1.1*	± *6.0*	± *2.0*
Test item										
1 mean	12	13	115	103	8	11	2	9	15	8
± *SD*	± *2.5*	± *0.5*	± *32.5*	± *9.8*	± *5.1*	± *4.5*	± *0.5*	± *4.0*	± *3.0*	± *1.5*
3 mean	17	16	114	94	6	10	4	6	14	8
± *SD*	± *5.1*	± *1.0*	± *11.3*	± *8.1*	± *1.5*	± *4.1*	± *1.7*	± *2.0*	± *1.5*	± *2.0*
10 mean	12	13	100	116	3	6	4	9	14	7
± *SD*	± *5.0*	± *2.5*	± *7.5*	± *9.6*	± *1.2*	± *1.5*	± *2.6*	± *3.5*	± *2.5*	± *1.0*
100 mean	15	11	114	101	5	8	4	11	11	8
± *SD*	± *3.6*	± *2.8*	± *13.0*	± *7.5*	± *0.5*	± *2.0*	± *1.1*	± *3.7*	± *4.5*	± *3.2*
1000 mean	12	14	85	**177***	6	7	5	7	12	7
± *SD*	± *2.5*	± *3.4*	± *3.0*	± *13.4*	± *2.0*	± *0.5*	± *3.2*	± *1.5*	± *1.5*	± *1.1*
Positive control										
§ mean	95	135	396	420	171	372	85	128	75	76
± *SD*	± *22.9*	± *13.2*	± *15.3*	± *20.0*	± *7.6*	± *7.5*	± *6.4*	± *7.0*	± *12.8*	± *15.0*

§ Information on respective positive control is reported in Material and Method section A.2

* A statistically significant increase in the number of his+ revertant colonies of about 40 % was observed for strain TA100 at the top concentration of 1000 µg/plate in the presence of metabolic activation (trial 1/2 combined), but as the increase was less than

Table 5.4.1-30: Study of the ability of the test article glyphosate to induce gene mutations in strains of *Salmonella typhimurium* ([redacted], 1993), second experiment

Experiment 2: Standard plate test (SPT)										
Strain	**TA 98**		**TA 100**		**TA 1535**		**TA 1537**		**TA 1538**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**

two-fold compared to the number of revertant colonies induced by the solvent control, the observation was considered incidental

III. CONCLUSION

Based on the experimental results, glyphosate technical did not induce a positive response in the number of revertant colonies in any experiment in any tester strain and was therefore considered negative for mutagenicity in bacteria, both, in the presence and absence of metabolic activation.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Negative for gene mutation in bacteria (*S. typhimurium* TA 98, TA 100, TA 1535, TA 1537 and TA1538) with and without metabolic activation.

The study was considered as supporting information as it was conducted in 4 valid strains only. Strains like *S. typhimurium* TA102 or *E. coli* WP2 enabling the detection of cross-linking mutagens were not included. Further deviations were of minor degree and considered to not compromise the validity of the study.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.4.1/019
Report author	[redacted]
Report year	1993
Report title	Mutagenicity evaluation of glyphosate in *Salmonella* / microsomal reversion assay (Ames test)
Report No	87BMA012-E
Document No	Not reported
Guidelines followed in study	No guideline followed. The test was conducted similarly to OECD 471 (1997).
GLP	Yes
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	*S. typhimurium* strains TA 98 and TA 100 were exposed to glyphosate isopropylamine salt (SN-750721, batch: not reported, purity: 64.5 %) in the presence and absence of metabolic activation (Aroclor 1254-induced rat liver S9 fraction). A single experiment was performed, using the plate-incorporation method and five replicates per condition. Both bacterial strains were exposed to test

	item concentrations in the range of 0.01 – 100 µg/plate in the presence and absence of S9 mix. Untreated, solvent (DMSO) and positive controls were included. After 2 days of incubation at 37 °C, the number of revertant colonies per plate was determined. Evaluation of precipitation and cytotoxicity were not included in the study report.
Short description of results:	When evaluating cytotoxicity based on the number of revertant colonies in comparison to those of negative controls, treatment with glyphosate isopropylamine salt revealed no evidence for cytotoxicity. There was a statistically significant increase in the frequency of revertant colonies noted for strain TA 100 at 0.01, 0.1, 1 and 100 µg/plate in the presence of metabolic activation, however, the increases were less than two-fold of those of untreated / solvent controls and without a clear dose-response relationship, therefore the findings were considered to be incidental. There were no statistically significant increases in the number of revertant colonies observed upon treatment with the test item in strain TA98. The number of revertant colonies obtained by the untreated and solvent controls were in the expected range. The positive controls strongly increased the number of revertant colonies of more than twice than those of untreated controls, demonstrating the functionality of the S9 mix and the sensitivity of the test. Based on the experimental results, glyphosate isopropylamine salt was not mutagenic in the Ames plate incorporation test, neither with nor without metabolic activation.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	The study was considered not acceptable as it was conducted in two strains of *S. typhimurium* only (TA 98 and TA 100), with a test material of only 64 % purity. In addition, it was not clear whether the given purity refers to the contents of glyphosate in the formulation or the salt. A single experiment using 5 replicates per conditions was conducted, but a confirmatory experiment was not included. Test item concentrations up to 100 µg/mL were used, but a justification for the selection of concentrations was not given. In addition, no historical control data were provided.
Reasons why the study report is not available for submission	
Category study in AIR5 dossier (L-docs)	Category 3b

1. Information on the study

Data point	CA 5.4.1/020
Report author	[redacted]
Report year	1991
Report title	Mutagenicity test: Ames Salmonella Assay with Glyphosate, batch 206-JaK-25-1
Report No	12323
Document No	Not reported
Guidelines followed in study	OECD 471 (1983), US EPA FIFRA 84-2
Deviations from current	The test was conducted in 4 valid strains only, strains like *S. typhimurium*

test guideline OECD 471 (1997)	TA102 or *E. coli* WP2 enabling the detection of cross-linking mutagens were not included. In addition, 2-aminoanthracene was used as sole positive control substance in the presence of S9 mix and historical control data were not provided. Acceptance and evaluation criteria were not specified in the study report.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

S. typhimurium strains TA 98, TA 100, TA 1535 and TA 1537 were exposed to glyphosate (batch: 206-JaK-25-1, purity: 98.6 %) in the presence and absence of metabolic activation (Aroclor 1254-induced rat liver S9 fraction). A preliminary cytotoxicity test was performed in strain TA98. Cytotoxicity was evident at 1670 and 5000 µg/plate in the absence of S9 mix only, but not in the presence of S9 mix. Based on these observations, the concentrations for the main mutagenicity study were selected. In the main mutation assay two independent experiments were performed, using the plate incorporation (first experiment) and the pre-incubation method (second experiment) with concentrations in the range of 160 - 2500 µg/plate in the absence of S9 mix and concentrations in the range of 310 – 5000 µg/plate in the presence of S9 mix. Negative and positive controls were included in each experiment.

Precipitation of the test item was not reported. Cytotoxicity, evident as a reduction in the mean number of spontaneous revertant colonies, was noted solely for strain TA100 at 2500 µg/plate in the absence of S9 mix in both experiments, at 5000 µg/plate in the first experiment with S9 mix and at 2500 and 5000 µg/plate in the second experiment with S9 mix.

There was no statistically significant increase in the number of his$^+$ revertant colonies observed in any experiment for any strain at any concentration, neither in the presence nor in the absence of S9 mix. The number of revertant colonies induced by the negative and the positive controls were within the expected range, displaying the sensitivity of the test and the functionality of the metabolic activation system.

Based on the results of the present study and under the experimental conditions chosen, glyphosate is not mutagenic in the Ames test with and without metabolic activation.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material: Glyphosate

Identification: Not specified

Description: White powder

Lot/Batch #: 206-JaK-25-1

Purity: 98.6 %

Stability of test compound:	The stability of tehe test item at storage conditions (at room temperature in the dark) or in solvent (vehicle) was not specified.
Solvent (vehicle) used:	Distilled water

2. Control materials:

Negative control:	Untreated controls were included in each experiment.
Solvent (vehicle) control:	Not examined.
Solvent (vehicle)/final concentration:	0.3 mL per plate.
Positive controls:	Please refer to the table below.

Strain	Metabolic activation	Mutagen	Conc. [µg/plate]
S. typhimurium strains			
TA 100	-S9	Sodium azide	0.5
	+S9	2-Aminoanthracene	1.25
TA 1535	-S9	Sodium azide	1.0
	+S9	2-Aminoanthracene	1.25
TA 98	-S9	2-Nitrofluorene	0.6
	+S9	2-Aminoanthracene	1.25
TA 1537	-S9	2-Nitrofluorene	0.6
	+S9	2-Aminoanthracene	1.25

3. Metabolic activation:

S9 mix was obtained from livers of Wistar Mol:WIST rats weighing approx. 200 g. The animals received a single intraperitoneal injection of Aroclor 1254 at a dose of 500 mg/kg bw. The livers were prepared 5 days after treatment. The S9 mix was thawed prior to each experiment and co-factor was immediately added to prepare S9 mix containing the following components:

S9 mix component	Concentration	Unit
Phosphate buffer (pH 7.4)	100	mM
KCl	33	mM
NADPH-generating system		
Glucose 6-phosphate	5	mM
NADP	4	mM
$MgCl_2$	8	mM
S9 Standard plate test (plate-incorporation) Pre-incubation test	 4 7	 % (v/v) % (v/v)

4. Test organisms:

Tester strains				Bacteria batch checked for	
S. typhimurium		***E. coli***			
TA 98	✓	WP2 uvrA		deep rough character (rfa)	✓
TA 100	✓	WP2 uvrA (pKM101)		ampicillin resistance (R factor plasmid)	✓
TA 1535	✓			UV-light sensitivity (absence of uvrB and uvrA genes in *S. typhimurium* and *E. coli* strains, respectively)	✓
TA 1537	✓				
TA 1538				Histidine auxotrophy (automatically via the spontaneous rate)	✓

5. Test concentrations:

(a) Preliminary cytotoxicity assay:

Plate incorporation test ± S9 mix:	
Concentrations:	*560, 1670 and 5000 µg/plate*
Tester strains:	TA 98
Replicates:	*Triplicates*

(b) Mutation assays:

Plate incorporation test ± S9 mix:	
Concentrations: -S9 mix +S9 mix	*160, 310, 630, 1300 and 2500 µg/plate* *310, 630, 1300, 2500 and 5000 µg/plate*
Tester strains:	TA 1535, TA 1537, TA 98 and TA 100
Replicates:	*Triplicates*
Pre-incubation test ± S9 mix:	
Concentrations: -S9 mix +S9 mix	*160, 310, 630, 1300 and 2500 µg/plate* *310, 630, 1300, 2500 and 5000 µg/plate*
Tester strains:	TA 1535, TA 1537, TA 98 and TA 100
Replicates:	*Triplicates*

B: STUDY DESIGN AND METHODS

1. Finalisation date: 10 Sep 1991

2. Standard plate test (plate-incorporation test, SPT):
An aliquot of 0.3 mL test solution or vehicle/positive control, an aliquot of 0.1 mL fresh bacterial culture and 0.5 mL S9 mix (in tests with metabolic activation only) were added to 2 mL of molten top agar (supplemented with 0.5 mM L-histidine + 0.5 mM biotin). After whirl mixing, the mixture was spread on a Vogel-Bonner agar plate and incubated for 48 – 72 hours at 37 °C. Each concentration and the controls were tested in triplicates. Following incubation, the bacterial background lawn was examined and the number of his^+ revertant colonies was counted.

3. Pre-incubation test (PIT):
0.3 mL of test solution or vehicle/positive control, 0.1 mL pre-cultured bacterial suspension and 0.5 mL of S9 mix (in tests with metabolic activation only) were mixed and pre-incubated in a test tube for 30 minutes at 37 °C under gentle shaking. After pre-incubation, 2.0 mL of top agar was added, the mixture

was whirl-mixed and spread on a Vogel-Bonner agar plate. After an incubation period of 48 – 72 hours at 37 °C the bacterial background lawn was examined and the number of his^+ revertant colonies was counted.

4. Cytotoxicity

Toxicity was detected by a

- reduction in the number of spontaneous revertants
- clearing or diminution of the background lawn (= reduced his^- or trp^- background growth)

and recorded for all test groups both with and without S9 mix in all experiments.

5. Statistics

Statistical analysis of the negative control versus test data was performed using the Analysis of Variance method. Statistical analysis of the negative versus positive control data was performed using the Student's t-test.

6. Acceptance criteria

Acceptance criteria were not specified in the study report.

7. Evaluation criteria

Evaluation criteria were not specified in the study report.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Analytical determinations have not been performed in the study, as not required by the test guideline.

B. CYTOTOXICITY

In the preliminary toxicity test in strain TA98, cytotoxicity was observed in the absence of S9 mix at 1670 and 5000 µg/plate. There was no cytotoxicity noted in the presence of S9 mix. Based on these findings, the top concentrations for the main mutagenicity test were chosen to be 2500 µg/plate without S9 mix and 5000 µg/plate with S9 mix.

In the main mutagenicity assay, no clear depression of the bacterial background growth was observed at any concentration with or without S9 mix. However, cytotoxicity evident as a reduction in the mean number of spontaneous revertants was observed for strain TA100 in both experiments at 2500 µg/plate in the absence of S9 mix, at 5000 µg/plate in the first experiment (standard plate test, plate incorporation method) with S9 mix and at 2500 and 5000 µg/plate in the second experiment (pre-incubation test) with S9 mix. There was no relevant cytotoxicity observed in any other tester strain in the presence or absence of metabolic activation.

C. SOLUBILITY

Precipitation was not evaluated in the study report.

D. MUTATION ASSAY

There was no statistically significant increase in the number of his^+ revertant colonies observed in any experiment for any strain at any concentration, neither in the presence nor in the absence of S9 mix. The number of revertant colonies induced by the negative and the positive controls were within the expected range, displaying the sensitivity of the test and the functionality of the metabolic activation system.

Table 5.4.1-31: Mutagenicity test: Ames Salmonella Assay with Glyphosate ([redacted], 1991), first experiment

Experiment 1: Standard plate test (SPT)								
Strain	**TA100**		**TA 98**		**TA 1537**		**TA 1535**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Negative control								
mean	175.7	198.3	33.0	36.0	11.0	13.0	20.0	20.7
± *SD*	± *14.4*	± *10.4*	± *12.5*	± *4.4*	± *1.0*	± *1.0*	± *1.0*	± *2.9*
Test item [µg/plate]								
160 mean	153.0	/	30.3	/	10.7	/	23.0	/
± *SD*	± *5.3*	/	± *0.6*	/	± *3.2*		± *0.0*	/
310 mean	182.3	182.3	33.0	38.7	11.0	10.3	19.7	25.7
± *SD*	± *11.5*	± *27.5*	± *5.6*	± *7.7*	± *1.7*	± *3.2*	± *5.0*	± *4.0*
630 mean	151.3	187.7	30.3	42.7	10.0	12.3	19.3	20.0
± *SD*	± *14.7*	± *20.4*	± *4.5*	± *5.5*	± *1.0*	± *2.1*	± *5.1*	± *3.0*
1300 mean	97.7	124.3	27.3	35.0	10.3	11.7	13.3	21.3
± *SD*	± *7.8*	± *10.0*	± *5.7*	± *6.6*	± *2.3*	± *2.1*	± *1.5*	± *4.9*
2500 mean	75.7#	104.0	23.0	35.7	9.3	13.0	13.0	16.3
± *SD*	± *18.6*	± *8.7*	± *2.6*	± *5.5*	± *0.6*	± *5.0*	± *1.0*	± *0.6*
5000 mean	/	76.3#	/	26.0	/	8.7	/	13.3
± *SD*	/	± *12.6*	/	± *3.5*	/	± *0.5*	/	± *3.5*
Positive control								
§ mean	880.0**	1120.0**	317.3**	1076.7**	127.7**	147.3**	1053.3**	218.7**
± *SD*	± *98.5*	± *158.2*	± *12.1*	± *326.5*	± *22.1*	± *6.4*	± *225.0*	± *16.7*

§ Information on respective positive control is reported in Material and Method section I.A.2

** Statistically significant at 1 % level (analysis of variance)

Cytotoxicity observed

Table 5.4.1-32: Mutagenicity test: Ames Salmonella Assay with Glyphosate ([redacted] 1991), second experiment

Experiment 2: Pre-incubation Test (PIT)								
Strain	**TA100**		**TA 98**		**TA 1537**		**TA 1535**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Negative control								
mean	182.3	197.0	44.3	20.3	13.7	5.3	26.3	14.7
± *SD*	± *9.5*	± *18.2*	± *7.5*	± *1.5*	± *2.1*	± *0.6*	± *4.9*	± *0.6*
Test item [µg/plate]								

Table 5.4.1-32: Mutagenicity test: Ames Salmonella Assay with Glyphosate ([redacted], 1991), second experiment

Experiment 2: Pre-incubation Test (PIT)								
Strain	**TA100**		**TA 98**		**TA 1537**		**TA 1535**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
160 mean	195.7	/	52.0	/	15.7	/	28.7	/
± *SD*	± *6.8*	/	± *4.0*	/	± *1.5*	/	± *4.5*	/
310 mean	185.0	182.7	49.3	23.3	14.0	7.3	30.3	15.7
± *SD*	± *19.1*	± *18.8*	± *11.9*	± *7.5*	± *6.1*	± *2.1*	± *3.8*	± *2.1*
630 mean	133.3	169.7	44.0	22.3	8.3	6.0	32.7	15.0
± *SD*	± *10.5*	± *8.0*	± *9.2*	± *0.6*	± *3.1*	± *1.0*	± *7.5*	± *1.0*
1300 mean	110.0	122.0	35.0	25.0	16.3	6.0	29.3	14.3
± *SD*	± *14.0*	± *4.0*	± *2.6*	± *2.6*	± *2.9*	± *1.0*	± *3.1*	± *0.6*
2500 mean	54.3#	94.0#	31.7	19.7	11.3	6.0	20.7	15.0
± *SD*	± *14.5*	± *6.0*	± *9.0*	± *0.6*	± *2.3*	± *1.0*	± *1.5*	± *1.0*
5000 mean	/	76.7#	/	19.7	4.7	/	14.7	
± *SD*	/	± *11.4*	/	± *1.5*	/	± *0.6*	/	± *1.2*
Positive control								
§ mean	616.7**	680.0**	299.3**	986.7**	143.7**	145.7**	1020.0**	165.0**
± *SD*	± *58.1*	± *17.3*	± *23.9*	± *220.3*	± *4.5*	± *10.2*	± *166.8*	± *47.7*

§ Information on respective positive control is reported in Material and Method section I.A.2

** Statistically significant at 1 % level (analysis of variance)

Cytotoxicity observed

III. CONCLUSION:

Based on the results of the present study and under the experimental conditions of the test, glyphosate was found to be non-mutagenic in the Ames test in the presence and absence of metabolic activation.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Negative for gene mutation in bacteria (*S. typhimurium* TA 100, TA 98, TA 1535 and TA 1537) with and without metabolic activation.

The study was performed under GLP conditions and according to OECD guideline 471 (1983). When compared to the currently valid OECD guideline 471 (1997), some deviations became evident. The test was conducted with 4 valid strains only, strains like *S. typhimurium* TA 102 or *E. coli* WP2 enabling the detection of cross linking agents were not included. Further deviations to OECD guideline 471 (1997) were considered to be of minor degree and to not compromise the validity of the study. The study was considered to provide supporting information.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.4.1/021
Report author	[redacted]
Report year	1990
Report title	Agrichem Glyphosate Active: Reverse Mutation Assay "Ames Test" Using *Salmonella Typhimurium*
Report No	300/1
Document No	Not reported
Guidelines followed in study	OECD 471 (1983), EEC Commission Directive 84/449/EEC (1984)
GLP	Yes
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	An Ames test was conducted with glyphosate active (batch: 0190A, purity: not reported) in *S. typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 in the presence and absence of metabolic activation (Aroclor 1254-induced rat liver S9 fraction). Based on the results of a preliminary toxicity test in strain TA 100, in which no cytotoxicity was evident up to 5000 µg/plate, concentrations for the main mutagenicity test were selected. In the main mutagenicity study, two independent experiments were performed using the plate incorporation method (standard plate test). Triplicate cultures were exposed to test item concentrations in the range of 8 – 5000 µ/plate (first experiment) and 312.5 – 5000 µg/plate (second experiment) with and without S9 mix. Untreated, vehicle (sterile distilled water) and appropriate positive controls were included in each experiment.
Short description of results:	Precipitation of the test material in culture medium was not observed, neither in the presence nor absence of S9 mix. Cytotoxicity, evident as a reduction of the bacterial background lawn, was not observed in any experiment. However, there was a concentration-related reduction in the frequency of revertant colonies observed for most strains. In the first experiment, the frequency of revertant colonies was ≥ 50 % reduced at ≥ 1000 µg/plate for tester strain TA 1538 in the absence of metabolic activation and for tester strains TA 98, TA 100 and TA 1535 at 5000 µg/plate in the absence of metabolic activation. In the second experiment, in the presence of S9 mix, there was a ≥ 50 % reduction in the number of revertant colonies noted for strain TA1537 at ≥ 1250 µg/plate, for strain TA 100 at ≥ 2500 µg/plate and for strain TA 1535 at 5000 µg/plate. There was no statistically significant increase in the number of revertant colonies observed in any experiment for any strain at any concentration, neither in the presence nor in the absence of metabolic activation. The number of revertant colonies induced by the vehicle control was found to be in the expected range. A strong increase in the number of revertant colonies was observed for the

	appropriate positive controls, demonstrating the functionality of the metabolic activation system and the validity of the test. Under the conditions of the test, glyphosate active was considered negative for gene mutation in bacteria (Ames test) in the presence and absence of metabolic activation.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	The study was considered not acceptable due to large number of deviations to current guidelines. The test was conducted in 4 valid strains only. Strains like *S. typhimurium* TA102 or *E. coli* WP2 enabling the detection of cross-linking mutagens were not included. The purity of the test material was not reported. In addition, 2-aminoanthracene was used as sole positive control substance in the presence of S9 mix and historical control data were not provided.
Reasons why the study report is not available for submission	
Category study in AIR5 dossier (L-docs)	Category 3b

1. Information on the study

Data point:	CA 5.4.1/022
Report author	[redacted]
Report year	1986
Report title	Report on mutagenicity tests with glyphosate (technical) of Excel Industries Limited, Bombay. Ames bacterial test.
Report No	Not reported
Document No	Not reported
Guidelines followed in study	No guideline followed. The study was conducted according to the main criteria of OECD 471 (1983).
GLP	No, not conducted under GLP/ Officially recognised testing facilities. When the study was conducted, GLP was not compulsory.
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	Glyphosate technical (batch: not reported, purity: not reported) was evaluated for its potential to induce gene mutations in bacteria in an Ames test conducted with in *S. typhimurium* strains TA 98, TA 100, TA 1535 and TA 1537 and *E. coli* strain WP2 uvrA. A single experiment was performed according to the pre-incubation method. Triplicate cultures were exposed to the test item, negative and positive controls in the presence and absence of metabolic activation (Aroclor 1254-induced rat liver S9 fraction). Three concentrations of glyphosate technical were tested, covering a concentration range of 10 – 1000 µg/plate. After at least 48 hours of incubation at 37 °C, the number of revertant colonies were counted for each plate.
Short description of results:	Evaluation of precipitation and cytotoxicity were not included in the study report. When evaluating cytotoxicity based on the number of revertant colonies in comparison to those of negative controls, treatment with glyphosate technical revealed no evidence for cytotoxicity. There was no statistically significant increase in the number of revertant colonies observed for any tester strain up to the highest

	tested concentration, neither in the presence nor in the absence of metabolic activation. For all conditions, the number of revertant colonies was comparable to those of negative controls. Treatment with the positive controls revealed a strong increase in the number of revertant colonies, demonstrating the functionality of the S9 mix and the sensitivity of the bacteria strains to respond to mutagenic agents. Based on the experimental results, glyphosate technical did not induce gene mutations in bacteria, neither in the presence nor in the absence of metabolic activation.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	The study was considered not acceptable due to a large number of guideline deviations. No details on the experimental performance were given and information on the test item regarding purity, batch no. and solvent were not reported. A single experiment was performed, which was not repeated in a confirmatory experiment. Further, only three concentrations were tested with an interval of factor 10 and a maximum concentration of 1000 µg/plate. There was no justification for the highest concentration given. In addition, positive controls were not included for all strains, but only for strains TA98, TA1537 and WP2uvrA. Although negative controls were included in the experiment, it was not clear whether these controls represented untreated controls or solvent controls. Further, no historical control data were provided.
Reasons why the study report is not available for submission	
Category study in AIR5 dossier (L-docs)	Category 3b

1. Information on the study

Data point:	CA 5.4.1/023
Report author	[redacted]
Report year	1981
Report title	Mutagenic testing of glyphosate active principle and of glyphosate product: Ames- and host mediated test
Report No	Not reported
Document No	Not reported
Guidelines followed in study	No guideline followed.
GLP	No, not conducted under GLP/ Officially recognised testing facilities. When the study was conducted, GLP was not compulsory.
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	Glyphosate active principle (batch and purity not reported) was assessed for gene mutation in bacteria (Ames test) in *S. typhimurium* strains his-G46, TA 1537 and TA 1538. A single experiment using triplicate cultures was performed (spot test). Glyphosate was dissolved in DMSO and concentrations in the range of 1 – 1000 µg/plate were put into the central point of the agar plates. Solvent (DMSO) and positive controls (streptozotocin, 2.5 µg/plate and ICR 191, 10 µg/plate) were included. After 48 hours incubation at 37 °C, the number of revertant colonies per plate was determined.

Short description of results:	Based on the experimental findings, glyphosate active principal did not induce the frequency of revertant colonies in strains his-G46, TA 1537 and TA 1538. The positive controls streptozotocin and ICR 191 produced a marked increase in the number of revertant colonies.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	The study was considered not acceptable. The description of material and methods was so poor that an evaluation of the reliability of the results obtained (the test substance was considered non-mutagenic) was not possible. Further, the test was conducted in two valid strains only and only a single experiment was performed. The experimental procedure is not in line with current standard methods.
Reasons why the study report is not available for submission	
Category study in AIR5 dossier (L-docs)	Category 3b

1. Information on the study

Data point	CA 5.4.1/024
Report author	[redacted]
Report year	1978
Report title	The report of mutagenic study with bacteria for CP 67573
Report No	ET-78-241
Document No	Not reported
Guidelines followed in study	No guideline followed, the study was conducted similarly to OECD 471 (1983)
Deviations from current test guideline OECD 471 (1997)	A single experiment using duplicate plating was performed but a justification for the missing confirmatory experiment or the missing third replicate was not provided. Instead of *E. coli* strain WP2 uvrA, strain WP2 hcr was used. 2-Aminoanthracene was used as sole positive control substance in the presence of S9 mix. Historical control data were not reported. Evaluation of cytotoxicity and precipitation were not reported, but concentrations were tested up to limit concentrations.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, not conducted under GLP/ Officially recognised testing facilities. When the study was performed, GLP was not compulsory.
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Glyphosate (CP67573, batch: XHJ-46, purity: 98.4 %) was tested in an Ames test conducted with *S. typhimurium* strains TA 98, TA 100, TA 1535, TA1537 and TA 1538, and *E. coli* strain WP2 hcr in the presence and absence of metabolic activation (Aroclor-induced rat liver S9 fraction). A single experiment (plate-incorporation method) was performed, using test item concentrations in the range of 10 – 5000 µg/plate. Solvent (water) and appropriate positive controls were included. After incubation at 37 °C for 48 hours, the number of bacterial colonies (his^+ or trp^+ revertants) were counted.

Evaluation of precipitation and cytotoxicity were not provided in the study report. Cytotoxicity was re-evaluated by comparing the presented mean numbers of revertant colonies induced by the test item to the mean number of spontaneous revertants observed in the corresponding vehicle control. Based on these criteria, cytotoxicity was noted at 5000 µg/plate in strains WP2 hcr, TA 100, TA 1537 and TA 98 in the absence of S9 mix and at 5000 µg/plate in strain TA 100 in the presence of S9 mix.

There was no significant increase in the number of his^+ or trp^+ revertants observed in any tester strain at any concentration when compared to solvent controls, neither in the presence nor in the absence of metabolic activation. In contrast, a strong increase in reverse mutations was observed for all positive control compounds in all tester strains, demonstrating the sensitivity of the test and the functionality of the S9 fraction.

Based on the experimental results of the present study, glyphosate is not mutagenic in the Ames plate incorporation test with and without metabolic activation.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material: Glyphosate

Identification:	CP67573
Description:	Not provided
Lot/Batch #:	XHJ-46
Purity:	98.4 %
Stability of test compound:	The stability of the test item at storage conditions or in solvent (vehicle) was not specified.
Solvent (vehicle) used:	Water

2. Control materials:

Negative control:	Not examined
Solvent (vehicle) control:	Water
Solvent (vehicle)/final concentration:	0.1 mL per plate.
Positive controls:	Please refer to the table below.

Strain	Metabolic activation	Mutagen	Conc. [µg/plate]
S. typhimurium strains			
TA 100	-S9	2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2)	0.05
	+S9	2-Aminoanthracene	10.0
TA 1535	-S9	β-Propiolactone	50.0
	+S9	2-Aminoanthracene	10.0
TA 98	-S9	2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2)	0.1
	+S9	2-Aminoanthracene	10.0
TA 1537	-S9	9-Aminoacridine	200.0
	+S9	2-Aminoanthracene	10.0
TA 1538	-S9	2-Nitrofluorene	50.0
	+S9	2-Aminoanthracene	10.0
E. coli strain			
WP2 hcr	-S9	2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2)	0.25
	+S9	2-Aminoanthracene	10.0

3. Metabolic activation:

S9 mix was obtained from livers of 11 weeks old male Sprague-Dawley rats with an average body weight of approx. 364 g. The animals received a single intraperitoneal injection of 500 mg/kg bw Aroclor 1254. The livers were prepared five days after treatment. Co-factor was immediately added to prepare S9 mix containing the following components:

S9 mix component	Concentration	Unit
Sodium phosphate buffer (pH 7.4)	100	mM
KCl	33	mM
NADPH-generating system		
Glucose 6-phosphate	5	mM
$NADP^+$	4	mM
$MgCl_2$	8	mM
S9	33	% (v/v)

4. Test organisms:

Tester strains				Bacteria batch checked for	
S. typhimurium		***E. coli***			
TA 98	✓	WP2 hcr	✓	deep rough character (rfa)	Not specified
TA 100	✓	WP2 uvrA (pKM101)		ampicillin resistance (R factor plasmid)	Not specified
TA 1535	✓			UV-light sensitivity (absence of uvrB and uvrA genes in *S. typhimurium* and *E. coli* strains, respectively)	Not specified
TA 1537	✓				
TA 1538	✓			Histidine and tryptophan auxotrophy (automatically via the spontaneous rate)	✓

5. Test concentrations:

Plate incorporation test ± S9 mix:	
Concentrations:	*10, 50, 100, 500, 1000 and 5000 µg/plate*
Tester strains:	TA 1538, TA 1535, TA 1537, TA 98, TA 100 and WP2 hcr

Replicates:		*Duplicates in a single experiment*	

B: STUDY DESIGN AND METHODS

1. Finalisation date: 20 Jul 1978

2. Standard plate test (plate-incorporation test, SPT):
An aliquot of 0.1 mL test solution or vehicle/positive control, an aliquot of 0.1 mL fresh bacterial culture and 0.5 mL S9 mix (in tests with metabolic activation only) were added to 2 mL of molten agar (supplemented with 0.5 mM L-histidine + 0.5 mM biotin or 0.5 mM L-tryptophan) and spread onto minimal agar plates with modified Vogel-Bonner E medium. Each concentration and the controls were tested in duplicates. After incubation at 37 °C for 48 hours, the number of bacterial colonies (his^+ or trp^+ revertants) were counted.

3. Cytotoxicity
Criteria for the evaluation of cytotoxicity have not been specified in the study report. Cytotoxicity was indicated only in case no revertant colonies were detected on a plate. Cytotoxicity was evaluated retrospectively by comparing the presented mean numbers of revertant colonies induced by the test item to the mean number of spontaneous revertants observed in the corresponding vehicle control. A cytotoxic effect was evident when the number of spontaneous revertant colonies was 50 % or less of the value in corresponding vehicle controls.

4. Statistics
Results were judged without statistical analysis.

5. Acceptance criteria
Acceptance criteria were not specified in the study report.

6. Evaluation criteria
Evaluation criteria were not specified in the study report.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS
Analytical determinations were not performed in the study, as not required by the test guideline.

B. CYTOTOXICITY
Although cytotoxicity was noted in the mutagenicity study, detailed observations on cytotoxicity in individual strains were not reported. Cytotoxicity evident as no bacterial background lawn at all was observed at 5000 µg/plate in *E. coli* strain WP2 hcr in the absence of metabolic activation. Cytotoxicity was re-evaluated by comparing the presented mean numbers of revertant colonies induced by the test item to the mean number of spontaneous revertants observed in the corresponding vehicle control. A cytotoxic effect was evident when the number of spontaneous revertant colonies was 50 % or less of the value in corresponding vehicle controls. Based on these criteria, cytotoxicity was noted at 5000 µg/plate in strains WP2 hcr, TA 100, TA 1537 and TA 98 in the absence of S9 mix and at 5000 µg/plate in strain TA 100 in the presence of S9 mix.

C. SOLUBILITY
Evaluation of precipitation was not reported.

D. MUTATION ASSAY

There was no significant increase in the number of his$^+$ or trp$^+$ revertants observed in any tester strain at any concentration when compared to solvent controls, neither in the presence nor in the absence of metabolic activation.

In contrast, a strong increase in reverse mutations was observed for all positive control compounds in all tester strains, demonstrating the sensitivity of the test and the functionality of the S9 fraction.

Table 5.4.1-33: The report of mutagenic study with bacteria ([redacted], 1978)

Standard plate test (SPT)[X]												
Strain	**WP2 hcr**		**TA 1535**		**TA 100**		**TA 1537**		**TA 1538**		**TA 98**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Vehicle control												
Water mean	22.0	19.5	10.0	5.5	148.0	139.5	9.5	6.0	11.5	9.5	23.5	19.0
Test item [µg/plate]												
10 mean	21.5	43.0	3.5	2.5	145.0	122.5	5.0	3.0	20.5	13.5	27.5	21.0
50 mean	18.5	24.5	5.0	7.0	155.0	127.0	5.5	8.0	15.0	15.0	36.5	23.5
100 mean	19.0	25.0	4.5	6.0	151.5	122.0	8.0	8.5	20.5	16.0	20.0	14.5
500 mean	23.5	29.0	2.0	3.0	130.5	124.5	10.0	8.5	11.0	11.0	27.5	22.5
1000 mean	16.5	26.5	10.5	7.5	103.5	92.5	10.0	9.0	15.0	15.5	22.0	19.0
5000 mean	#	29.5	6.0	6.0	72.5[T]	43.5[T]	3[T]	4.5	6.5	13.0	6.5[T]	20.5
Positive control												
[§] mean	88.5	1972.0	355.5	336.5	>3000	1087.0	379.0	>10,000	>3000	>3000	>3000	311.0

[X] = The table shows mean values of duplicate plates which were be lately calculated based on the raw data given in study report.

[§] Information on respective positive control is reported in Material and Method section I.A.2

Toxicity, no revertant colonies on the plate; T: cytotoxicity, re-evaluation as described in Material and Method section.

III. CONCLUSION:

In conclusion, based on the results of the present study and under the experimental conditions chosen, glyphosate is not mutagenic in bacteria (Ames test) with and without metabolic activation.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Negative for gene mutation in bacteria (*S. typhimurium* TA 100, TA 98, TA 1535, TA 1537 and TA 1538 and *E. coli* WP2 hcr) with and without metabolic activation.

The study was performed similar to OECD guideline 471 (1997) but not GLP-compliant. It was considered supplementary, as a number of deviations to the currently valid guideline became evident. A single experiment using duplicate plating was performed but a justification for the missing confirmatory experiment or the missing third replicate was not provided. Instead of *E. coli* strain WP2 uvrA, strain WP2 hcr was used. In addition, 2-aminoanthracene was used as sole positive control

substance in the presence of S9 mix and historical control data were not reported. Evaluation of cytotoxicity and precipitation were not reported, but concentrations were tested up to limit concentrations. In addition, no acceptance or evaluation criteria were specified.

Assessment and conclusion by RMS:

Cytogenicity and gene mutation in mammalian cells

Five *in vitro* chromosome aberration tests have been conducted with glyphosate (Table 5.4.1-). Study CA 5.4.1/029 is considered invalid and studies CA 5.4.1/025 and CA 5.4.1/028 are supportive only due to deficiencies identified in comparison to OECD 473 (2016). However, data from two valid (reliable with restrictions) studies are available that have been conducted with either Chinese hamster lung cells or human lymphocytes. Although some minor deficiencies were identified when the studies are compared to OECD 473 (2016), these deviations are considered to not affect the validity or integrity of the data. Overall, there are sufficient, reliable data to allow thorough evaluation of this data requirement. There is no evidence that glyphosate causes chromosome aberrations or polyploidy in mammalian cells.

Two mouse lymphoma assays (MLA) and one HPRT assay have been conducted with glyphosate (Table 5.4.1-31). These studies are all considered to be valid (reliable with restrictions). Although some minor deficiencies were identified when the studies are compared to either OECD 490 (2016) or OECD 476 (2016), as applicable, these deviations are considered to not affect the validity or integrity of the data. Overall, there are sufficient, reliable data to allow thorough evaluation of this data requirement. There is no evidence that glyphosate causes gene mutations in mammalian cells.

Table 5.4.1-34: Summary of *in vitro* genotoxicity testing with glyphosate acid: Cytogenicity and gene mutation in mammalian cells

Annex Point	Study	Study type Test system Exposure and Harvest	Substance Dose levels# Purity	Status	Result
CA 5.4.1/025	[redacted], 1998	Chromosome aberration test Human peripheral lymphocytes - S9: exposure: 20 and 44 h; harvest: 20 and 44 h + S9: exposure: 3 h, harvest: 20 and 44 h	Glyphosate acid - S9: 100 - 1250 µg/mL + S9: 100 - 1250 µg/mL Purity: 95.6 %	Supportive, Category 2a	negative
CA 5.4.1/026	[redacted], 1996	Chromosome aberration test Chinese hamster lung cells - S9: exposure: 24 and 48 h; harvest: 24 and 48 h + S9: exposure: 6 h, harvest: 24 h	Technical glyphosate - S9: 312.5 - 1250 µg/mL + S9: 312.5 - 1250 µg/mL Purity: 95.3 %	Valid, Category 2a	negative
CA 5.4.1/027	[redacted] 1995	Chromosome aberration test Chinese hamster lung cells - S9: exposure: 6, 24 and 48 h; harvest: 24 and 48 h + S9: exposure: 6 h, harvest: 24 h	Glyphosate technical - S9: 250 - 2000 µg/mL (6 h harvest), 125 - 1000 µg/mL (24 h harvest) and 62.5 - 500 µg/mL (48 h harvest)	Valid, Category 2a	negative

			+ S9: 250 - 2000 µg/mL Purity: 95.68 %		
CA 5.4.1/028	[redacted], 1995	Chromosome aberration test Human peripheral lymphocytes - S9: exposure: 24 and 48 h; harvest: 24 and 48 h + S9: exposure: 3 h, harvest: 24 and 48 h	Glyfosaat - S9: 33 - 237 µg/mL (24 h harvest, 1st exp.), 33 - 333 µg/mL (24 h harvest, 2nd exp.), 237 µg/mL (48 h harvest) + S9: 237 - 562 µg/mL (24 h harvest, 1st exp.), 333 - 562 µg/mL (24 h harvest, 2nd exp.), 562 µg/mL (48 h harvest) Purity: 96 %	Supportive, Category 2a	negative
CA 5.4.1/029	[redacted], 1989	Chromosome aberration test Chinese hamster ovary cells - S9: exposure: 3 h§; harvest: 21 h + S9: exposure: 3 h§, harvest: 21 h	Glyphosate - S9: 250 - 1000 µg/mL + S9: 62.5 - 250 µg/mL Purity: not reported	Invalid, Category 3b	negative
CA 5.4.1/030	[redacted], 1996	Mouse lymphoma assay Mouse Lymphoma L5178Y TK+/- cells - S9: exposure: 4 h + S9: exposure: 4 h	Glyphosate acid - S9: 444 – 1500 µg/mL (exp I, highest concentration not evaluated for mutagenicity; 296 - 1000 µg/mL (exp II) + S9: 296 - 1000 µg/mL Purity: 95.6 %	Valid, Category 2a	negative
CA 5.4.1/031	[redacted] 1991	Mouse lymphoma assay Mouse Lymphoma L5178Y TK+/- cells - S9: exposure: 4 h + S9: exposure: 3 h	Glyphosate technical - S9: 630 - 5000 µg/mL + S9: 520 - 4200 µg/mL Purity: 98.6 %	Valid, Category 2a	negative
CA 5.4.1/032	[redacted], 1983	HGPRT assay Chinese hamster ovary cells - S9: exposure: 3 h + S9: exposure: 3 h	Glyphosate - S9: 2000 - 20000 µg/mL + S9: 5000 - 25000 µg/mL Purity: 98.7 %	Valid, Category 2a	negative

Only dose levels listed which were investigated for chromosome aberrations (Chromosome aberrations test) or for mutagenicity (Mouse lymphoma assay and HGPRT assay)
HGPRT: Hypoxanthine guanine phoyphoribosyltransferase
§ Inconsistencies in study report on the duration of exposure. It was assumed that the cells were exposed for 3 hours, followed by 18 hours of incubation (including 2 hours of treatment with colchicine) and chromosome preparation 21 hours after start of exposure.

1. Information on the study

Data point	CA 5.4.1/025
Report author	[redacted]
Report year	1998
Report title	Glyphosate Acid: *In Vitro* Cytogenetic Assay In Human Lymphocytes
Report No	CTL/P/6050
Document No	Not reported
Guidelines followed in study	OECD 473 (1983), Commission Directive 67/548/EEC (1992), Commission Directive 92/69/EEC B.10 (1992)
Deviations from current test guideline OECD 473 (2016)	Only 200 instead of 300 cells in metaphase were evaluated per condition for test item treated cultures and only 25 metaphases were evaluated for the corresponding positive control. There are no information on numerical chromosome aberrations given (polyploidy index could not be determined). Data on the laboratory's historical control range were not provided. Positive controls were only included for the 20 hour sampling time point. A short-term exposure in the absence of metabolic activation as recommended by OECD 473 (2016) was not included. Although pH changes were observed, there was no attempt to buffer the pH change. Acceptance criteria were not specified and evaluation criteria were inconsistent with test guideline OECD 473 (2016).
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Glyphosate acid (batch: P24, purity: 95.6 %) was evaluated for its clastogenic potential *in vitro* in human lymphocytes. Cells from two different donors were treated in the presence and absence of metabolic activation (phenobarbital and β-naphthoflavone-induced rat liver S9 fraction). Duplicate cultures were exposed to test item concentrations in the range of 50 to 2000 µg/mL. Due to reductions of the pH in the culture medium at a test item concentration of 1250 µg/mL (-0.57 units) and above, the maximum concentration for the cytogenicity assay was 1250 µg/mL. Cultures treated with glyphosate acid at 100, 750 and 1250 µg/mL were selected for chromosomal aberration analysis along with the appropriate solvent (culture medium) and positive control cultures (mitomycin C without S9 mix and cyclophosphamide with S9 mix).

The cells from the first donor (male) were exposed for 20 hours in the absence of S9 mix and for 3 hours in the presence of S9 mix followed by 17 hours of incubation in test item-free medium. Sampling for chromosome preparations were made 20 hours after start of exposure in the presence and absence of S9 mix, corresponding to 68 hours after cell culture establishment. The cells from the second donor (female) were exposed for 20 and 44 hours in the absence of S9 mix, for 3 hours in the presence of S9 mix followed by 17 hours of incubation in test item-free medium and for 3 hours in the presence of metabolic activation followed by 41 hours of incubation in test item-free medium. Cells exposed for 3 hours in the presence of S9 mix were sampled 20 and 44 hours after start of exposure (68 and 92 hours after cell culture establishment). Cells exposed for 20 and 44 hours in the absence of S9 mix were sampled immediately following exposure (68 and 92 hours after cell culture establishment, respectively).

A total of 200 metaphase cells per condition were scored for structural chromosome aberrations and cytotoxicity was assessed as mitotic index (MI) and evaluated for 1000 cells per culture.

Cytotoxicity was evident at 1250 µg/mL in the absence of metabolic activation only, at the 20 hour sampling time point. In both donors, the mean mitotic activity was slightly reduced (-37 % and -33 %, respectively). There was no cytotoxicity at the 44 hour sampling time point or in the presence of metabolic activation.

Treatment with glyphosate acid did not induce a statistically or biologically significant increase in the percentage of aberrant metaphases in none of the experiments, neither in the presence nor in the absence of S9 mix. Frequencies of aberrant metaphases of solvent control cultures remained within the range of the laboratory's historical control data. The positive controls showed a clear clastogenic effect and markedly induced the number of aberrant metaphases, demonstrating the sensitivity of the test and the functionality of the S9 mix.

Based on the results of the present study, glyphosate acid is not clastogenic to cultured human lymphocytes with and without metabolic activation.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material: Glyphosate acid

Identification:	Y04707/034
Description:	White solid
Lot/Batch #:	P24
Purity:	95.6 %
Stability of test compound:	From information supplied by the sponsor, the test substance was stable during the period of the study.
Solvent (vehicle) used:	Culture medium

2. Control Materials:

Negative control:	The negative control corresponded to the solvent control.
Solvent (vehicle) control:	Culture medium (RPMI-1640)
Positive control:	-S9 mix: Mitomycin C (MCC): 0.2 µg/mL
	+S9 mix: Cyclophosphamide (CP): 50 µg/mL

3. Metabolic activation:

S9 mix was obtained from the livers of male Sprague-Dawley rats. The animals received intraperitoneal injections of phenobarbital (80 mg/kg bw) and β-naphthoflavone (100 mg/kg bw/day) for three consecutive days. The animals were sacrificed on the day following the third dose. The S9 mix was prepared on the day of culture treatment by mixing S9 fraction and co-factor solution as a 1:1 mixture.

S9 mix component	Concentration	Unit
Sodium phosphate buffer (pH 7.4)	150	mM
KCl	49.5	mM
NADPH-generating system		
Glucose 6-phosphate	7.5	mM
NADP Na salt	6	mM
$MgCl_2$	8	mM
S9	25	% (v/v)

4. Test organism:

Human blood samples were obtained by venepuncture in lithium heparin tubes from healthy, non-smoking donors, one male (donor 1) and one female (donor 2). Both donors had a previously established

low incidence of chromosomal aberrations in their peripheral blood lymphocytes.

5. Cell culture:

Medium: RPMI-1640 medium (Dutch modification) supplemented with approx. 10 % foetal bovine serum (FBS), 1.0 IU/mL heparin, 100 IU/mL penicillin and 100 µg/mL streptomycin.

Incubation: At 37 °C

Cell culture establishment prior to exposure: 0.5 mL of whole blood was added to 9.0 mL of culture medium and 5 % (v/v) phytohaemagglutinin. Cells were maintained at approximately 37 °C for 48 hours with gentle daily mixing where possible.

6. Test concentrations and number of replicates:

Exp.	Donor	Metabolic activation	Duration of exposure	Concentrations	Replicates
1	male	(- S9 mix)	20 h	50, 100*, 250, 500, 750*, 1000, 1250*, 1500 and 2000 µg/mL	Duplicate
1	male	(+ S9 mix)	3 h[$]	50, 100*, 250, 500, 750*, 1000, 1250*, 1500 and 2000 µg/mL	Duplicate
2	female	(- S9 mix)	20 h	50, 100*, 250, 500, 750*, 1000, 1250*, 1500 and 2000 µg/mL	Duplicate
2	female	(+ S9 mix)	3 h[$]	50, 100*, 250, 500, 750*, 1000, 1250*, 1500 and 2000 µg/mL	Duplicate
2	female	(- S9 mix)	44 h[#]	50, 100, 250, 500, 750, 1000, 1250*, 1500 and 2000 µg/mL	Duplicate
2	female	(+ S9 mix)	3 h[§]	50, 100, 250, 500, 750, 1000, 1250*, 1500 and 2000 µg/mL	Duplicate

[#] The culture medium was changed after 68 h of culture initiation (= 20 hours after start of treatment)

[$] The cells were exposed for 3 hours, followed by an incubation period of 17 hours (sampling time: 20 hours after exposure

[§] The cells were exposed for 3 hours, followed by an incubation period of 41 hours (sampling time: 44 hours after exposure)

* Dose levels selected for assessment of chromosome aberration

B: STUDY DESIGN AND METHODS

1. Dates of experimental work: 03 Jul 1995 – 26 Aug 1998
Finalisation date: 29 Oct 1998

2. Preliminary cytotoxicity test:

A preliminary cytotoxicity test was not performed in this study.

3. Main cytogenicity test:

Treatment: Approximately 48 hours after cell culture establishment, 8 mL aliquots of test item formulations in medium were administered to each culture. Duplicate cultures per condition were exposed to concentrations in the range of 50 to 2000 µg/mL.

The cells from the first donor (male) were treated for 20 hours in the absence of S9 mix and for 3 hours in the presence of S9 mix, followed by 17 hours of

incubation in test item-free medium. Sampling for chromosome preparations were made 20 hours after start of exposure in the presence and absence of S9 mix, corresponding to 68 hours after cell culture establishment
The cells from the second donor (female) were exposed for 20 and 44 hours in the absence of S9 mix, for 3 hours in the presence of S9 mix followed by 17 hours of incubation in test item-free medium and for 3 hours in the presence of metabolic activation followed by 41 hours of incubation in test item-free medium. Cells exposed for 3 hours in the presence of S9 mix were sampled 20 and 44 hours after start of exposure (68 and 92 hours after cell culture establishment, corresponding to 3-hour exposure followed by 17 and 41 hours of test item-free exposure). Cells exposed for 20 and 44 hours in the absence of S9 mix were sampled immediately following exposure (68 and 92 hours after cell culture establishment, respectively).
In cell cultures of the latter sampling time point (44 hours after start of exposure, 92 hours after cell culture establishment) a medium change was performed after 20 hours of exposure.
Corresponding solvent (culture medium) and positive controls (MMC without S9 mix and CP with S9 mix) were included for the 3 hours exposure period with S9 mix and for the 20 hours exposure period without S9 mix.

Spindle inhibition: Colcemid (0.4 µg/mL) was added to the cultures two hours before harvest.

Cell harvest: The cells were harvested by centrifugation and the pellets were re-suspended in 0.075 M potassium chloride solution at room temperature for approximately 10 minutes. Afterwards, the cells were centrifuged again and fixed in methanol:glacial acetic acid fixative (3:1, v/v). the fixative was removed following centrifugation and replaced with freshly prepared fixative. The process was repeated at least twice prior to slide preparation.

Slide preparation: Fixed cells were dropped on clean, moist labelled microscope slides. The slides were air-dried, stained in filtered Giemsa stain (10 % Gurr's R66) for 7 minutes, rinsed with water, air-dried again and mounted with coverslips in DPX.

Metaphase analysis The slides were coded prior to analysis and 100 cells in metaphase (200 metaphases per condition in total), where possible, were analysed from each culture for the incidence of structural chromosomal damage. Recording of chromosomal aberrations was performed according to Scott et al. (1990), including the type and frequency of observed aberrations. Chromosome gaps, breaks and minutes, multiple damage, interchanges and e.g. re-arrangements were noted. For each condition, frequencies of aberrant metaphases were calculated including and excluding gap-type aberrations.

Cytotoxicity: Mitotic indices were determined by examining 1000 lymphocytes per culture and calculating the percentage of cells in metaphase.

4. Statistics

The statistically evaluation of the percentage of metaphases showing aberrations (excluding cells with only gap-type aberrations) was performed using the (one-sided) Fisher's Exact Probability Test. Data from each treatment group in the presence and absence of S9 mix was compared to the respective solvent control value.

5. Acceptance criteria

Acceptance criteria were not specified in the study report.

6. Evaluation criteria

A test substance was judged negative if the following criteria were met:

- There was no statistically significant increase in the percentage of aberrant cells at any concentration above concurrent solvent control values.
- A statistically significant increase in the percentage of aberrant cells above concurrent solvent controls fell into the range of the laboratories historical control data.

A test substance was judged positive if there was an increase in the percentage of aberrant cells, at least at one concentration, which is substantially greater than the laboratory's historical solvent control values.

II. RESULTS AND DISCUSSION

A. ANALYTCAL DETERMINATIONS

Analytical determinations of the test substance in the solvent were not performed, as not required by the test guideline.

B. CYTOTOXICITY

Small reductions in the mean mitotic activity when compared to the respective solvent control were noted at 1250 µg/mL for the 20-hour sampling time point (68 hours after cell culture establishment) in the absence of metabolic activation only. At 1250 µg/mL, which was the highest concentration selected for chromosomal aberration analysis, in the absence of S9 mix, the mitotic indices were reduced by -37 % and -33 % for cultures of donors 1 and 2, respectively. There was no cytotoxicity observed at the 44 hour sampling time point (92 hours after cell culture establishment) in the absence of metabolic activation. In addition, there were no significant reductions in mitotic activity in all approaches in the presence of S9 mix.

Treatment with glyphosate acid caused a concentration-related reduction in the pH of the culture medium. The highest concentration of glyphosate acid selected for chromosomal aberration analysis was 1250 µg/mL, in which the pH of the culture medium was reduced by 0.57 units. Cultures treated with concentrations of glyphosate acid higher than 1250 µg/mL were therefore considered not to be suitable for chromosomal aberration analysis.

C. SOLUBILITY

Evaluation of test item precipitation was not provided in the study report. However, there was a concentration-related reduction in the pH of the culture medium in glyphosate acid treated cultures. Treatment of the culture medium with test item concentrations up to 2000 µg/mL had no significant effect on osmolality.

D. CYTOGENICITY

There was no statistically or biologically significant increase in the percentage of aberrant metaphases observed in none of the experiments, neither in the presence nor in the absence of S9 mix. Frequencies of aberrant metaphases of solvent control cultures remained within the range of the laboratory's historical control data. The positive controls mitomycin C and cyclophosphamide showed a clear clastogenic effect and markedly induced the number of aberrant metaphases, demonstrating the sensitivity of the test and the functionality of the S9 mix.

Table 5.4.1-35: Glyphosate Acid: In Vitro Cytogenetic Assay In Human Lymphocytes ([redacted], 1998), first experiment (cells from Donor 1)

Compound	Concentration [µg/mL]	No. of metaphases scored	Genotoxicity				Judge	Mitotic index [%]
			no. structural aberrant cells[x]		% structural aberrant cells			
			incl. gaps	**excl. gaps**	incl. gaps	**excl. gaps**		
Without metabolic activation; 20-hour treatment and sampling (68 hours after cell culture establishment)								
Solvent (culture medium)	800 µL/mL	200	1.00	**1.00**	0.50	**0.50**	negative	15.1
Test item	100.0	200	4.00	**3.00**	2.00	**1.50**	negative	14.70
	750.0	200	2.00	**2.00**	1.00	**1.00**	negative	12.40
	1250.0	200	2.00	**2.00**	1.00	**1.00**	negative	9.50
MMC	0.20	25	9.00	**9.00****	36.00	**36.00****	positive	7.00 ∞
With metabolic activation; 3-hour treatment, 17-hour incubation, sampling after 20 hours (68 hours after cell culture establishment)								
Solvent (culture medium)	800 µL/mL	200	4.00	**3.00**	2.00	**1.50**	negative	14.6
Test item	100.0	200	4.00	**1.00**	2.00	**0.50**	negative	13.60
	750.0	200	2.00	**0.00**	1.00	**0.00**	negative	13.80
	1250.0	200	8.00	**4.00**	4.00	**2.00**	negative	14.30
CP	50.0	25	6.00	**5.00****	24.00	**20.00****	positive	9.70 ∞

MI mitotic index: number of cells in mitosis/ number of cells, based on 1000 cells per culture
MMC Mitomycin C, positive control without S9 mix; CP:cyclophosphamide, positive control with S9 mix
∞ Mitotic index and percentage of aberrant cells were determined from a single culture
a Mitotic index not required for selection of concentrations for chromosomal aberration analysis
** Statistically significant increase in the percentage of aberrant cells at $p < 0.01$ using the Fisher's Exact Test (one-sided)
x Total number of chromosome aberrations from 200 metaphases scored

Table 5.4.1-36: Glyphosate Acid: In Vitro Cytogenetic Assay In Human Lymphocytes ([redacted], 1998), second experiment (cells from Donor 2)

Compound	Concentration [µg/mL]	No. of metaphases scored	Genotoxicity				Judge	Mitotic index [%]
			no. structural aberrant cells[x]		% structural aberrant cells			
			incl. gaps	**excl. gaps**	incl. gaps	**excl. gaps**		
Without metabolic activation; 20-hour treatment and sampling (68 hours after cell culture establishment)								
Solvent (culture medium)	800 µL/mL	200	0.00	**0.00**	0.00	**0.00**	negative	11.1
Test item	100.0	200	2.00	**2.00**	1.00	**1.00**	negative	10.40
	750.0	200	2.00	**2.00**	1.00	**1.00**	negative	9.00
	1250.0	201	2.00	**1.00**	1.00	**0.50**	negative	7.40
MMC	0.20	25	10.00	**9.00****	40.00	**36.00****	positive	6.70 ∞
With metabolic activation; 3-hour treatment, 17-hour incubation, sampling after 20 hours (68 hours after cell culture establishment)								

Table 5.4.1-36: Glyphosate Acid: In Vitro Cytogenetic Assay In Human Lymphocytes ([redacted], 1998), second experiment (cells from Donor 2)

Compound	Concentration [µg/mL]	No. of metaphases scored	Genotoxicity					Mitotic index [%]
			no. structural aberrant cells[x]		% structural aberrant cells		Judge	
			incl. gaps	excl. gaps	incl. gaps	excl. gaps		
Solvent (culture medium)	800 µL/mL	200	2.00	**2.00**	1.00	**1.00**	negative	11.2
Test item	100.0	200	5.00	**5.00**	2.50	**2.50**	negative	10.70
	750.0	200	2.00	**2.00**	1.00	**1.00**	negative	10.80
	1250.0	200	3.00	**3.00**	1.50	**1.50**	negative	11.00
CP	50.0	25	8.00	**7.00***	32.00	**28.00****	positive	4.60 ∞
With metabolic activation; 3-hour treatment, 41-hour incubation, sampling after 44 hours (92 hours after cell culture establishment)								
Solvent (culture medium)	800 µL/mL	200	0.00	**0.00**	0.00	**0.00**	negative	10.1
Test item	1250.0	200	1.00	**1.00**	0.50	**0.50**	negative	9.50
Without metabolic activation; 44-hour treatment and sampling (92 hours after cell culture establishment)								
Solvent (culture medium)	800 µL/mL	200	4.00	**4.00**	2.00	**2.00**	negative	11.2
Test item	1250.0	200	3.00	**3.00**	1.50	**1.50**	negative	11.30

MI mitotic index: number of cells in mitosis/ number of cells, based on 1000 cells per culture
MMC Mitomycin C, positive control without S9 mix; CP cyclophosphamide, positive control with S9 mix
∞ Mitotic index and percentage of aberrant cells were determined from a single culture
a Mitotic index not required for selection of concentrations for chromosomal aberration analysis
** Statistically significant increase in the percentage of aberrant cells at $p < 0.01$ using the Fisher's Exact Test (one-sided)
[x] Total number of chromosome aberrations from 200 metaphases scored

III. CONCLUSION:

In conclusion, glyphosate acid was not clastogenic to human lymphocytes *in vitro*, either in the presence or absence of metabolic activation.

3. Assessment and conclusion

Assessment and conclusion by applicant:
Negative for cytogenicity in peripheral human lymphocytes with and without metabolic activation.
The study was performed under GLP conditions and in accordance with OECD guideline 473 (1983). When compared to the currently valid guideline OECD 473 (2016), a number of deviations were noted. Only 200 metaphase cells were investigated and numerical chromosome aberrations were obviously not included in the evaluation. A short-term exposure in the absence of S9 mix was not included in the experiment and positive controls were only included for the 20-hour sampling time point.

Further deviations were considered to be of minor degree and to not compromise the outcome of the study. The study is considered to provide supporting information

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.4.1/026
Report author	[redacted]
Report year	1996
Report title	Technical glyphosate: Chromosome aberration test in CHL cells *in vitro*
Report No	434/015
Document No	Not reported
Guidelines followed in study	Not specified, experimental procedure similar to OECD 473 (2016)
Deviations from current test guideline OECD 473 (2016)	Only 200 cells in metaphase were evaluated, whereas the evaluation of 300 metaphases is recommended according to OECD guideline 473 (2016). No historical control data from the testing laboratory provided, but values were compared to published control values. Although pH changes were observed and the maximum concentration was limited based on pH changes, there was no attempt to buffer the pH change. Cytotoxicity was measured by counting the number of cells at the end of the culture period relative to control, whereas OECD 473 (2016) recommends to measure the relative population doubling or the relative increase in cell count. Acceptance and evaluation criteria were inconsistent with test guideline OECD 473 (2016).
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Glyphosate technical (batch: H95D161A, purity: 95.3 %) was tested for clastogenic effects *in vitro* in Chinese hamster lung (CHL) cells in the presence and absence of metabolic activation (Aroclor 1254-induced rat liver S9 fraction). Test item concentrations were selected based on the results of a preliminary cytotoxicity test. Due to reductions of the pH in culture medium at test item concentrations > 1250 µg/mL, the maximum concentration for the main mutagenicity assay was 1250 µg/mL. Duplicate cultures were exposed to the test substance at concentrations of 39, 78.1, 156.25, 312.5, 625 and 1250 µg/mL for 24 and 48 hours in the absence of metabolic activation and for 6 hours in the presence and absence of metabolic activation. Concurrent vehicle (culture medium) and positive controls (mitomycin C without S9 mix and cyclophosphamide with S9 mix) were included for experiments with and without metabolic activation, respectively.

The cells were sampled after 24 and 48 hours of exposure without S9 mix and 24 h after start of exposure for the 6 hour exposure with and without S9 mix. A total of 200 metaphases per condition were scored for structural and numerical chromosome aberrations. Cytotoxicity was assessed as percentage of growth inhibition when compared to solvent controls.

There was no cytotoxicity observed at any concentration of any sampling time point, neither in the presence, nor in the absence of metabolic activation.

There was no statistically significant increase in the frequency of cells with chromosomal aberrations at any dose level in any treatment group. In addition, the test material did not induce a significant increase in the numbers of polyploid metaphase cells in any of the tested conditions with or without S9 mix. Frequencies of aberrant metaphases of vehicle control cultures were within the expected range. The positive controls gave highly significant increases in the frequency of aberrant metaphase cells, indicating that the metabolic activation system was satisfactory and that the test method itself was operating as expected.

Based on the results of the present study, there is no evidence for induction of chromosome aberrations by glyphosate technical with and without metabolic activation.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material: Technical Glyphosate

Identification:	Not specified
Description:	White powder
Lot/Batch #:	H95D161A
Purity:	95.3 %
Stability of test compound:	The stability of the test item at storage conditions (room temperature) was guaranteed for > 2 years. The stability of the test item in the solvent (vehicle) was at the responsibility of the sponsor and not further specified.
Solvent (vehicle) used:	Culture medium (Eagle's Minimal Essential medium with Earle's Salts)

2. Control Materials:

Negative control:	The negative control corresponded to the solvent control.
Solvent (vehicle) control:	Culture medium (Eagle's Minimal Essential medium with Earle's Salts)
Positive control:	-S9 mix: Mitomycin C (MCC): 0.05 µg/mL +S9 mix: Cyclophosphamide (CP): 10 µg/mL

3. Metabolic activation:

S9 mix was obtained from the livers of male Sprague-Dawley rats, weighing approx. 200 g. The animals received a single intraperitoneal injection of Aroclor 1254. Five days after administration, the S9 fraction was prepared. Prior to the experiment, an aliquot of S9 mix fraction was mixed with standard co-factors.

S9 mix component	Concentration	Unit
Sodium phosphate buffer (pH 7.4)	30	mM
KCl	33	mM
NADPH-generating system		
Glucose 6-phosphate	5	mM
NADP	5	mM
$MgCl_2$	8	mM
S9	5	% (v/v)

4. Test organism:

CHL cells were used, established from the lung of Chinese hamster. The cells have an average generation time of approximately 11 hours. It was not reported whether the cells were screened for mycoplasma contamination.

5. Cell culture:

Medium: Eagle's MEM medium supplemented with 10 % foetal calf serum

	and antibiotics
Incubation:	At 37 °C with 5 % CO_2 in air
Cell culture establishment prior to exposure:	The cells were seeded approx. 48 hours prior to treatment in 25 cm^2 flasks. 0.15 x 10^6 cells were seeded per flask for the 6 and 24 h cultures and 0.075 x 10^6 cells were seeded per flask for the 48 h cultures.

6. Test concentrations and number of replicates:

(a) Preliminary cytotoxicity assay:

Metabolic activation	Duration of exposure	Concentrations	Replicates
-S9 mix	24 h	19.5, 39.1, 78.13, 156.25, 312.5, 625, 1250, 2500 and 5000 µg/mL	Duplicate
-S9 mix	48 h	19.5, 39.1, 78.13, 156.25, 312.5, 625, 1250, 2500 and 5000 µg/mL	Duplicate
± S9 mix	6 h	19.5, 39.1, 78.13, 156.25, 312.5, 625, 1250, 2500 and 5000 µg/mL	Duplicate

(b) Main cytogenicity test:

Metabolic activation	Duration of exposure	Concentrations	Replicates
-S9 mix	24 h	39, 78.1, 156.25, 312.5*, 625* and 1250* µg/mL	Duplicate
-S9 mix	48 h	39, 78.1, 156.25, 312.5*, 625* and 1250* µg/mL	Duplicate
± S9 mix	6 h	39, 78.1, 156.25, 312.5*, 625* and 1250 µg/mL	Duplicate

* Dose levels selected for assessment of chromosome aberration

B: STUDY DESIGN AND METHODS

1. Dates of experimental work: 30 Aug 1995 – 04 Jan 1996
Finalisation date: 18 Jan 1996

2. Preliminary cytotoxicity test:
In a preliminary test, CHL cells were treated at concentrations in the range of 19.5 to 5000 µg/mL each with and without metabolic activation. Duplicate cell cultures were exposed to the test item or solvent. The cells were exposed for 24 and 48 hours without metabolic activation and for 6 hours with and without metabolic activation, followed by an 18-hour recovery period in treatment-free medium. Growth inhibition was estimated by counting the number of cells at the end of the culture period and expressing the cell count as a percentage of the concurrent vehicle control value. In addition, slides were prepared from the cells in order to check for the presence of cells in metaphase.

3. Main cytogenicity test:
Treatment: After the 48 hour pre-incubation period, treatment for the main mutagenicity test was initiated. Based on the results of the preliminary cytotoxicity test, duplicate cultures were exposed to test item concentrations in the range of 39 to 1250 µg/mL for 24 and 48 hours in the absence of metabolic activation and for 6 hours in the presence and absence of metabolic activation. Corresponding solvent and positive controls (MMC without S9 mix and CP with S9 mix) were included.
In the absence of S9 mix, chromosome preparations were made at 24 and 48 hours after start of treatment. In case of 6 hour exposure in the presence and absence of S9 mix, the cells were incubated for 18 hours following exposure

and chromosome preparations were made at 24 h after start of exposure.

Spindle inhibition: Colcemid (0.1 µg/mL) was added to the cultures two hours before harvest.

Cell harvest: The cells were harvested by centrifugation of trypsinised cultures and re-suspended in hypotonic potassium chloride solution (0.075 M) for 10 minutes. Afterwards, the cells were centrifuged again and fixed in methanol:glacial acetic acid fixative (3:1, v/v). The fixative was changed several times and the cells stored at 4 °C for sufficient time to ensure complete fixation.

Slide preparation: Fixed cells were dropped onto clean, wet microscope slides and left to air dry. Each slide was labelled with the appropriate identification data. Air-dried slides were stained in 2 % Gurrs Giemsa R66 for 5 minutes, rinsed, dried and coverslipped in mounting medium.

Metaphase analysis Where possible, 100 consecutive well-spread metaphases were examined per culture (200 metaphase cells per condition in total), using microscopic assessment. Concentrations in the range of 312.5 to 1250 µg/mL were evaluated for the number of chromosomes, chromosome gaps, breaks or re-arrangements. Cells with 28-31 chromosomes were scored as aneuploidy cells. In addition, the % incidence of polyploid cells (defined as metaphase cells with > 31 chromosomes) was noted. The percentage of cells showing structural chromosome aberrations (gaps, breaks and exchanges) were calculated including and excluding gap-type aberrations.

Cytotoxicity: Growth inhibition was estimated by counting the number of cells at the end of the culture period and expressing the cell count as a percentage of the concurrent vehicle control value.

4. Statistics

Statistical significance on the number of aberrant metaphases and polyploid cells when compared with those of corresponding solvent controls was identified using the Fisher's Exact test.

5. Acceptance criteria

The cytogenicity test was considered acceptable if the percentage of cells with chromosomal aberrations was within the range of aberration frequencies acceptable for control cultures, which are commonly in the range of 0 – 3 % (Ishidate (1987))[12].

6. Evaluation criteria

A positive response was recorded for a particular treatment if the percentage of cells with aberrations (gaps included) was ≥ 10 %. For polyploid cells, an incidence > 10 % is generally considered as positive.
An equivocal response was recorded for values between 5 and 10 %.
A negative response was obtained if the percentage of cells with aberrations was < 5 %.

II. RESULTS AND DISCUSSION

A. ANALYTCAL DETERMINATIONS

Analytical determinations of the test substance in the solvent were not determined as not required by the test guideline.

[12] Ishidate (1987): Data Book of Chromosomal Aberration Test *in Vitro*

B. CYTOTOXICITY

Preliminary toxicity test:

In the preliminary cytotoxicity test cytotoxicity was observed at ≥ 2500 µg/mL after 24 hours of exposure and at ≥ 1250 µg/mL after 48 hours of exposure, both in the absence of metabolic activation. After 6 hours of exposure in the absence of S9 mix, cytotoxicity was evident at 5000 µg/mL. Growth inhibition was also observed at lower doses in the range of 19.5 – 1250 µg/mL, however, there was no dose-response relationship and no cytotoxicity at 2500 µg/mL. There was no cytotoxicity after 6 hours of exposure in the presence of S9 mix.

After 6 hours of exposure, metaphase cells were observed in the presence and absence of S9 mix up to a concentration of 5000 µg/mL. After 24 and 48 hours of exposure in the absence of metabolic activation, metaphases were present at concentrations up to 2500 µg/mL.

In addition, there was a dose-related reduction in pH value. At 2500 and 5000 µg/mL, the pH was reduced by ≥ 1 unit.

Based on the observations in pH changes in the preliminary test, the maximum dose level selected for the main study was 1250 µg/mL.

Main mutagenicity test

In the main mutagenicity test there was no cytotoxicity observed up the highest tested concentration, both in the presence and absence of metabolic activation.

C. SOLUBILITY

Evaluation of test item precipitation was not provided in the study report. However, there was a dose-related decrease in the pH value noted in all experiments. At 2500 and 5000 µg/mL, the pH was reduced by ≥ 1 unit.

D. CYTOGENICITY

There was no statistically significant increase in the frequency of cells with chromosomal aberrations at any dose level in any treatment group. In addition, the test material did not induce a significant increase in the numbers of polyploid metaphase cells in any of the tested conditions with or without S9 mix. Frequencies of aberrant metaphases of vehicle control cultures were within the expected range. The positive controls mitomycin C in the absence of S9 mix and cyclophosphamide in the presence of S9 mix gave highly significant increases in the frequency of aberrant metaphase cells. No statistically significant increase was observed for cyclophosphamide after 6 hours of exposure in the absence of S9 mix. The test results indicate that the metabolic activation system was functioning satisfactory and that the test method itself was operating as expected.

Table 5.4.1-37: Technical glyphosate: Chromosome aberration test in CHL cells in vitro ([redacted] 1996), 24 and 48 h incubation without metabolic activation

Compound	Concentration [µg/mL]	No. of metaphases scored	Genotoxicity						Growth inhibition [%]
			no. structural aberrant cells[x]		% structural aberrant cells		PI	Judge	
			incl. gaps	**excl. gaps**	incl. gaps	**excl. gaps**	Polyploid cells		
Without metabolic activation; 24-hour treatment and sampling									
Solvent (culture medium)		200	3.00	**3.00**	1.50	**1.50**	1.00	negative	100.0
HCD[#] range					0-3				
Test item	312.5	200	2.00	**0.00**	1.00	**0.00**	1.00	negative	94.00
	625.0	200	1.00	**1.00**	0.50	**0.50**	0.00	negative	115.00
	1250.0	200	2.00	**1.00**	1.00	**0.50**	0.00	negative	108.00

Table 5.4.1-37*:* Technical glyphosate: Chromosome aberration test in CHL cells in vitro ([redacted] 1996), 24 and 48 h incubation without metabolic activation

Compound	Concentration [µg/mL]	No. of metaphases scored	Genotoxicity						Growth inhibition [%]
			no. structural aberrant cells[x]		% structural aberrant cells		PI	Judge	
			incl. gaps	**excl. gaps**	incl. gaps	**excl. gaps**	Polyploid cells		
MMC	0.05	200	37***	**32*** **	18.50	**16.00**	1.00	positive	97.00
Without metabolic activation; 48-hour treatment and sampling									
Solvent (culture medium)		200	3.00	**2.00**	1.50	**1.00**	1.00	negative	100.0
HCD[#] range					0-3				
Test item	312.5	200	7.00	**5.00**	3.50	**2.50**	3.00	negative	110.00
	625.0	200	6.00	**5.00**	3.00	**2.50**	3.00	negative	107.00
	1250.0	200	6.00	**4.00**	3.00	**2.00**	1.00	negative	100.00
MMC	0.05	200	61***	**56*** **	40.70	**37.30**	0.00	positive	84.00

[#] HCD Historical control data from published literature cited in the study report

MMC Mitomycin C, positive control without S9 mix; CP:cyclophosphamide, positive control with S9 mix

[x] Total number of chromosome aberrations from 200 metaphases scored

*** $p < 0.001$ with Fisher's Exact Test

Table 5.4.1-38: **Technical glyphosate: Chromosome aberration test in CHL cells in vitro ([redacted] 1996), 6 h incubation with and without metabolic activation**

Compound	Concentration [µg/mL]	No. of metaphases scored	Genotoxicity						Growth inhibition [%]
			no. structural aberrant cells[x]		% structural aberrant cells		PI	Judge	
			incl. gaps	**excl. gaps**	incl. gaps	**excl. gaps**	Polyploid cells		
With metabolic activation; 6-hour treatment, 18-hour incubation, sampling after 24-hours									
Solvent (culture medium)		200	6.00	**4.00**	3.00	**2.00**	1.00	negative	100.0
HCD[#] range					0-3				
Test item	312.5	200	1.00	**1.00**	0.50	**0.50**	0.00	negative	97.00
	625.0	200	5.00	**2.00**	2.50	**1.00**	0.00	negative	95.00
	1250.0	200	5.00	**3.00**	2.50	**1.50**	0.00	negative	103.00
CP	10	200	57***	**48*** **	38.00	**32.00**	0.00	positive	66.00
Without metabolic activation; 6-hour treatment, 18-hour incubation, sampling after 24-hours									
Solvent (culture medium)		200	1.00	**1.00**	0.50	**0.50**	3.00	negative	100.0
HCD[#] range					0-3				
Test item	312.5	200	2.00	**2.00**	1.00	**1.00**	0.00	negative	103.00

Table 5.4.1-38: **Technical glyphosate: Chromosome aberration test in CHL cells in vitro ([redacted] 1996), 6 h incubation with and without metabolic activation**

Compound	Concentration [µg/mL]	No. of metaphases scored	Genotoxicity						Growth inhibition [%]
			no. structural aberrant cells[x]		% structural aberrant cells		PI	Judge	
			incl. gaps	**excl. gaps**	incl. gaps	**excl. gaps**	Polyploid cells		
	625.0	200	2.00	**2.00**	1.00	**1.00**	0.00	negative	101.00
	1250.0	200	2.00	**1.00**	1.00	**0.50**	2.00	negative	114.00
CP	10	200	3.00	**3.00**	1.50	**1.50**	0.00	negative	113.00

[#] HCD Historical control data from published literature cited in the study report

MMC Mitomycin C, positive control without S9 mix; CP:cyclophosphamide, positive control with S9 mix

[x] Total number of chromosome aberrations from 200 metaphases scored

*** $p < 0.001$ with Fisher's Exact Test

III. CONCLUSION:

In conclusions, Technical Glyphosate did not induce any statistically significant, dose-related increase in the frequency of cells with chromosome aberrations or in the frequency of polyploid cells, neither in the presence nor absence of a liver enzyme metabolising system or after various exposure times. Technical Glyphosate is therefore considered to be non-c1astogenic to CHL cells in vitro.

3. Assessment and conclusion

Assessment and conclusion by applicant:
Negative for cytogenicity in Chinese hamster lung cells with and without metabolic activation.

The study was performed under GLP conditions and the experimental procedure was similar to OECD guideline 473 (2016), except for some minor deviations. Only 200 metaphase cells were investigated, which was the number of metaphases to be analysed according to previous OECD guidelines 473. In addition, cytotoxicity was evaluated by counting the number of cells at the end of the culture period relative to control, whereas the currently valid guideline (2016) recommends the evaluation based on relative population doubling or relative increase of the cell count. These and further deviations were considered to not compromise the validity of the study. The study is therefore considered valid and acceptable.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.4.1/027
Report author	[redacted]
Report year	1995
Report title	HR-001: *In vitro* cytogenicity test
Report No	IET 94-0143
Document No	Not reported
Guidelines followed in	OECD 473 (1983), U.S. EPA FIFRA Guidelines, Subdivision F (1991) and

study	Japanese MAFF (1985)
Deviations from current test guideline OECD 473 (2016)	Only 200 cells in metaphase were evaluated, whereas the evaluation of 300 metaphases is recommended according to OECD guideline 473 (2016). Historical control data was provided for untreated and solvent control cultures only, but not for the positive control substances. pH measurements were not performed. Cytotoxicity was evaluated based on mitotic indices, whereas OECD 473 (2016) recommends to measure the relative population doubling or the relative increase in cell count. Acceptance and evaluation criteria were inconsistent with those specified in test guideline OECD 473 (2016).
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Glyphosate technical (HR-001, batch: 940908-1, purity: 95.68 %) was tested *in vitro* for its potential to induce chromosomal aberrations in Chinese hamster lung (CHL) cells in the presence and absence of metabolic activation (phenobarbital and 5,6 benzoflavone-induced rat liver S9 fraction). Dose levels were chosen based on the results of a preliminary cytotoxicity test, in which cytotoxicity was observed after 48 hours of treatment at 1000 µg/mL in the absence of S9 mix and after 6 hours of treatment at 2000 µg/mL in the presence of S9 mix.

Duplicate cultures were exposed to the test substance at concentrations of 125, 250, 500 and 1000 µg/mL for 24 hours without S9 mix, at concentrations of 62.5, 125, 250 and 500 µg/mL for 48 hours without S9 mix and at concentrations of 250, 500, 1000 and 2000 µg/mL for 6 hours in the presence and absence of S9 mix. Untreated cultures, solvent controls (Hanks´ balanced salt solution) and positive controls (mitomycin C without S9 mix and benzo(a)pyrene with S9 mix) were included for experiments with and without metabolic activation, respectively.

The cells were sampled after 24 and 48 hours of exposure without S9 mix and 24 h after start of exposure for the 6 hour exposure with and without S9 mix. A total of 200 metaphases per condition were scored for structural and numerical chromosome aberrations. Cytotoxicity was assessed as mitotic index (MI) and evaluated for 1000 cells per culture.

Cytotoxicity was observed after 48 hours of treatment at 1000 µg/mL in the absence of S9 mix and after 6 hours of treatment at 2000 µg/mL in the presence of S9 mix. Due to the high cytotoxicity, no cells for chromosome preparations were obtained at these concentrations.

Treatment with the test item did not induce a significant increase in the number of aberrant metaphases in any of the tested conditions. After 24 and 48 hours of treatment in the absence of metabolic activation and after 6 hours exposure in the presence and absence of metabolic activation, the number of chromosome aberrations with and without gaps was comparable to those of controls. In addition, there was no increase in the frequencies of polyploid metaphases in any of the tested conditions with or without S9 mix. Frequencies of aberrant metaphases of untreated and solvent control cultures remained within the range of the laboratory's historical control data. The positive controls showed a clear clastogenic effect and markedly increased the number of aberrant metaphases when compared to untreated and solvent control cultures, demonstrating the validity of the test system.

Based on the results of the present study, there is no evidence for a clastogenic potential of glyphosate technical with and without metabolic activation.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material: Glyphosate technical

Identification:	HR-001
Description:	White crystals
Lot/Batch #:	940908-1
Purity:	95.68 %
Stability of test compound:	The stability of the test item at storage conditions (at room temperature) or in the solvent (vehicle) were not specified.
Solvent (vehicle) used:	Hank's balances salt solution (HBSS) and culture medium

2. Control Materials:

Negative control:	Untreated cell cultures were included.
Solvent (vehicle) control:	Hank's balance salt solution (10 % final concentration in medium)
Positive control:	-S9 mix: Mitomycin C (MCC): 0.1 µg/mL in physiological saline +S9 mix: Benzo(a)pyrene (B(a)P): 40 µg/mL in DMSO

3. Metabolic activation:

S9 mix was purchased from [redacted]). The homogenate was obtained from the livers of 7 weeks old male Sprague-Dawley rats, weighing 192 – 229 g. The animals received intraperitoneal injections of phenobarbital (30 mg/kg bw on Day 1, each 60 mg/kg bw on Days 2, 3 and 4) and 5,6 benzoflavone on Day 3. The S9 mix was prepared immediately before the experiment by mixing S9 fraction and co-factor.

S9 mix component	Concentration	Unit
Sodium phosphate buffer (pH 7.4)	100	mM
KCl	33	mM
NADPH-generating system		
Glucose 6-phosphate	5	mM
NADH	4	mM
NADP	4	mM
$MgCl_2$	8	mM
S9	30	% (v/v)

4. Test organism:

CHL cells were used, established from the lung of Chinese hamster. Stocks of passage number 11 were stored in liquid nitrogen and thawed immediately before use. Each batch used for mutagenicity testing was screened for mycoplasma contamination.

5. Cell culture:

Medium:	Eagle's MEM medium supplemented with 10 % newborn calf serum
Incubation:	At 37 °C in a humidified atmosphere of 5 % CO_2

Cell culture establishment prior to exposure

Preliminary cytotoxicity assay:	CHL cells were seeded at density of 1.0 x 10^5 cells in 5 mL of medium and incubated for 48 h
Main cytogenicity test:	CHL cells were seeded at density of 2.0 x 10^5 cells in 10 mL of

medium and incubated for 48 h

6. Test concentrations and number of replicates:

(a) Preliminary cytotoxicity assay (growth inhibition test):

Metabolic activation	Duration of exposure	Concentrations	Replicates
-S9 mix	24 h	3.9, 7.8, 15.6, 31.3, 62.5, 125, 250, 500 and 1000 µg/mL	Duplicate
-S9 mix	48 h	3.9, 7.8, 15.6, 31.3, 62.5, 125, 250, 500 and 1000 µg/mL	Duplicate
+ S9 mix	6 h	3.9, 7.8, 15.6, 31.3, 62.5, 125, 250, 500 and 1000 µg/mL	Duplicate
+ S9 mix	6 h*	1000, 2000, 3000, 4000 and 5000 µg/mL	Duplicate

* Repeated growth inhibition test with metabolic activation at higher concentrations

(b) Main cytogenicity test:

Metabolic activation	Duration of exposure	Concentrations	Replicates
-S9 mix	24 h	125, 250, 500 and 1000 µg/mL	Duplicate
-S9 mix	48 h	62.5, 125, 250 and 500 µg/mL	Duplicate
± S9 mix	6 h	250, 500, 1000 and 2000 µg/mL	Duplicate

B: STUDY DESIGN AND METHODS

1. Dates of experimental work: 13 Mar – 09 May 1995
Finalisation date: 29 May 1995

2. Preliminary cytotoxicity test:
In a preliminary test, CHL cells were seeded at density of 1.0 x 10^5 cells in 5 mL of medium and incubated for 48 h to establish the cell culture. The cells were treated at concentrations in the range of 3.9 to 1000 µg/mL in the presence and absence of metabolic activation. Without S9 mix (direct method) duplicate cultures each were exposed for 24 and 48 hours and relative cell growth was measured by comparing with the staining density in the concurrent solvent control. In the presence of S9 mix, duplicate cell cultures were exposed for 6 hours, followed by an incubation period of 18 hours. About 24 hours after start of exposure, the relative cell growth was determined. As no reduction of cell growth was observed in the presence of metabolic activation, a second growth inhibition test was performed using test item concentrations in the range of 1000 – 5000 µg/mL in the presence of S9 mix.

3. Main cytogenicity test:

Treatment: CHL cells were seeded at a density of 2.0 x 10^5 cells with 10 mL of medium and incubated for 48 h to establish the cell culture. Based on the results of the preliminary cytotoxicity test, the duplicate cultures were exposed to test item concentrations of 125 – 1000 µg/mL for 24 hours in the absence of metabolic activation, at concentrations of 62.5 – 500 µg/mL for 48 h in the absence of metabolic activation and at concentrations of 250 – 2000 µg/mL for 6 hours in the presence and absence of metabolic activation. Corresponding untreated negative control, solvent and positive controls (MMC without S9 mix for 24 and 48 hour treatment and B(a)P with S9 mix) were included.
In the absence of S9 mix, chromosome preparations were made at 24 and 48 hours after start of treatment. In case of 6 hour exposure, the cells were incubated for 18 hours following exposure and chromosome preparations were made at 24 h after start of exposure. The 6-hour experiments in the presence

and absence of S9 mix were set-up as parallel experiments with the one without S9 being a concurrent control experiment against S9 mix treatment (all conditions the same as the exept for the adding S9 mix; in both settings, B(a)P was used as positive control).

Spindle inhibition: Colchicine (0.5 µg/mL) was added to the cultures two hours before harvest.

Cell harvest: The cells were detached by 0.25 % trypsin and swollen with a hypotonic potassium chloride solution (0.075 mM) for 15 minutes at room temperature. Afterwards the cells were fixed with Carnoy's solution (methanol:acetic acid = 3:1) and dropped on glass slides.

Slide preparation: Fixed cells were dropped on glass slides and air-dried. Two slides were prepared per culture and labelled with code numbers. Air-dried slides were stained with 2 % Giemsa solution for 15 minutes at room temperature.

Metaphase analysis A total number of 200 metaphase cells per condition (100 metaphase cells per culture) were examined by light microscopy. Diploid metaphase cells which possessed the typical karyotype of CHL cells and polyploid metaphase cells were analysed for chromosomal aberrations. The following data were recorded:

- Number and frequency of polyploid cells
- Number and frequency of each structural chromosome aberrations
- Number and frequency of metaphase cells with structural chromosome aberrations. Chromosome gaps, -breaks, -exchanges and chromatid breaks and -exchanges, fragmentation and other structural chromosome aberrations such as multiple aberration were recorded.
- Numerical chromosome aberrations. Only polyploid cell having 3 or more copies of haploid number of chromosomes was scored as a numerical chromosome aberration cell.

Cytotoxicity: Mitotic indices were calculated based on the number of cells in metaphase observed per 1000 cells scored.

4. Statistics

The number of aberrant metaphases and polyploid cells at each dose were statistically compared with those of corresponding solvent controls using a chi-square test

5. Acceptance criteria

The cytogenicity test was considered valid if the following criteria were met:

- The frequencies of the aberrant metaphases in the solvent control groups were within the range of the laboratory's historical negative control range (mean ± 3x SD).
- The frequency of the aberrant metaphases in the positive control groups were 10 % or more.

6. Evaluation criteria

A test substance was judged negative if there was no significant increase in the frequencies of aberrant metaphases or polyploid cells at any dose. A test substance was judged positive if reproducible and significant increases in the frequencies of aberrant metaphases or polyploid cells were observed with a dose-related response. Both biological and statistical significance were considered together in a final evaluation.

II. RESULTS AND DISCUSSION

A. ANALYTCAL DETERMINATIONS

Analytical determinations of the test substance in the solvent were not performed, as not required by the test guideline.

B. CYTOTOXICITY

Preliminary cytotoxicity test

In the preliminary cytotoxicity test, cytotoxicity was observed in the presence and absence of metabolic activation. Without S9 mix (24 and 48 h treatment), a reduction in relative cell growth by 50 % or more were observed at 500 and 1000 µg/mL, respectively. With S9 mix, no cell growth inhibition effect was observed in the first growth inhibition test up to 1000 µg/mL. A second growth inhibition test with S9 mix was performed using concentrations up to 5000 µg/mL. Cell growth inhibition of over 50 % was observed at 2000 µg/mL and above. No reduction in relative cell growth by 50 % or more was observed at any concentration in cells exposed for 6 hours without S9 mix.

It was noticed that in both experiments the color of the culture medium was turned to yellow at 500 µg/mL or more, indicating a decline of pH.

Results obtained in this preliminary cytotoxicity test were used to select concentrations for the main cytogenetic assay. The highest test compound concentration was expected to reduce mitotic index to approximately 50 % compared to corresponding solvent controls. Based on the findings of the preliminary test, the highest concentrations for the main cytogenicity assay were chosen to be 1000 µg/mL in the absence of S9 mix and 2000 µg/mL in the presence of S9 mix.

Table 5.4.1-39: Preliminary growth inhibition test

Concentration (µg/mL)	Relative cell growth (%)			
	-S9 mix 24 h	-S9 mix 48 h	+S9 mix 6-18 h (1st exp.)	+S9 mix 6-18 h (2nd exp.)
Solvent control (HBSS)	100	100	100	100
3.9	100	100	100	-
7.8	97	101	99	-
15.6	100	101	108	-
31.3	96	110	104	-
62.5	100	106	97	-
125	102	99	103	-
250	92	82	106	-
500	74	46	112	-
1000	22	9	106	100
2000	-	-	-	22
3000	-	-	-	38
4000	-	-	-	27
5000	-	-	-	25

Main mutagenicity test

In the main mutagenicity test, excessive cytotoxicity was observed after 48 hour treatment at 1000 µg/mL in the absence of S9 mix and after 6 hours treatment at 2000 µg/mL in the presence of S9 mix. Due to the high cytotoxicity, no cells for chromosome preparations were obtained at these concentrations.

It was noticed that in both treatments (with and without S9 mix) pH of the culture medium of the cultures

treated at 500, 1000 and 2000 µg/mL went down.

C. SOLUBILITY

Precipitation of the test item was not reported up to the highest tested concentrations, neither in the presence, nor in the absence of metabolic activation. However, a decrease in pH was noted in all experiments at ≥ 500 µg/mL with and without S9 mix, indicated by a medium color change to yellow. As there was no significant increase in the number of aberrant metaphases at any concentration in any test, this decrease in pH does not have any impact.

D. CYTOGENICITY

There was no significant increase in the number of aberrant metaphases observed in none of the experiments, neither in the presence nor in the absence of S9 mix. After 24 and 48 hours of treatment in the absence of metabolic activation and after 6 hours exposure in the presence and absence of metabolic activation, the number of chromosome aberrations with and without gaps was comparable to those of controls. In addition, there was no increase in the frequencies of polyploid metaphases in any of the tested conditions with or without S9 mix. Frequencies of aberrant metaphases of untreated and solvent control cultures remained within the range of the laboratory's historical control data. The positive controls mitomycin C (- S9) and benzo(a)pyrene (+ S9) showed a clear clastogenic effect and markedly increased the number of aberrant metaphases when compared to untreated and solvent controls, demonstrating the sensitivity of the test and the functionality of the S9 mix. In the 6-hour treatment without S9 (concurrent control experiment against S9 mix treatment), benzo(a)pyrene showed no significant increase in chromosome aberrations (as expected), as this requires metabolic activation.

Table 5.4.1-40: *In vitro* cytogenicity test [redacted], 1995), 24 and 48 h incubation without metabolic activation

Compound	Concentration [µg/mL]	No. of metaphases scored	Genotoxicity						Mitotic index [%]
			no. structural aberrant cells[x]		% structural aberrant cells		PI (%)	Judge	
			incl. gaps	**excl. gaps**	incl. gaps	**excl. gaps**	Polyploid cells		
Without metabolic activation; 24-hour treatment and sampling									
Untreated	0.0 %	200	3.00	**1.00**	1.50	**0.50**	0.00	negative	6.1
HCD[#] mean	0.0 %				1.12	**0.50**	0.37		
± SD					± 0.93	**± 0.59**	± 0.56		
Solvent (HBSS)	10.0 %	200	0.00	**0.00**			1.00	negative	6.0
HCD[#] (DMSO) mean	0.5 %				1.32	**0.61**	0.43		
± SD					± 0.75	**± 0.55**	± 0.52		
Test item	125.0	200	1.00	**1.00**	0.50	**0.50**	1.00	negative	6.70
	250.0	200	3.00	**1.00**	1.50	**0.50**	1.00	negative	5.90
	500.0[b]	200	4.00	**1.00**	2.00	**0.50**	0.00	negative	5.60
	1000.0[bc]	200							
MMC	0.1	200	101.00	**98.00**	50.50	**49.00**	1.00	positive	3.30
Without metabolic activation; 48-hour treatment and sampling									
Untreated	0.0 %	200	3.00	**2.00**	1.50	**1.00**	1.00	negative	2.5

Table 5.4.1-40: *In vitro* cytogenicity test ([redacted], 1995), 24 and 48 h incubation without metabolic activation

Compound	Concentration [µg/mL]	No. of metaphases scored	Genotoxicity						Mitotic index [%]
			no. structural aberrant cells[x]		% structural aberrant cells		PI (%)	Judge	
			incl. gaps	**excl. gaps**	incl. gaps	**excl. gaps**	Polyploid cells		
HCD[#] mean	0.0 %				1.18	**0.50**	0.48		
± SD					± 1.22	**± 0.66**	± 0.51		
Solvent (HBSS)	10.0 %	200	0.00	**0.00**	0.00	**0.00**	1.00	negative	3.4
HCD[#] (DMSO) mean	0.5 %				1.18	**0.65**	0.47		
± SD					± 0.86	**± 0.61**	± 0.49		
Test item	62.5	200	3.00	**0.00**	1.50	**0.00**	0.00	negative	3.00
	125.0	200	2.00	**1.00**	1.00	**0.50**	0.00	negative	2.90
	250.0	200	5.00	**2.00**	2.50	**1.00**	2.00	negative	3.10
	500.0[b]	200	5.00	**2.00**	2.50	**1.00**	0.00	negative	3.00
MMC	0.1	200	144.00	**141.00**	72.00	**70.50**	2.00	positive	2.60

[#] HCD Historical control data based on 39 cytogenicity tests performed in the laboratory from December 1988 to December 1994

MI mitotic index: number of cells in mitosis/ number of cells, based on 1000 cells per culture
PI: polyploid index: (number of polyploid + endoreduplicated cells)/number of cells in mitosis, based on 200 metaphases
MMC Mitomycin C, positive control without S9 mix; B(a)P: Benzo(a)pyrene, positive control with S9 mix

[b] The color of the culture medium turned yellow after addition of the test substance

[c] No chromosome preparations due to cytotoxicity

[x] Total number of chromosome aberrations from 200 metaphases scored

Table 5.4.1-41: *In vitro* cytogenicity test ([redacted], 1995), 6 h incubation with and without metabolic activation

Compound	Concentration [µg/mL]	No. of metaphases scored	Genotoxicity						Mitotic index [%]
			no. structural aberrant cells[x]		% structural aberrant cells		PI (%)	Judge	
			incl. gaps	**excl. gaps**	incl. gaps	**excl. gaps**	Polyploid cells		
With metabolic activation; 6-hour treatment, 18-hour incubation, sampling after 24-hours									
Untreated	0.0 %	200	4.00	**1.00**	2.00	**0.50**	0.00	negative	4.9
HCD[#] mean	0.0 %				1.38	**0.52**	0.48		
± SD					± 1.22	**± 0.69**	± 0.70		
Solvent (HBSS)	10.0 %	200	3.00	**1.00**	1.50	**0.50**	0.00	negative	6.3
HCD[#] (DMSO) mean	0.5 %				1.41	**0.72**	0.43		

Table 5.4.1-41: *In vitro* cytogenicity test [redacted], 1995), 6 h incubation with and without metabolic activation

Compound	Concentration [µg/mL]	No. of metaphases scored	Genotoxicity						Mitotic index [%]
			no. structural aberrant cells[x]		% structural aberrant cells		PI (%)	Judge	
			incl. gaps	**excl. gaps**	incl. gaps	**excl. gaps**	Polyploid cells		
± SD					± 0.98	**± 0.67**	± 0.38		
Test item	250.0	200	2.00	**2.00**	1.00	**1.00**	0.00	negative	6.70
	500.0[b]	200	4.00	**2.00**	2.00	**1.00**	0.00	negative	5.60
	100.00[b]	200	3.00	**1.00**	1.50	**0.50**	1.00	negative	7.20
	2000.0[bc]	200							
B(a)P	40.0	200	79.00	**77.00**	39.50	**38.50**	0.00	positive	3.80
Without metabolic activation; 6-hour treatment, 18-hour incubation, sampling after 24-hours									
Untreated	0.0 %	200	4.00	**3.00**	2.00	**1.50**	0.00	negative	5.3
Solvent (HBSS)	10.0 %	200	2.00	**0.00**	1.00	**0.00**	2.00	negative	5.7
Test item	250.0	200	4.00	**2.00**	2.00	**1.00**	0.00	negative	5.10
	500.0	200	2.00	**1.00**	1.00	**0.50**	0.00	negative	4.90
	1000.0[b]	200	2.00	**1.00**	1.00	**0.50**	1.00	negative	5.70
	2000.0[bc]	200							
B(a)P	40.0	200	1.00	**1.00**	0.50	**0.50**	1.00		4.70

[#] HCD Historical control data based on 39 cytogenicity tests performed in the laboratory from December 1988 to December 1994

MI mitotic index: number of cells in mitosis/ number of cells, based on 1000 cells per culture
PI: polyploid index: (number of polyploid + endoreduplicated cells)/number of cells in mitosis, based on 200 metaphases

MMC Mitomycin C, positive control without S9 mix; B(a)P: Benzo(a)pyrene, positive control with S9 mix

[b] The color of the culture medium turned yellow after addition of the test substance

[c] No chromosome preparations due to cytotoxicity

[x] Total number of chromosome aberrations from 200 metaphases scored

III. CONCLUSION:

In the chromosome aberration test, there was no significant increase in the frequencies of abnormal metaphases with structural chromosome aberrations or polyploid metaphases in the treated groups compared to the control group whatever were the tested concentrations. Based on the results obtained, it was concluded that, under the conditions of this study, the test substance HR-001 did not induce chromosome aberrations in Chinese hamster CHL cells with or without the metabolic activation system.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Negative for cytogenicity in Chinese hamster lung cells with and without metabolic activation.
The study was performed under GLP conditions and in accordance with OECD guideline 473 (1983). There were only minor deviations when compared to the currently valid OECD 473 (2016). The number of metaphases was only 200, which was the number to be investigated recommended by the previous OCED 473 (1997). In addition, cytotoxicity was evaluated based on mitotic indices instead of

measuring the relative population doubling or the relative increase in cell count.

Historical control data were not provided and evaluation criteria of the test were inconsistent with those specified in the current guideline which requires that for a positive response: a. at least one of the test concentrations exhibits a statistically significant increase compared with the concurrent negative control, b. the increase is dose-related when evaluated with an appropriate trend test, c. any of the results are outside the distribution of the historical negative control data (e.g. Poisson based 95 % control limits).

However, as the increase in the frequencies of abnormal metaphases with structural chromosome aberrations or polyploid metaphases in the treated groups were similar to those of the negative control, the deviations were considered to not compromise the validity of the study. The study is therefore considered valid and acceptable.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.4.1/028
Report author	[redacted]
Report year	1995
Report title	Evaluation of the ability of glyfosaat to induce chromosome aberrations in cultured peripheral human lymphocytes (with independent repeat)
Report No	141918
Document No	Not reported
Guidelines followed in study	OECD 473 (1983), EEC Directive 92/69
Deviations from current test guideline OECD 473 (2016)	Only 200 cells in metaphase were evaluated, whereas the currently valid OECD 473 (2016) recommends the evaluation of 300 metaphase cells per condition. A short-term exposure in the absence of metabolic activation as recommended by OECD 473 (2016) was not included. There was no historical control data provided for the positive control compounds and historical control data obtained in the testing laboratory were not provided. pH measurements were not performed. Acceptance and evaluation criteria specified in the testing protocol differed from those recommended by OECD 473 (2016).
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

A chromosome aberration test in human peripheral lymphocytes was conducted to investigate the potential of glyphosate (batch: 22021, purity: 96 %) to induce chromosomal aberrations *in vitro* in the presence and absence of metabolic activation (Aroclor 1254-induced rat liver S9 fraction). Concentrations were selected

based on the results of a preliminary cytotoxicity test, in which a reduction of the mitotic index of ≥ 50 % was observed at 333 µg/mL in the presence of S9 mix and at 333 and 1000 µg/mL in the absence of S9 mix (24- and 48-hours sampling time point).

Two independent experiments were performed. In each experiment, corresponding solvent (DMSO) and positive controls (mitomycin C in the absence of S9 mix and cyclophosphamide in the presence of S9 mix) were included.

In the first experiment, the cultures were treated for 3 hours in the presence of S9 mix at concentrations in the range of 33 - 562 µg/mL, for 24 hours in the absence of S9 mix with concentrations in the range of 33 - 237 µg/mL and for 48 hours in the absence of S9 mix with concentrations in the range of 56 – 333 µg/mL. The cells treated in the presence of S9 mix were sampled 24 and 48 hours after start of exposure, whereas the cells treated in the absence of S9 mix were prepared directly following end of exposure.

In the second experiment, the cultures were exposed for 3 hours in the presence of S9 mix at concentrations in the range of 100 – 562 µg/mL and for 24 hours in the absence of S9 mix at concentrations in the range of 33 – 333 µg/mL. Chromosome preparations in the second experiment were made 24 hours after start of treatment only.

In each experiment, a total of 200 metaphases per condition was scored for structural and numerical chromosome aberrations. Cytotoxicity was assessed as mitotic index (MI) and evaluated for 1000 cells per culture.

Precipitation of the test item in culture medium was noted at concentrations of 562 µg/mL and above, both in the presence and absence of S9 mix. Cytotoxicity, evident as a reduction in the mitotic index of ≥ 50 %, was observed for the 24-hours sampling time point in the absence of metabolic activation at ≥ 133 µg/mL (first experiment) and for the 24-hours sampling time point in the absence of metabolic activation at 178 and 333 µg/mL (second experiment). Cytotoxicity was further observed after tTreatment for 48 hours in the absence of metabolic activation and after treatment for 3 hours in the presence of metabolic activation (24-hours sampling time point), however, the mitotic indices were reduced by < 50 %.

After treatment with glyphosate, there was no statistically significant increase in the number of cells with chromosomal aberrations observed in any experiment at any concentration when compared to solvent controls, neither in the presence nor in the absence of metabolic activation.
In addition, there was no increase in the frequencies of polyploid metaphases in any of the tested conditions with or without S9 mix.

The number of aberrant metaphases found in the solvent control cultures were within the range of the laboratory's historical control data. The positive controls mitomycin C and cyclophosphamide produced statistically significant increases in the frequency of aberrant cells, demonstrating the sensitivity of the test and the functionality of the S9 mix.

Based on the experimental findings there is no evidence for a clastogenic potential for glyphosate in peripheral human lymphocytes, neither in the presence nor in the absence of metabolic activation.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material:

	Glyfosaat
Identification:	Not specified
Description:	White powder
Lot/Batch #:	22021
Purity:	96 %
Stability of test compound:	The stability of the test item under storage conditions

	(at room temperature in the dark) was guaranteed until the mentioned expiry date 01 Jan 1998. The stability of the test item in solvent was not specified.
Solvent (vehicle) used:	Dimethylsulfoxide (DMSO), final concentration 0.9 % (v/v)

2. Control Materials:

Negative control:	A negative control was not employed in this study.
Solvent (vehicle) control:	DMSO (0.9 % (v/v) final concentration in medium)
Positive control:	-S9 mix: Mitomycin C (MMC): 0.1 and 0.2 µg/mL in Hank's Balanced Salt solution (HBSS) for 24- and 48-hours sampling, respectively +S9 mix: Cyclophosphamide (CP): 15 µg/mL in Hank's Balanced Salt solution (HBSS)

3. Metabolic activation:

S9 mix was routinely prepared from the livers of adult male Wistar rats, which received a single intraperitoneal injection of 500 mg/kg bw Aroclor 1254. Five days after treatment, the animals were sacrificed and the S9 homogenates were isolated. The S9 mix was prepared immediately before the experiment by mixing S9 fraction and co-factor.

S9 mix component	Concentration	Unit
HEPES	4.0	mM
KCl	33.0	mM
NADPH-generating system		
Glucose 6-phosphate	6.5	mM
NADP	4.6	mM
$MgCl_2$	5.0	mM
S9	50	% (v/v)

4. Test organism:

Human peripheral blood was obtained by venepuncture from a healthy, male volunteer and collected in heparinised vessels.

5. Cell culture:

Medium:	Ham's F10 medium without thymidine and hypoxanthine, supplemented with 20 % (v/v) fetal calf serum, 2 mM L-glutamine, penicillin/streptomycin (50 U/mL and 50 µg/mL, respectively), 1.2 g/L sodium bicarbonate and 30 U/mL heparin.
Incubation:	*In a humidified atmosphere (80 – 95 %) containing 5 % CO_2 in the dark.*
Cell culture establishment prior to exposure	*0.5 mL of whole blood was cultured with 5 mL culture medium and 0.1 mL of 9 mg/mL phytohaemagglutinin for 48 hours prior to treatment.*

6. Test concentrations and number of replicates:

(a) Preliminary cytotoxicity assay

Metabolic activation	Duration of exposure (Fixation time)	Concentrations	Replicates
-S9 mix	24 h (24 h)	10, 33, 100, 333 and 1000 µg/mL	Single culture
-S9 mix	48 h (48 h)	10, 33, 100, 333 and 1000 µg/mL	Single culture
+S9 mix	3 h (24 h)	10, 33, 100, 333 and 1000 µg/mL	Single culture

(b) Main cytogenicity test:

Metabolic activation	Duration of exposure (Fixation time)	Concentrations	Replicates
First experiment			
-S9 mix	24 h (24 h)	33*, 56, 100*, 133, 178 and 237* µg/mL	Duplicate
-S9 mix	48 h (48 h)	56, 100, 133, 178, 237* and 333 µg/mL	Duplicate
+S9 mix	3 h (24 h)	33, 100, 133, 178, 237*, 333* and 562* µg/mL	Duplicate
+S9 mix	3 h (48 h)	33, 100, 133, 178, 237, 333 and 562* µg/mL	Duplicate
Second experiment			
-S9 mix	24 h (24 h)	33*, 100, 133, 178, 237* and 333* µg/mL	Duplicate
+S9 mix	3 h (24 h)	100, 333*, 422* and 562* µg/mL	Duplicate

* Samples analysed for chromosomal aberrations

B: STUDY DESIGN AND METHODS

1. Dates of experimental work: 15 Mar – 28 May 1995
Finalisation date: 30 Jun 1995

2. Preliminary cytotoxicity test:
In a preliminary cytotoxicity test, human lymphocytes were treated with the test item at concentrations of 10 to 1000 µg/mL both, with and without metabolic activation under the same conditions as in the main mutagenicity test (described below). The highest concentration was selected based on the solubility of the test substance in culture medium. One single cell culture per condition was exposed to the test item for 3 hours in the presence of S9 mix or for 24 and 48 hours in the absence of S9 mix. Cells treated in the presence of S9 mix were prepared about 24 hours after start of exposure while cells treated in the absence of S9 mix were prepared immediately following end of exposure. Slides were prepared and the mitotic index of each culture was determined by counting the number of 1000 metaphases per culture.

Based on the results of the preliminary test, the concentrations for the main cytogenicity assay were selected. The concentrations of the test substance that was expected to show clear indication of toxicity with a mitotic index of ≥ 50 % was selected for scoring of chromosome aberrations.

3. Main cytogenicity test:
Treatment: Following cell culture establishment, duplicate cultures per condition were exposed to the test item, solvent or positive control in the presence and absence of metabolic activation. Two independent experiments were performed. Corresponding solvent (DMSO) and positive controls (mitomycin C in the absence of S9 mix and cyclophosphamide in the presence of S9 mix) were included in each experiment.

In the first experiment, the cultures were treated for 3 hours in the presence of S9 mix at concentrations in the range of 33 - 562 µg/mL, for 24 hours in the absence of S9 mix with concentrations in the range of 33 - 237 µg/mL and for 48 hours in the absence of S9 mix with concentrations in the range of 56 – 333 µg/mL. The cells treated in the presence of S9 mix were prepared for chromosome analysis 24 and 48 hours after start of exposure. After 3 hours of exposure with S9 mix, the cells were rinsed once with Hank's buffered salt solution (HBSS) to remove the test compound, re-suspended in medium and incubated for another 20 – 22 hours (24-hours fixation time) or for 44 - 46 hours (48-hours fixation time). The cells treated in the absence of S9 mix were prepared directly following end of exposure.
In the second experiment, the cultures were exposed for 3 hours in the presence of S9 mix at concentrations in the range of 100 – 562 µg/mL and for 24 hours in the absence of S9 mix at concentrations in the range of 33 – 333 µg/mL. Chromosome preparations in the second experiment were made 24 hours after start of treatment only.

Spindle inhibition: During the last 3 hours of the culture period, cell division was arrested by addition of 0.5 µg/mL colchicine (0.5 µg/mL) to each culture.

Cell harvest: The cell cultures were centrifuged and the remaining cell pellet swollen in 0.56 % hypotonic potassium chloride solution for 5 minutes at 37 °C. Afterwards, the cells were fixed with 3 changes of methanol : acetic acid fixative (3:1 v/v).

Slide preparation: Fixed cells were dropped on glass slides which were immersed for 24 hours in a 1:1 mixture of 96 % (v/v) ethanol/ether and cleaned with a tissue. Two slides were prepared per culture. Slides were allowed to dry, stained for 10 – 30 minutes with 5 % (v/v) Giemsa solution, rinsed with tap water, allowed to dry again and cleared in xylene before embedding in DePeX and mounting.

Metaphase analysis A total number of 200 metaphase cells per condition (100 metaphase cells per culture) were examined by light microscopy. In case the number of aberrant cells (gaps excluded) was ≥ 25 in 50 metaphases no more metaphases were examined. Only metaphases containing 46 chromosomes were analysed. The following data were recorded:

- The number of cells with chromosomal aberrations.

The number and type of structural chromosomal aberrations, Chromosome gaps, -breaks, -exchanges, -intrachanges, chromatid gaps, -breaks and -deletions, centromere state, ring chromosomes, and other structural chromosome aberrations such as multiple aberration.

- Numerical variations such as endoreduplication and polyploidy.

Cytotoxicity: The mitotic index of each culture was calculated based on the number of cells in metaphase observed per 1000 cells scored.

4. Statistics

The number of cells with chromosomal aberrations at each dose were compared with those of corresponding solvent controls and statistically evaluated using a chi-square test

5. Acceptance criteria

The chromosome aberration test was considered acceptable if the following criteria were met:

- The numbers of chromosome aberrations found in the solvent control cultures should reasonably be within the laboratories historical control data range.
- The positive control substances should produce a statistically significant increase in the number

of cells with chromosomal aberrations.

6. Evaluation criteria

A test substance was considered positive (clastogenic) in the chromosome aberration test if the following criteria were met:

- It induced a dose-related statistically significant increase in the number of cells with chromosome aberrations.
- A statistically significant increase in the frequency of aberrations was observed in the absence of a clear dose-response relationship.

A test substance was considered negative (not clastogenic) in the chromosome aberration test if none of the tested concentrations induced a statistically significant increase in the number of cells with chromosomal aberrations.

II. RESULTS AND DISCUSSION

A. ANALYTCAL DETERMINATIONS

Analytical determinations of the test substance in the solvent have not been performed, as not required by the test guideline.

B. CYTOTOXICITY

A detailed description on cytotoxic effects at individual concentrations was not provided in the study report. Description of cytotoxicity in the following section is based on re-evaluation of the raw data provided in the study report. A cytotoxic effect was considered evident when a reduction of the mitotic index of ≥ 50 % was observed.

Preliminary cytotoxicity test

In the preliminary cytotoxicity test, cytotoxicity was observed at 333 µg/mL in the presence of S9 mix and at 333 and 1000 µg/mL in the absence of S9 mix (24- and 48-hours sampling time point). For the 24-hours sampling time point at 333 and 1000 µg/mL in the absence of S9 mix and for the 48-hours sampling time point in the absence of S9 mix no metaphase cells at all were detected.

The results obtained in the preliminary cytotoxicity test were used to select concentrations for the main mutagenicity assay. The highest test compound concentration was expected to reduce mitotic index by ≥ 50 % compared to corresponding solvent controls. Therefore, for the main cytogenetic test concentrations of 237 and 333 µg/mL were selected as highest concentrations in the absence of S9 mix (24- and 48-hours sampling time point, respectively) and 562 µg/mL was chosen as highest concentration in the presence of S9 mix.

Main mutagenicity test

In the main mutagenicity test, cytotoxicity evident as a reduced mitotic index of ≥ 50 % was observed for the 24-hours sampling time point in the absence of metabolic activation at ≥ 133 µg/mL (first experiment) and for the 24-hours sampling time point in the absence of metabolic activation at 178 and 333 µg/mL (second experiment). There was also evidence of toxicity for the 48-hours sampling time point in the absence of metabolic activation (first experiment) and for the 24-hours sampling time point in the presence of metabolic activation (second experiment), but the mitotic indices were < 50 % (please refer to Table 5.4.1-42 and **Error! Reference source not found.**).

C. SOLUBILITY

Precipitation of the test item was reported in the preliminary cytotoxicity test as well as the main cytogenicity test, at 562 µg/mL and at higher concentrations, both in the presence and absence of S9 mix.

D. CYTOGENICITY

There was no statistically significant increase in the number of cells with chromosomal aberrations observed in any experiment at any concentration when compared to solvent controls, neither in the presence nor in the absence of metabolic activation.

In addition, there was no increase in the frequencies of polyploid metaphases in any of the tested conditions with or without S9 mix.

The number of aberrant metaphases found in the solvent control cultures were within the range of the laboratory's historical control data. The positive controls mitomycin C and cyclophosphamide produced statistically significant increases in the frequency of aberrant cells, demonstrating the sensitivity of the test and the functionality of the S9 mix.

Table 5.4.1-42: Evaluation of the ability of glyfosaat to induce chromosome aberrations in cultured peripheral human lymphocytes ([redacted], 1995), 24 and 48 h exposure (-S9), 3 h exposure (+S9)

Compound	Conc. [µg/mL]	No. of metaphases scored	Genotoxicity								MI [%]
			no. structural aberrant cells		% structural aberrant cells		no. structural aberrations		PI	Judge	
			incl. gaps	excl. gaps	incl. gaps	excl. gaps	incl. gaps	excl. gaps	Poly ploid cells		
Without metabolic activation; 24-hours treatment and sampling											
Solvent (DMSO)	0	200	4.0	**3.0**	2.0	**1.5**	4.0	**3.0**	2	negative	100
HCD# mean ± SD	0	100	1.0 ± 1.1								
Test item	33	200	4.0	**2.0**	2.0	**1.0**	6.0	**4.0**	2	negative	96
	100	200	2.0	**1.0**	1.0	**0.5**	2.0	**1.0**	0	negative	78
	237	200	5.0	**3.0**	2.5	**1.5**	5.0	**3.0**	1	negative	47
MMC	0.2	200	71.0*	**50.0***	35.5*	**25.0***	93.0*	**55.0***	0	positive	44
Without metabolic activation; 48-hours treatment and sampling											
Solvent (DMSO)		200	4.0	**1.0**	2.0	**1.0**	5.0	**2.0**	0	negative	100
HCD# mean ± SD	0	100	1.0 ± 1.1								
Test item	237	200	1.0	**0.0**	0.5	**0.0**	1.0	**0.0**	1	negative	65
MMC	0.1	200	81.0***	**67.0***	40.5*	**33.5***	126.0*	**84.0***	0	positive	83
With metabolic activation; 3-hours treatment, 24 h sampling time											
Solvent (DMSO)		200	6.0	**4.0**	3.0	**2.0**	6.0	**4.0**	1	negative	100
HCD# mean ± SD	0	100	0.8 ± 0.9								
Test item	237	200	2.0	**0.0**	1.0	**0.0**	2.0	**0.0**	0	negative	101
	333	200	3.0	**2.0**	1.5	**1.0**	3.0	**2.0**	2	negative	89
	562	200	4.0	**4.0**	2.0	**2.0**	7.0	**6.0**	1	negative	55
CP	15	200	89.0*	**53.0***	44.5*	**26.5***	150.0*	**81.0***	1	positive	33

Table 5.4.1-42: Evaluation of the ability of glyfosaat to induce chromosome aberrations in cultured peripheral human lymphocytes ([redacted], 1995), 24 and 48 h exposure (-S9), 3 h exposure (+S9)

Compound	Conc. [µg/mL]	No. of metaphases scored	Genotoxicity								MI [%]
			no. structural aberrant cells		% structural aberrant cells		no. structural aberrations		PI	Judge	
			incl. gaps	**excl. gaps**	incl. gaps	**excl. gaps**	incl. gaps	**excl. gaps**	Poly ploid cells		
With metabolic activation; 3-hours treatment, 48 h sampling time											
Solvent (DMSO)		200	1.0	**0.0**	0.5	**0.0**	1.0	**0.0**	0	negative	100
HCD[#] mean ± SD	0	100	0.8 ± 0.9								
Test item	562	200	0.0	**0.0**	0.0	**0.0**	0.0	**0.0**	0	negative	121

[#] HCD Historical control data from the laboratory's historical control range (time period for data generation not specified); MI Mitotic index
MMC Mitomycin C, positive control without S9 mix; CP:cyclophosphamide, positive control with S9 mix

* $p < 0.001$ with Chi-square test

Table 5.4.1-43: Evaluation of the ability of glyfosaat to induce chromosome aberrations in cultured peripheral human lymphocytes ([redacted], 1995), 24 h exposure (-S9), 3 h exposure (+ S9)

Compound	Conc. [µg/mL]	No. of metaphases scored	Genotoxicity								MI [%]
			no. structural aberrant cells		% structural aberrant cells		no. structural aberrations		PI	Judge	
			incl. gaps	**excl. gaps**	incl. gaps	**excl. gaps**	incl. gaps	**excl. gaps**	Poly ploid cells		
Without metabolic activation; 24-hours treatment and sampling											
Solvent (DMSO)	0	200	1.0	**0.0**	0.5	**0.0**	1.0	**0.0**	0	negative	100
HCD[#] mean ± SD	0	100	1.0 ± 1.1								
Test item	33	200	2.0	**0.0**	1.0	**0.0**	2.0	**0.0**	0	negative	84
	237	200	5.0	**3.0**	2.5	**1.5**	5.0	**3.0**	0	negative	61
	333	200	4.0	**2.0**	2.0	**1.0**	4.0	**2.0**	0	negative	34
MMC	0.2	200	51.0*	**51.0***	25.5*	**25.5***	63.0*	**60.0***	0	positive	43
With metabolic activation; 3-hour treatment, 24 h sampling time											
Solvent (DMSO)		200	4.0	**3.0**	2.0	**1.5**	4.0	**3.0**	0	negative	100
HCD[#] mean ± SD	0	100	0.8 ± 0.9								
Test item	333	200	7.0	**5.0**	3.5	**2.5**	7.0	**5.0**	1	negative	93
	422	200	4.0	**2.0**	2.0	**1.0**	4.0	**2.0**	0	negative	78
	562	200	3.0	**1.0**	1.5	**0.5**	3.0	**1.0**	0	negative	85
CP	15	200	53.0*	**53.0***	26.5*	**26.0***	89.0*	**88.0***	0	positive	36

Table 5.4.1-43: Evaluation of the ability of glyfosaat to induce chromosome aberrations in cultured peripheral human lymphocytes ([redacted], 1995), 24 h exposure (-S9), 3 h exposure (+ S9)

Compound	Conc. [µg/mL]	No. of metaphases scored	Genotoxicity								
			no. structural aberrant cells		% structural aberrant cells		no. structural aberrations		PI	Judge	MI [%]
			incl. gaps	excl. gaps	incl. gaps	excl. gaps	incl. gaps	excl. gaps	Poly ploid cells		

[#] HCD Historical control data from the laboratory's historical control range (time period for data generation not specified); MI Mitotic index
MMC Mitomycin C, positive control without S9 mix; CP:cyclophosphamide, positive control with S9 mix

* $p < 0.001$ with Chi-square test

III. CONCLUSION:

Based on the experimental findings there is no evidence for a clastogenic potential for glyphosate in peripheral human lymphocytes, neither in the presence nor in the absence of metabolic activation.

3. Assessment and conclusion

Assessment and conclusion by applicant:
Negative for cytogenicity (Chromosome Aberration test) in peripheral human lymphocytes with and without metabolic activation.

The study was performed under GLP conditions and in accordance with OECD guideline 473 (1983). When compared with the currently valid OECD guideline 473 (2016), several deviations became evident. The dose levels investigated were rather low when compared to other studies that were provided for the evaluation of glyphosate. In addition, a short-term exposure in the absence of S9 mix was not included in the experiment. Accordingly, the study was considered to provide supporting information. Further guideline deviations were considered to not impact the validity of the study.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.4.1/029
Report author	[redacted]
Report year	1989
Report title	Report on the possible chromosome damaging effect of glyphosate in Chinese hamster ovary cells
Report No	Not reported
Document No	Not reported
Guidelines followed in study	The study was conducted according to Natarajan et al.[13] (1967), according to guidelines and recommendations of the United

[13] Natarajan, A.T., Tates, A.B., van Buul, P.W., Meijery, M. and De Vogel, N.: Cytogenic effects of mutagens/carcinogens after activation in microsomal system in vitro. I. Induction of chromosome aberration and sister chromatid exchange by diethylnitrosamine (DEN) and dimethylnitrosamine (DMN) in CHO cells in the presence of rat liver microsome. Mut. Res., 37, 83-90 (1976)

	Kingdom Environmental Mutagen Society (UKEMS) and similar to OECD 473 (2016).
GLP	No, not conducted under GLP/ Officially recognised testing facilities. When the study was conducted, GLP was not compulsory.
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	Glyphosate (batch: 978, purity: not specified) was assessed for its ability to induce structural chromosome aberrations in Chinese hamster ovary (CHO) cells *in vitro*. The test was conducted in the presence and absence of metabolic activation (Aroclor 1254-induced rat liver S9 fraction). Glyphosate concentrations were selected based on the results of a preliminary toxicity test, in which cytotoxicity was observed at ≥ 2500 µg/mL in the presence of S9 mix and at ≥ 1250 µg/mL in the absence of S9 mix. Two independent experiments were performed. In each experiment, solvent (medium) and positive controls (ethylmethane sulfonate, 1 µg/mL without S9 mix and cyclophosphamide, 25 µg/mL with S9 mix were included. Tested glyphosate concentrations were 250 – 1000 µg/mL in the absence of S9 mix and 62.5 – 250 µg/mL in the presence of S9 mix. In both experiments, the cells were exposed for 3 hours in the presence and absence of S9 mix, followed by two washing steps and re-incubation for further 18 hours[14]. Chromosomes were prepared about 21 hours after start of exposure[14]. In each experiment, a total of 200 metaphases per condition was scored for structural chromosome and chromatid aberrations.
Short description of results:	Precipitation of the test item in culture medium was not reported. Cytotoxicity was not monitored in the cytogenicity experiments, as the concentrations selected were chosen to be non-cytotoxic. In the first experiment, there was a mathematically significant increase in the incidence of aberrant cells and the incidence of total chromosome aberrations at 62.5 µg/mL in the presence of metabolic activation. As the effect was noted at the lowest concentration and as a dose-dependency was lacking, the observation was considered incidental. In the second experiment, there was no statistically significant increase in the number of cells with chromosomal aberrations or the total number of aberrations at any concentration when compared to solvent controls, neither in the presence, nor in the absence of metabolic activation. The positive controls induced statistically significant aberrations in both experiments, demonstrating the functionality of the metabolic activation system and the sensitivity of the test. Based on the experimental findings and under the conditions of the test, glyphosate has no clastogenic potential in CHO cells *in vitro*, neither in the presence nor in the absence of metabolic activation.
Reasons for why the study is not considered relevant/reliable or not considered as key	The study is considered not acceptable due to the large number of deviations when compared to the currently valid OECD guideline 473 (2016). Only 100 – 200 metaphase cells were evaluated and only structural chromosome aberrations but no numerical

[14]: There are inconsistencies in the study report regarding the duration of exposure (2 or 3 hours) and the duration of post-exposure incubation (16 - 18 hours incubation followed by 2 hours incubation with colchicine or 16 hours incubation in total) prior to metaphase analysis. It was assumed that the cells were exposed for 3 hours, followed by 18 hours of incubation (including 2 hours of treatment with colchicine) and chromosome preparation 21 hours after start of exposure.

study:	aberrations were considered. In addition, the maximum concentration did not meet the cytotoxicity recommendations specified in the current guideline. Cytotoxicity was not measured in the main cytogenicity test, but evaluated only in a preliminary toxicity test based on cell morphology. Besides a number of further guideline deviations, there were inconsistencies regarding the duration of exposure and reporting deficiencies regarding the source and purity of the test material. The study was therefore considered not valid.
Reasons why the study report is not available for submission	
Category study in AIR5 dossier (L-docs)	Category 3b

1. Information on the study

Data point	CA 5.4.1/030
Report author	[redacted]
Report year	1998
Report title	Glyphosate Acid: L5178Y TK$^{+/-}$ Mouse Lymphoma Gene Mutation Assay
Report No	CTL/P/4991
Document No	Not reported
Guidelines followed in study	OECD 476 (1984), US EPA OPPTS 870.5300 (1998), Council Directive 2000/32/EEC B.17 (2000)
Deviations from current test guideline OECD 490 (2016)	The newly introduced cytotoxicity parameters RTG (relative total growth), SG (suspension growth) and RSG (relative suspension growth) could not be re-calculated, since no data on suspension growth were available. In the present study, cytotoxicity was evaluated based on cloning efficiency, in accordance with the previous guideline version. Historical control data were only provided for negative/vehicle controls but not for the positive control. pH changes observed upon compound addition were not buffered. Acceptance and evaluation criteria were inconsistent with those specified in OECD 490 (2016).
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Glyphosate acid (batch: P24, purity: 95.6 %) was tested *in vitro* for its ability to induce forward mutations in mammalian cells by assessing the mutation of the TK locus in Mouse Lymphoma L5178Y TK$^{+/-}$ cells. Two independent experiments with a treatment duration of 4 hours were conducted in the presence or absence of metabolic activation (phenobarbital/β-naphthoflavone-induced rat liver S9 fraction) using two parallel cultures each. Based on the results of a preliminary range-finding experiment, in which the pH value was excessively reduced at a concentration of 2000 µg/mL, concentrations in the range of 296 to 1000 µg/mL were used in the main mutation assay (1500 µg/mL included in experiment I, but not evaluated for mutagenicity; 296 µg/mL included in experiment II). Solvent controls and appropriate positive controls (ethylmethane sulphonate (EMS), without S9 mix and n-nitrosodimethylamine (NDMA), with S9 mix)

were included in each experiment.

After 4 hours of exposure, the cells were incubated for 48 hours to allow expression of the mutant phenotype. The expression period was followed by a selection period, where cells were incubated in selection medium containing trifluorothymidine (TFT) for 10-13 days.

Precipitation of the test substance was not reported. A reduction in pH of the treatment medium was observed at 1000 µg/mL and above, however, only little cytotoxicity was observed at the concentrations tested, both in the presence and in the absence of metabolic activation. A biologically relevant and dose-dependent increase in the mean number of mutant colonies was not observed up to the highest test item dose, independent of the presence or absence of metabolic activation. Appropriate reference mutagens (EMS and NDMA) used as positive controls showed a distinct increase in induced mutant colonies, indicating the test-system to be sensitive and valid. The negative controls gave mutant frequencies within the range of historical control data.

Based on the results of the present study, glyphosate acid did not induce forward mutations in the mouse lymphoma assay with L5178Y $TK^{+/-}$ cells in the absence or presence of metabolic activation. Thus, the test item is considered not mutagenic in mammalian cells *in vitro*.

I. MATERIALS AND METHODS

J.

A: MATERIALS

1. Test material: Glyphosate acid

Identification:	
Description:	White solid
Lot/Batch number:	P24
Purity:	95.6 % (w/w)
Stability of test compound:	The stability of the test item at storage conditions (in an anti-static bag at ambient temperature) and in solvent (vehicle) were not specified.

2. Control material:

Negative control:	A negative control was not employed in this study
Solvent (vehicle) control:	Dimethylsulfoxide (DMSO), final concentration 1 %
Positive control:	- S9 mix: Ethylmethane sulphonate (EMS), 750 µg/mL in DMSO
	+ S9 mix N-nitrosodimethylamine (NDMA), 600 µg/mL in DMSO

3. Metabolic activation:

S9 mix was prepared from the livers of male Sprague-Dawley rats, that received oral doses of phenobarbital (80 mg/kg bw) and β-naphthoflavone (100 mg/kg bw) on three consecutive days. The treated animals were sacrificed on the day following the last dose. The S9 mix was prepared immediately before the experiment by mixing S9 fraction and co-factors. The co-factor solution was prepared as a stock solution of 75 mM NADP (disodium salt) and 1200 mM glucose-6-phosphate (monosodium salt) in RPMI 1640 culture medium with a final pH adjusted to 7.5. S9 fraction was added at 5 % (1 mL S9 added to the 20 mL cell culture) and co-factors at 1 % (200 µL to the 20 mL cell culture).

S9-mix component	Concentration	Unit
RPMI 1640 culture medium (pH 7.5)		
NADPH-generating system		
Glucose 6-phosphate Na salt	12	mM
NADP Na_2 salt	0.75	mM
S9	5	% (v/v)

4. Test organism:
L5178Y $TK^{+/-}$ mouse lymphoma cells were used. Stocks were maintained in liquid nitrogen and thawed immediately before use. Each batch used for mutagenicity testing was screened for mycoplasma contamination.

5. Cell culture media:

Cultivation medium: RPMI 1640 medium with Hepes, supplemented with 4 mM L-glutamine, 200 IU/mL penicillin and 200 µg/mL streptomycin

Growth medium: Cultivation medium supplemented with 10 % horse serum (in case of microwell cultivation 20 % horse serum

Treatment medium (± S9:) Cultivation medium supplemented with 5 % horse serum

Selection medium: Growth medium supplemented with 20 % horse serum and 4 µg/mL trifluorothymidine (TFT)

Incubation: At 37 °C, 5 % CO_2 and 98 % humidity

6. Locus examined Thymidine kinase (TK)

7. Test concentrations and number of replicates:

(a) Preliminary range-finding assay

Metabolic activation	Duration of exposure	Concentrations	Replicates
-S9 mix	4 h	125, 250, 296, 444, 500, 667, 1000, 1500 and 2000 µg/mL	Duplicate
+S9 mix	4 h	125, 250, 296, 444, 500, 667, 1000, 1500 and 2000 µg/mL	Duplicate

(b) Main mutation assay:

Metabolic activation	Duration of exposure	Concentrations	Replicates
-S9 mix	4 h	296*, 444*, 667*, 1000* and 1500 µg/mL	Duplicate
+S9 mix	4 h	296*, 444*, 667*, 1000* and 1500 µg/mL	Duplicate

* Samples evaluated for mutagenicity

B: STUDY DESIGN AND METHODS

1. Dates of experimental work: 22 Nov 1995 – 12 Mar 1996
Finalisation date: 24 May 1996

2. Preliminary cytotoxicity test:
A preliminary dose-range finding study was performed to determine the concentrations of the test item to be used in the main mutation assay. Toxicity was evaluated based on changes in the pH of the treatment medium and on osmolality. Data of the range-finding test were not included in the study report. Based on the observations in pH changes during the preliminary toxicity experiment, concentrations for the mutation assay were selected.

3. Main mutation assay:

Pre-treatment of cells:

A bulk cell culture with a density in the range of 1 - 1.2 x 10^6 cells per mL was prepared prior to each experiment. The culture was then diluted 50:50 (v/v) with serum-free medium to obtain a reduced serum content of 5 % at treatment time. Each 20 mL treatment culture (5 - 6 x 10^5) cells were used from the bulk culture for each test condition.

Treatment:

Just prior to treatment, the medium was supplemented with S9 mix in the appropriate cell cultures with metabolic activation. Two cultures were treated in parallel for each group. Aliquots of the test substance, solvent or positive control were added and the cultures were exposed for 4 hours, rotating on a roller apparatus at 37 °C. At the end of the treatment period, the cells were centrifuged and the cell pellets were re-suspended in 50 mL fresh culture medium.

Expression period:

After treatment, all cell cultures were incubated for a two days expression period. To maintain exponential growth during the expression time, each culture was counted daily and the cell numbers were adjusted to 2 x 10^5 cells/mL in 50 mL medium, thereby ensuring approximately 10^7 cells at each subculture.

After 48 hours expression period, the cell density of each culture was determined and each culture was divided into two series of dilutions. The first was used for the selection of mutants, the second was to assess the viability of the cultures in the absence of selection medium.

Selection period:

For the selection of mutants, each post-expression culture was diluted to give 50 mL at 1 x 10^4 cells/mL. Trifluorothymidine (TFT) was added and each culture was then dispensed at 200 µL per well into two 96 well microtiter plates (2000 cells/well). After incubation for 10 - 13 days cell growth was assessed. All plates were scored for the number of negative wells (no cell growth) and the number of wells containing small or large colonies. Small colonies were considered to be associated with clastogenic effects, large colonies were considered to be associated with gene mutation effects.

4. Cytotoxicity:

Cloning efficiency CE_1 (survival)

At the end of the exposure period, a sample of each cell culture was collected to assess survival. The cultures were diluted to give 50 mL at 8 cells/mL and dispensed at 200 µL/well into two 96-well plates (1.6 cells/well). After an incubation of 10 - 13 days the plates were scored for empty wells.

Cloning efficiency CE_2 (viability)

After the expression period, 2 days after end of exposure, the cultures were divided into two series of dilutions. The first was used for the selection of mutants, the second was to assess the viability of the cultures in the absence of selection medium. A sample from each mutation culture was diluted to give 50 mL at 8 cells/mL and dispensed at 200 µL per well into two 96 well microtiter plates (1.6 cells/well). After incubation for 10 - 13 days the plates were scored for empty wells.

5. Evaluation:

Cytotoxicity (cloning efficiency CE)

The number of empty wells of the two seeded 96-well plates was scored and recorded.

CE_1 (survival)

The cytotoxicity of the test substance after the exposure period was determined for each test group and is indicated as absolute and relative cloning efficiency (CE_1 and RCE_1, respectively).

CE$_2$ (viability)
The cytotoxicity of the test substance at the end of the expression period was determined for each test group and is given as absolute and relative cloning efficiency (CE$_2$ and RCE$_2$, respectively).

The cloning efficiency (CE, %) was calculated for each test group as follows:

$$CE_x = \frac{-\ln \frac{\text{total number of empty wells}}{\text{total number of seeded wells (96)}}}{\text{number of seeded cells per well (1.6)}} \times 100$$

$$RCE_x = \frac{CE_x \text{ of the test group}}{CE_x \text{ of the negative or vehicle control}} \times 100$$

Mutant frequency (MF)
The number of empty wells and the number of wells containing colonies were scored and reported. The colonies are classified into large colonies (indication of gene mutation) and small colonies (indication of chromosome breakage). Large colonies are defined as more than 25 % of the diameter of the well. A large colony should have shown less densely packed cells, especially around the edges of the well. Small colonies are defined as less than 25 % of the diameter of the well. A small colony should have shown a dense clonal morphology. Any well which contained more than one small colony was scored as a small colony. Any well which contained more than one large colony was scored as a large colony. Any well which contained a combination of large and small colonies was scored as a large colony. An empty well was one which contained no cell growth.

Uncorrected mutant frequency:
The uncorrected mutant frequency per 10^6 cells (MF_{uncorr}) was calculated for each test group as follows:

$$MF_{uncorr.} = \frac{-\ln \frac{\text{total number of empty wells}}{\text{total number of seeded wells (96)}}}{\text{number of seeded cells per well (2000)}} \times 10^6$$

Corrected mutant frequency
The corrected mutation frequency (MF_{corr}) was calculated regarding the values of CE$_2$:

$$MF\ corrected = \frac{MF\ uncorrected}{CE_2} \times 100$$

Determination of borderline mutant frequency based on GEF
The GEF (global evaluation factor) method requires that the MF exceeds a value based on the global distribution of the background MF of the test method. This value is defined as the mean of the negative/vehicle MF distribution plus one standard deviation.
Based on a large data base (n = 493 experiments) from six laboratories a GEF of 126 mutant colonies per 10^6 cells [mean $MF_{corr} = 99 \times 10^{-6}$ colonies; standard deviation = 27×10^{-6} colonies] was calculated for the microwell method. To be judged positive, the mutation frequency has to exceed a threshold of 126 colonies per 10^6 cells (GEF) above the concurrent negative/vehicle control value. The borderline mutant frequency was calculated for each experiment separately as follows:
Borderline MF = $MF_{vehicle\ control\ corr}$ + GEF (126×10^{-6})
The borderline MF was not evaluated as part of the present study, but was determined retrospectively for this evaluation.

6. Statistics:
Statistical analysis was not performed in the study.

7. Acceptance criteria:
The assay was considered valid if the following criteria were met:

- Post-expression cloning efficiencies of 50 % or greater were achieved for the solvent control viability plates.
- Spontaneous mutant frequencies of the solvent controls were within the range of the laboratories historical control data.
- Results obtained with the positive controls gave an unequivocal positive response in the presence and absence of S9 mix.

8. Evaluation criteria:

A substance was considered to be mutagenic if the following criteria are met:

- There was a statistically significant and dose-related increase in mutant frequency, but not only at concentrations eliciting cytotoxicity.
- The increase in mutant frequency was above those of solvent controls and reproducible in an independent experiment.

A substance was considered to be negative for mutagenicity if there was no reproducible statistically significant dose-related increase in mutant frequency observed. When reproducible significant increases in mutant frequency were seen only at levels of excessive toxicity, or when such increases were not accompanied by an increase in absolute numbers of mutants over solvent control values, consideration was given to such factors as statistical significance of the difference between treated and control cultures, and dose response relationships in order to clarify the response. Failing this, results from an independent experiment were obtained to attempt to clarify the result.

II. RESULTS AND DISCUSSION

1. ANALYTICAL DETERMINATIONS

In view of the short-term nature of the study, no analysis of stability, homogeneity or achieved concentrations were carried out on the preparation of the test item or positive control substance formulations either prior to or after addition to the cell cultures. Analytical determinations are not required by the test guideline.

2. CYTOTOXICITY

In the preliminary cytotoxicity test, toxicity was evaluated based on changes in pH of the treatment medium. A concentration of 2000 µg/mL in the presence and absence of S9 mix was found to produce an excessive reduction in the pH of the treatment medium. Based on these findings, a maximum concentration of 1500 µg/mL with and without S9 mix was considered appropriate for the main mutation assay.

In the main mutation assay, 1500 µg/mL was found to produce an excessive reduction in the pH of the treatment medium (0.99 units), whereas reductions in pH seen at 1000 µg/mL (0.59 and 0.40 units in the first and second experiment, respectively) were considered acceptable and not to affect the outcome of the study. Very little cytotoxicity was seen at the concentrations tested.

Table 5.4.1-44: Glyphosate Acid: L5178Y TK+/- Mouse Lymphoma Gene Mutation Assay ([redacted], 1996), first experiment

Test group	Mutagenicity data	Toxicity data			
	Corrected Mutant[$] Frequency per 10^6 cells	Cloning efficiency[#] (CE_1 -survival)		Cloning efficiency[#] (CE_2 -viability)	
	total	absolute	relative (RCE_1)	absolute	relative (RCE_2)
Without metabolic activation; 4-hour exposure period					
DMSO control	111.00	45.56	100.00	68.17	100.00
MF threshold[§]	237.00				
Test item [µg/mL]					
444.00	60.00	34.31	75.31	44.73	65.62
667.00	91.00	43.66	95.83	62.18	91.21
1000.00	105.00	32.70	71.77	79.29	116.31
1500.00	-	33.55	73.64	-	-
EMS 750 µg/mL	1254.00	12.31	27.02	23.68	34.74
IMF	*1143.00*				
With metabolic activation; 4-hour exposure period					
DMSO control	84.00	90.35	100.00	74.19	100.00
Test item [µg/mL]					
444.00	79.00	76.46	84.63	84.72	114.19
667.00	120.00	51.69	57.21	71.01	95.71
1000.00	128.00	49.86	55.19	107.03	144.26
1500.00	-	66.43	73.53	-	-
NDMA 600 µg/mL	425.00	57.17	63.28	27.87	37.57
IMF	*341.00*				

MF: mutant frequency

[$] Mutant frequency values for 10^6 cells. Values differ from those mentioned in study report, where the MF were given for 10^4 cells.

IMF: induced mutant frequency, should be $\geq 300 \times 10^{-6}$ for total MF or $\geq 150 \times 10^{-6}$ for small colonies

[§] $MF_{\text{vehicle control corr}} + GEF (126 \times 10^{-6})$, rounded

[#] Cloning efficiencies were re-calculated according to the formulas specified in OECD 490 (2016) based on raw data provided in study report

Table 5.4.1-45: Glyphosate Acid: L5178Y TK+/- Mouse Lymphoma Gene Mutation Assay ([redacted], 1996), second experiment

Test group	Mutagenicity data	Toxicity data			
	Corrected Mutant[$] Frequency per 10^6 cells	**Cloning efficiency[#] (CE_1 -survival)**		**Cloning efficiency[#] (CE_2 -viability)**	
	total	**absolute**	**relative (RCE_1)**	**absolute**	**relative (RCE_2)**
Without metabolic activation; 4-hour exposure period					
DMSO control	130.00	59.61	100.00	97.65	100.00
MF threshold[§]	256.00				
Test item [µg/mL]					
296.00	142.00	77.83	130.57	91.86	94.07
444.00	117.00	61.84	103.74	119.70	122.58
667.00	128.00	63.44	106.43	104.41	106.92
1000.00	222.00	91.38	153.30	122.14	125.08
EMS 750 µg/mL	1211.00	32.58	54.66	61.30	62.78
IMF	*1081.00*				
With metabolic activation; 4-hour exposure period					
DMSO control	157.00	62.75	100.00	89.24	100.00
Test item [µg/mL]					
296.00	148.00	79.67	126.96	91.41	102.43
444.00	160.00	67.00	106.77	91.07	102.05
667.00	123.00	62.40	99.44	85.41	95.71
1000.00	177.00	84.95	135.38	66.77	74.82
NDMA 600 µg/mL	601.00	69.02	109.99	26.89	30.13
IMF	*444.00*				

MF: mutant frequency

[$] Mutant frequency values for 10^6 cells. Values differ from those mentioned in study report, where the MF were given for 10^4 cells.

IMF: induced mutant frequency, should be ≥ 300 x 10^{-6} for total MF or ≥ 150 × 10^{-6} for small colonies

[§] $MF_{\text{vehicle control corr}}$ + GEF (126 × 10^{-6}), rounded

[#] Cloning efficiencies were re-calculated according to the formulas specified in OECD 490 (2016) based on raw data provided in study report

3. SOLUBILITY

Precipitation of the test item was not reported. pH measurements revealed a reduction of about >0.40 units at 1000 µg/mL and above. A significant effect on osmolality was not observed.

4. MUTANT FREQUENCY

There was no biologically relevant and reproducible increase in the number of mutant colonies observed upon treatment with the test item in both main experiments, neither in the presence, nor in the absence of metabolic activation. Mutant frequencies obtained for solvent controls remained within the range of the laboratory's historical control data. Exposure to the positive controls EMS and NDMA induced substantial increases in the mutation frequency in all experiments, demonstrating the sensitivity of the test system and the activity of the S9 mix.

III. CONCLUSION:

Glyphosate acid did not induce increased mutant frequencies in L5178Y TK$^{+/-}$ cells in the presence or absence of S9-mix. Based on the results of the present study and under the experimental conditions chosen, the test item is negative for mutagenicity in mammalian cells *in vitro*.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Negative for mutagenicity in L5178Y TK$^{+/-}$ cells with and without metabolic activation.

The study was performed under GLP conditions and in accordance with OECD guideline 476 (1984). A number of deviations were observed when compared to the currently valid OECD guideline 490 (2016). All deviations were considered to be of minor degree and to not compromising the validity of the study. Mutant frequency and toxicity data given in the study report included data from 10^4 cells. For this evaluation, data were calculated retrospectively for 10^6 cells. In addition, the borderline mutant frequency based on GEF was determined retrospectively. The study was considered valid and acceptable.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.4.1/031
Report author	[redacted]
Report year	1991
Report title	Mutagenicity test: *In vitro* Mammalian Cell Gene Mutation Test with Glyphosate, batch 206-JaK-25-1
Report No	12325
Document No	Not reported
Guidelines followed in study	OECD 476 (1983), US CFR part 700 (F) §798.5265 (1987)
Deviations from current test guideline OECD 490 (2016)	The newly introduced cytotoxicity parameters RTG (relative total growth), SG (suspension growth) and RSG (relative suspension growth) could not be re-calculated, since no data on suspension growth were available. In the present study, cytotoxicity was evaluated based on cloning efficiency, in accordance with the previous guideline version. Although the growth rates of the cultures were monitored, the data were not provided within the study report. The number of cells treated was below 6×10^6 cells, the number of cells recommended in OECD 490 (2016). No historical control data were provided for the negative and the positive controls. pH measurements were not performed. Acceptance criteria were not defined in the study report and evaluation criteria specified in OECD 490 (2016) were not applied.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially	Yes

recognised testing facilities	
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Glyphosate technical (batch: 206-JaK-25-1, purity: 98.6 %) was tested in a Mouse Lymphoma assay for its ability to induce forward mutations in mammalian cells *in vitro*. In two independent experiments, duplicate cultures of Mouse Lymphoma L5178Y $TK^{+/-}$ cells were exposed to the test item, medium or appropriate positive controls (100 µg/mL ethylnitrosourea (ENU) without S9 mix and 5-10 µg/mL dimethylbenzanthracene (DMBA) with S9 mix), both, in the presence and absence of metabolic activation (Aroclor 1254-induced rat liver S9 fraction). Based on the results of a preliminary toxicity test, in which no cytotoxicity was observed up to limit concentrations of 5.0 mg/mL glyphosate in the absence of S9 mix or 4.2 mg/mL glyphosate in the presence of S9 mix, the two experiments of the main mutagenicity assay were conducted at the same concentration ranges.

After 3 hours of exposure with S9 mix or 4 hours of exposure without S9 mix, the cells were incubated for 2-3 days to allow expression of the mutant phenotype. The expression period was followed by a selection period, in which the cells were cultivated in selection medium containing trifluorothymidine (TFT) for a period of 10 days. Cell survival and cell viability were assessed as cloning efficiency 1 and cloning efficiency 2 at the end of the exposure period and after the expression period, respectively.

Precipitation of the test item in the medium was not reported and there was no cytotoxicity observed at any of the tested concentrations, neither in the presence nor in the absence of S9 mix. There was no statistically significant increase in the number of mutant colonies observed upon treatment with glyphosate in both experiments at any of the tested concentrations, neither in the presence, nor in the absence of metabolic activation. Mutant frequencies of the medium control cultures were in the expected range.

A clear increase in mutant frequencies was observed for the positive control ENU in the absence of S9 mix, while the positive control DMBA in the presence of S9 mix showed a rather moderate increase in mutant frequency at 5.0 µg/mL. Thus, the second experiment was conducted with a higher DMBA concentration of 10 µg/mL. The higher dose revealed a much stronger response in mutant colony formation, demonstrating the sensitivity of the test system and the activity of the S9 mix.
Based on the results of the present study, glyphosate technical did not induce increased mutant frequencies in L5178Y $TK^{+/-}$ cells in the presence or absence of S9-mix and is therefore considered non-mutagenic for mammalian cells *in vitro*.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material: Glyphosate technical

Identification:	Not specified
Description:	White powder
Lot/Batch number:	206-JaK-25-1
Purity:	98.6 %
Stability of test compound:	The stability of the test item at storage conditions (at room temperature in the dark) or in the solvent (vehicle) was not specified.

2. Control material:

Negative control:	Untreated cell cultures which were cultivated in cultivation medium only were included in each experiment.

Solvent (vehicle) control: As culture medium was used as solvent for the test item, the solvent control represents actually the negative control.
Positive control: - S9 mix: Ethylnitrosourea (ENU), 100 µg/mL
+ S9 mix N-nitrosodimethylamine (DMBA), 5 and 10 µg/mL

3. Metabolic activation:

S9 mix was obtained from the livers of Wistar rats weighing approximately 200 g. The animals received a single intraperitoneal injection of Aroclor 1254 at a dose of 500 mg/kg bw. The animals were sacrificed 5 days after treatment following a 16-hour period of fasting until liver homogenates were prepared. The S9 mix was prepared immediately before the experiment by mixing S9 fraction and co-factor.

S9 mix component	Concentration	Unit
HEPES	20	mM
KCl	33	mM
NADPH-generating system		
Glucose 6-phosphate	3	mM
NADP	4	mM
$MgCl_2$	5	mM
S9	30	% (v/v)

4. Test organism:

L5178Y $TK^{+/-}$ mouse lymphoma cells were used. Stocks were maintained in liquid nitrogen and thawed immediately before use. Each batch used for mutagenicity testing was checked for general morphology, growth characteristics and absence of mycoplasma.

5. Cell culture media:

Cultivation medium: RPMI 1640 medium, supplemented with 10 % horse serum, 200 µg/mL sodium pyruvate and 50 µg/mL gentamycin

Pre-treatment medium A ("THMG medium"): Cultivation medium supplemented with 9 µg/mL hypoxanthine, 15 µg/mL methotrexate and 22.5 µg/mL glycine

Pre-treatment medium B + treatment medium ("THG medium"): Cultivation medium supplemented with 50 % conditioned medium, 9 µg/mL hypoxanthine and 22.5 µg/mL glycine

Selection medium: Cultivation medium, supplemented with 10 % horse serum and 4 µg/mL trifluorothymidine (TFT)

Incubation: *At 37 °C at 5 % CO_2*

6. Locus examined Thymidine kinase (TK)

7. Test concentrations and number of replicates:

(a) Preliminary range-finding assay

Metabolic activatio	Duration of exposure	Concentrations	Replicates

n			
-S9 mix	*4 h*	*0.63, 1.3, 2.5 and 5.0 mg/mL*	*Duplicate*
+S9 mix	3 h	0.52, 1.0, 2.1 and 4.2 mg/mL	Duplicate

(b) Main mutation assay:

Metabolic activation	Duration of exposure	Concentrations	Replicates
-S9 mix	*4 h*	*0.63, 1.3, 2.5 and 5.0 mg/mL*	*Duplicate*
+S9 mix	3 h	*0.52, 1.0, 2.1 and 4.2 mg/mL*	Duplicate

B: STUDY DESIGN AND METHODS

1. Dates of experimental work: 04 Apr – 13 May 1991
Finalisation date: 10 Sep 1991

2. Preliminary cytotoxicity test:

A preliminary dose-range finding study was performed to determine the concentrations of the test item to be used in the main mutation assay. Pre-treatment of cells and exposure with the test item was conducted under the same conditions as in the main mutagenicity assay.

A series of duplicate cultures was exposed for 4 hours at concentrations in the range of 0.63 – 5.0 mg/mL in the absence of S9 mix and for 3 hours at concentrations in the range of 0.52 – 4.2 mg/mL in the presence of S9 mix. Following treatment, the cultures were diluted and a sample of cells from each culture was seeded (2 cells/well) for the determination of cloning efficiency. In addition, the growth rates of the cultures were monitored for a period of 2 days after treatment. The microtiter plates were incubated for 8-10 days at 37 °C and 5 % CO_2, followed by counting the number of clones and determination of the cloning efficiency.

Based on the results of the preliminary toxicity test, the concentrations for the main mutagenicity assay were chosen. The highest concentration of the main mutagenicity assay was selected to induce 20 % cytotoxicity

3. Main mutation assay:

Pre-treatment of cells:

Thawed cells were maintained at a density of 2×10^5 - 1.5×10^6 in sterile NUNC plastic flasks and incubated at 37 °C and 5 % CO_2. Prior to treatment, spontaneous TK deficient mutants (TK-/- cells) were eliminated from the stock cultures by incubating the cells for one day in THMG medium (pre-treatment medium A), followed by a recovery period of 2-3 days in THG medium (pre-treatment medium B).

Treatment:

In two independent experiments using duplicate cultures per condition, glyphosate was tested at 4 concentrations with and without metabolic activation. Based on the results of the preliminary toxicity test, concentrations for the main mutagenesis assay were selected.

For treatment of cells in the absence of S9 mix, cell suspensions of about 6×10^5 cells/mL were mixed 1:1 with corresponding, 2-fold concentrated test item solutions at concentrations in the range of 0.63 – 5.0 mg/mL prepared in cultivation medium. All cultures were set up in final volumes of 15 mL and incubated under gentle shaking for 4 hours at 37 °C.

For treatment of cells in the presence of S9 mix, pre-treated cell cultures were centrifuged and for each culture 1.8×10^6 cells were re-suspended in 2.5 mL cultivation medium containing the test item at concentrations in the range of 0.52 – 4.2 mg/mL. 0.5 mL S9 mix were added to each culture, followed by incubation for 3 hours under gentle shaking.

In each experiment, negative and positive controls (100 µg/mL ethylnitrosourea in the absence of S9 mix and 5 or 10 µg/mL dimethylbenzanthracene in the presence of S9 mix) were included. At the end of the exposure period, the cells were centrifuged, re-suspended in 15 mL fresh medium and a small sample of cells from each culture was diluted and seeded in a microtiter plate at a density of 2 cells/well for determination of relative cell survival (cloning efficiency 1).

Expression period:

After the exposure period, the cells were incubated for a two days expression period, in which each culture was diluted daily and the growth rate was recorded. After the expression period, each culture was divided. One aliquot of each culture was plated to determine the cell viability (cloning efficiency 2) of the cultures, the other one was used for the selection of mutants.

Selection period:

For the selection of mutants, two microtiter plates were prepared from each post-expression culture, seeding 2000 cells per well in medium supplemented with 4 µg/mL trifluorothymidine (TFT). After an incubation period of 10 days, the number of cell clones was counted. The clones were differentiated into large clones and small dense clones. Small colonies were considered to be associated with clastogenic effects, large colonies were considered to be associated with gene mutation effects.

4. Cytotoxicity:

Cloning efficiency (CE_1 survival)

At the end of the exposure period, a sample of each cell culture was collected to assess cell survival. A full 96-well microtiter plate was seeded at a density of 2 cells/well for each culture. After 10 days of incubation, the number of colonies was counted.

CE_2 (viability)

After the expression period, 2-3 days after end of exposure, a sample of each cell culture was collected to assess cell viability. For each culture, a full 96-well microtiter plate was seeded at a density of 2

cells/well. After 10 days of incubation, the number of colonies was counted.

5. Evaluation:

Cytotoxicity (cloning efficiency CE)
The number of colonies divided by the number of cells plated was calculated for each sample. The absolute cloning efficiency was determined for each test group, as well as the relative cloning efficiency in comparison to the solvent control group.
CE_1 (survival)
The cytotoxicity of the test substance after the exposure period was determined for each test group and is indicated as absolute and relative cloning efficiency (CE_1 and RCE_1, respectively).

CE_2 (viability)
The cytotoxicity of the test substance at the end of the expression period was determined for each test group and is given as absolute and relative cloning efficiency (CE_2 and RCE_2, respectively).
The cloning efficiency (CE, %) was calculated for each test group as follows:

$$CE_x = -\frac{1}{2}\ln\frac{\text{Number of empty wells}}{\text{Number of wells seeded}}$$

$$RCE_x = \frac{CE_x \text{ of the test group}}{CE_x \text{ of the negative or vehicle control}} \times 100$$

Mutant frequency (MF)
The number of empty wells and the number of wells containing colonies were scored and reported. The colonies are classified into large colonies (indication of gene mutation) and small colonies (indication of chromosome breakage).

Uncorrected mutant frequency:
The uncorrected mutant frequency per 10^4 cells (MF_{uncorr}) was calculated for each test group as follows:

$$MF_{uncorr.} = -\frac{1}{2000}\ln\frac{\text{Number of empty wells}}{\text{Number of wells seeded}}$$

Corrected mutant frequency
The corrected mutation frequency (MF_{corr}) was calculated regarding the values of CE_2:

$$MF_{corr.} = \frac{MF_{uncorr.}}{CE_2} \times 100$$

Determination of borderline mutant frequency based on GEF
The GEF (global evaluation factor) method requires that the MF exceeds a value based on the global distribution of the background MF of the test method. This value is defined as the mean of the negative/vehicle MF distribution plus one standard deviation.
Based on a large data base (n = 493 experiments) from six laboratories a GEF of 126 mutant colonies per 10^6 cells [mean $MF_{corr} = 99 \times 10^{-6}$ colonies; standard deviation = 27×10^{-6} colonies] was calculated for the microwell method. To be judged positive, the mutation frequency has to exceed a threshold of 126 colonies per 10^6 cells (GEF) above the concurrent negative/vehicle control value. The borderline mutant frequency was calculated for each experiment separately as follows:
Borderline MF = $MF_{\text{vehicle control corr}}$ + GEF (126×10^{-6}).
The borderline MF was not evaluated as part of the present study, but was determined retrospectively for this evaluation.

6. Statistics:
Statistical analysis was conducted using the Analysis of Variance method on the corresponding test and control cultures.

7. Acceptance criteria:
Acceptance criteria were not defined in the study report.

8. Evaluation criteria:
A substance was considered to be mutagenic if the following criteria were met:

- There was a statistically significant and reproducible increase in the mutation frequency as compared to the negative control cultures.
- A dose-response was evident.
- The mutation frequency at the dose level where the highest effect was found more than twice the concurrent spontaneous mutant frequency.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS
Analytical determinations were not performed in the present study, as not required by the test guideline.

B. CYTOTOXICITY
In the preliminary cytotoxicity test, no cytotoxicity evident as ≥ 20 % reduction of cell survival was observed for any glyphosate concentration, neither in the presence, nor in the absence of metabolic activation. Growth rates of glyphosate treated cells were comparable to those of control cultures (data not provided in study report). Based on these findings, the concentrations used in the preliminary toxicity assay were also applied for the main mutagenicity test.

C. SOLUBILITY
Precipitation of the test item was not reported.

D. MUTANT FREQUENCY
There was no statistically significant increase in the number of mutant colonies observed upon treatment with glyphosate in both experiments at any of the tested concentrations, neither in the presence, nor in the absence of metabolic activation. Mutant frequencies of the medium control cultures were in the expected range.

A clear increase in mutant frequencies was observed for the positive control ethylnitrosourea (100 µg/mL) in the absence of S9 mix. The positive control dimethylbenzanthracene (DMBA) at 5.0 µg/mL showed a rather moderate increase in mutant frequency (1.88 fold induction when compared to medium control), therefore the second experiment was conducted with a higher DMBA concentration of 10 µg/mL. The higher dose revealed a much stronger response in mutant colony formation. Taken together the results of both experiments, the medium and positive controls demonstrated the sensitivity of the test system and the activity of the S9 mix.

Table 5.4.1-46: Mutagenicity test: *In vitro* Mammalian Cell Gene Mutation Test with Glyphosate ([redacted] 1991), first experiment

Test group	Mutagenicity data[$]			Toxicity data[$]			
	Corrected Mutant Frequency per 10^6 cells			Cloning efficiency (CE_1 -survival)		Cloning efficiency (CE_2 -viability)	
	total	small	large	absolute	relative (RCE_1)	absolute	relative (RCE_2)
Without metabolic activation; 4-hour exposure period							

Table 5.4.1-46: Mutagenicity test: *In vitro* Mammalian Cell Gene Mutation Test with Glyphosate ([redacted] 1991), first experiment

Test group	Mutagenicity data$^{\$}$			Toxicity data$^{\$}$			
	Corrected Mutant Frequency per 10^6 cells			Cloning efficiency (CE_1 -survival)		Cloning efficiency (CE_2 -viability)	
	total	**small**	**large**	**absolute**	**relative (RCE_1)**	**absolute**	**relative (RCE_2)**
Medium control	112	50	56	87.0	100.0	78.0	100.0
MF threshold§	238	176	182				
Test item [µg/mL]							
0.61	95	50	42	98.0	112.6	87.0	111.5
1.30	135	53	76	93.0	106.9	69.0	88.5
2.50	125	50	69	81.0	93.1	76.5	98.1
5.00	98	32	62	88.5	101.7	79.5	101.9
ENU 100 µg/mL	760	120	495	73.5	84.5	71.5	91.7
IMF	*648*	*71*	*440*				
With metabolic activation; 3-hour exposure period							
Medium control	155	69	77	81.5	100.0	76.0	100.0
MF threshold§	281	195	203				
Test item [µg/mL]							
0.52	145	53	84	90.0	110.4	74.0	97.4
1.00	145	59	80	88.5	108.6	69.0	90.8
2.10	230	62	66	93.5	114.7	76.0	100.0
4.20	160	63	72	78.0	95.7	76.0	100.0
DMBA 5 µg/mL	510	185	265	46.0	56.4	47.5	62.5
IMF	*355*	*117*	*188*				

IMF: Induced Mutant Frequency; an increase above vehicle MF, IMF should be $\geq 300 \times 10^{-6}$ for total MF or $\geq 150 \times 10^{-6}$ for small colonies

MF: Mutant frequency; § = $MF_{vehicle\ control\ corr}$ + GEF (126×10^{-6}), rounded

$^{\$}$ Mutant frequency values and toxicity data for 10^6 cells. Values differ from those mentioned in study report, where the MF and CE values were given for 10^4 cells.

ENU: Ethylnitrosourea, DMBA: Dimethylbenzanthracene

Table 5.4.1-47: Mutagenicity test: *In vitro* Mammalian Cell Gene Mutation Test with Glyphosate ([redacted] 1991), second experiment

Test group	Mutagenicity data$ Corrected Mutant Frequency per 10^6 cells			Toxicity data$ Cloning efficiency (CE_1 -survival)		Cloning efficiency (CE_2 -viability)	
	total	small	large	absolute	relative (RCE_1)	absolute	relative (RCE_2)
Without metabolic activation; 4-hour exposure period							
Medium control	225	90	120	64.0	100.0	62.5	100.0
MF threshold§	351	216	246				
Test item [µg/mL]							
0.61	260	113	108	61.5	96.1	52.5	84.0
1.30	285	109	155	65.0	101.6	55.0	88.0
2.50	270	107	145	62.5	97.7	50.0	80.0
5.00	290	104	165	61.5	96.1	53.0	84.8
ENU 100 µg/mL	1250	245	750	52.5	82.0	43.0	68.8
IMF	*1025*	*155*	*630*				
With metabolic activation; 3-hour exposure period							
Medium control	330	108	200	61.5	100.0	54.0	100.0
MF threshold§	456	234	326				
Test item [µg/mL]							
0.52	260	91	155	54.5	88.6	56.5	104.6
1.00	320	123	180	57.5	93.5	49.5	91.7
2.10	410	140	235	53.5	87.0	52.0	96.3
4.20	330	110	185	62.0	100.8	60.0	111.1
DMBA 10 µg/mL	4050	2750	2500	8.0	13.0	3.0	5.6
IMF	*3720*	*2642*	*2300*				

IMF: Induced Mutant Frequency, an increase above vehicle MF, IMF should be $\geq 300 \times 10^{-6}$ for total MF or $\geq 150 \times 10^{-6}$ for small colonies
MF: Mutant frequency; § = $MF_{vehicle\ control\ corr}$ + GEF (126×10^{-6}), rounded
$ Mutant frequency values and toxicity data for 10^6 cells. Values differ from those mentioned in study report, where the MF and CE values were given for 10^4 cells.
ENU: Ethylnitrosourea, DMBA: Dimethylbenzanthracene

III. CONCLUSION:

Based on the experimental findings, glyphosate technical did not induce increased mutant frequencies in L5178Y TK+/- cells in the presence or absence of S9-mix. Under the conditions of the test, the test item is negative for mutagenicity in mammalian cells *in vitro.*

3. Assessment and conclusion

Assessment and conclusion by applicant:

Negative for mutagenicity in L5178Y TK$^{+/-}$ cells with and without metabolic activation.

The study was conducted in compliance with GLP and in accordance with OECD guideline 476 (1983). When compared to the currently valid OECD guideline 490 (2016), a number of deviations became evident, all of them of minor degree and not compromising the validity of the study. Mutant frequency and toxicity data given in the study report included data from 10^4 cells. For this evaluation, data were calculated retrospectively for 10^6 cells. In addition, the borderline mutant frequency based on GEF was determined retrospectively. The study was considered valid and acceptable.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.4.1/032
Report author	[redacted]
Report year	1983
Report title	CHO/HGPRT Gene Mutation Assay with Glyphosate
Report No	ML-83-155
Document No	Not reported
Guidelines followed in study	No guideline followed. The study was conducted similarly to OECD 476 (1984)
Deviations from current test guideline OECD 476 (2016)	The newly introduced cytotoxicity parameter RS (relative survival) and the adjusted cloning efficiency were not re-calculated, since no data on the number of cells after treatment were provided. In the current study, cytotoxicity was evaluated based on cloning efficiency after treatment (CE_1, survival) and after selection (CE_2, viability), in accordance with the previous guideline version. The authors did not discriminate between large and small mutant colonies. Historical control data on the medium control and the positive control substances were not included. pH assessments were not performed. The number of cells treated was not reported and the number of cells plated for mutant selection was insufficient. Acceptance and evaluation criteria were not specified. Test results were evaluated inconsistent with the evaluation criteria specified in the current guideline.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, not concluded under GLP /Officially recognised testing facilities. When the study was conducted, GLP was not compulsory.
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Glyphosate (batch: XHJ-64, purity: 98.7 %) was tested for its ability to induce forward mutations in mammalian cells *in vitro* in a HGPRT assay. Chinese hamster ovary (CHO) cells were exposed the test item, medium and positive controls (ethylmethane sulfonate for cultures without S9 mix and benzo(a)pyrene for cultures with S9 mix) in the presence and absence of metabolic activation (Aroclor

1254-induced rat liver S9 fraction).

Based on the results of an initial range-finding test (data not provided in study report), in which approximately 90 % cell-killing was observed in the range of 20-25 mg/mL, concentrations for the mutagenicity test were selected. Two independent experiments, one preliminary cytotoxicity and mutagenicity test and one mutation assay were performed in the present study. In the preliminary cytotoxicity and mutagenicity test, glyphosate concentrations in the range of 5 – 22.5 mg/mL were applied, which were intended to yield approximately 100, 50 and 10 % relative survival of the cells. In addition, S9 mix concentrations in the range of 1-10 % were added to identify the optimal S9 mix concentration. In the main mutagenicity experiment, the cells were exposed to glyphosate concentrations in the range of 2 - 20 mg/mL in the absence of S9 mix and to concentrations in the range of 5 – 25 mg/mL in the presence of S9 mix. Based on the results of the preliminary test, the five test item concentrations for the main mutagenicity assay were chosen to yield 100, 70, 50, 20 and 10 % cell survival at the optimal S9 mix concentration.

After 3 hours of exposure, the cells were incubated for 7-9 days to allow expression of the mutant phenotype. The expression period was followed by a selection period, in which the cells were cultivated in 6-thioguanine-enriched medium for 8-12 days.

Precipitation of the test substance was not reported. In the preliminary cytotoxicity and mutagenicity test, glyphosate-related cytotoxicity was observed at all S9 concentrations tested. In both experiments, cytotoxicity was observed at ≥ 10 mg/mL.

In the preliminary experiment, none of the samples treated with glyphosate exhibited a statistically significant increased mutant frequency compared to the control cultures, neither in the presence nor in the absence of metabolic activation. In addition, mutant frequencies of test item-treated samples were comparable to those of control cultures at all S9 mix concentrations tested. Therefore, an S9 concentration of 5 % was chosen as representative S9 concentration in the main mutagenicity assay.

The negative results were confirmed in the main mutagenicity experiment, in which none of the glyphosate treated samples showed a statistically significant increase in the number of mutant colonies, neither in the presence nor in the absence of metabolic activation. In addition, there was no statistically significant dose-response relationship.

Mutant frequencies of the medium control cultures remained low as expected, whereas the positive control mutagens ethylmethane sulfonate and benzo(a)pyrene yielded large increases in mutant frequencies, demonstrating the sensitivity of the test and the functionality of the S9 mix.

Based on the experimental findings and under the conditions of the test, glyphosate did not induce gene mutations in the HGPRT locus, neither in the presence nor in the absence of metabolic activation and is therefore considered negative for mutagenicity in mammalian cells *in vitro*.

I. MATERIALS AND METHODS

A: MATERIALS

1. **Test material:** Glyphosate

Identification:	Not specified
Description:	White powder
Lot/Batch number:	XHJ-64
Purity:	98.7 %
Stability of test compound:	The stability of the test item at storage conditions (at room temperature) or in the solvent (vehicle) was not specified.

2. **Control material:**

Negative control: Untreated cell cultures which were cultivated in cultivation medium only were included in each experiment.
Solvent (vehicle) control: As culture medium was used as solvent for the test item, the solvent control represents actually the negative control.
Positive control: - S9 mix: Ethyl methane sulphonate (EMS), 200 µg/mL
+ S9 mix Benzo(a)pyrene (B(a)P), 2 µg/mL

3. Metabolic activation:

S9 mix was purchased from [redacted]. The liver homogenate was produced from Aroclor 1254-induced rats and prepared before the experiment by mixing S9 fraction and co-factor as follows:

S9 mix component	Concentration	Unit
Sodium phosphate buffer (pH 7.5)	50	mM
KCl	30	mM
NADPH-generating system		
Glucose 6-phosphate	5	mM
NADP	4	mM
$MgCl_2$	10	mg
$CaCl_2$	10	mM
S9*	5	% (v/v)

* In a preliminary experiment, the optimal S9 concentration was determined: 1, 2, 5 and 10 % (v/v) of S9 mix were added to the co-factor solutions. 5 % (v/v) of S9 fraction in the co-factor mix were used for the main mutagenicity study.

4. Test organism:

Chinese hamster ovary (CHO) cells (K1BH4) were used. The cells were routinely maintained as logarithmically growing monolayer cultures in Ham's F12 medium.

5. Cell culture media:

Cultivation medium: Ham's F12 medium, supplemented with 10 % newborn calf serum

Growth medium: Ham's F12 medium, supplemented with 10 % dialysed newborn calf serum

Treatment medium (± S9): *Ham's F12 medium*

Selection medium: Ham's F12 medium supplemented with 5 % dialysed newborn calf serum and 10 µM 6-thioguanine (6TG)

Incubation: *At 37.5 ± 2 °C, 5 % CO_2 and 95 % humidity*

6. Locus examined: Hypoxanthin guanine phosphoribosyltransferase (HGPRT)

7. Test concentrations and number of replicates:

(a) Preliminary cytotoxicity and mutagenicity test:

Metabolic activation	Duration of exposure	Concentrations	Replicates

±S9 mix	*3 h*	*5, 17.5 and 22.5 mg/mL*		*Duplicate*

(b) Main gene mutation test:

Metabolic activation	Duration of exposure	Concentrations	Replicates
-S9 mix	*3 h*	*2, 5, 10, 15 and 20 mg/mL*	*Duplicate*
+S9 mix	3 h	*5, 10, 15, 20 and 25 mg/mL*	Duplicate

B: STUDY DESIGN AND METHODS

1. **Dates of experimental work:** 21 Jun – 09 Sep 1983
Finalisation date: 20 Oct 1983

2. Preliminary cytotoxicity and mutagenicity test:

A preliminary test was performed to initially estimate the mutagenic potential of the test item and to identify the optimal S9 mix concentration.

Based on the results of an initial range-finding test (data not provided in study report), in which approximately 90 % cell-killing was observed in the range of 20 - 25 mg/mL, glyphosate concentrations in the range of 5 – 25 mg/mL were applied in the present preliminary cytotoxicity and mutagenicity test. The test item concentrations for the preliminary test were selected to yield approximately 100, 50 and 10 % relative survival of the cells.

The procedure of the preliminary test was the same as for the main mutation assay described below. The cells were treated under the same conditions as in the main mutagenicity assay at concentrations of 5, 17.5 and 22.5 mg/mL in the presence and absence of metabolic activation. To identify the optimal S9 mix concentration, 1, 2, 5 or 10 % of S9 mix were added to cultures with metabolic activation. Each two cultures per condition were exposed for 3 hours with and without S9 mix. Following exposure, cells were plated for determination of cell survival (cloning efficiency 1) and mutagenicity. Mutant cells were allowed for expression of the mutant phenotype for 7 - 9 days, followed by an 8 - 12 day selection period in 6-thioguanine-enriched medium. In addition, the cell viability (cloning efficiency 2) was determined for mutant cultures in the presence and absence of selection medium. Mutagenicity was assessed by calculation of the mutant frequency for all tested conditions.

Based on the results of the preliminary test, the five test item concentrations for the main mutagenicity assay were chosen to yield 100, 70, 50, 20 and 10 % cell survival at the optimal S9 mix concentration.

3. Main mutation assay:

Pre-treatment of cells:

For each test group, 0.5 x 10^6 cells per flask were seeded into 25 cm^2 flasks and incubated for 18 - 24 hours prior to treatment.

Treatment:

On the day of treatment, the medium was changed to serum-free treatment medium and, for treatment

with metabolic activation, 5 % S9 mix was added. The cells were exposed to glyphosate concentrations in the range of 2 - 20 mg/mL in the absence of S9 mix and to concentrations in the range of 5 – 25 mg/mL in the presence of S9 mix for 3 hours at 37.5 ± 2 °C. Solvent (medium) and the positive controls (ethylmethane sulfonate for cultures without S9 mix and benzo(a)pyrene for cultures with S9 mix) were included. After exposure, the cells were washed with Hank's Buffered Salt Solution (HBSS), trypsinised and counted. 200 cells per sample were seeded for the determination of survival (cloning efficiency 1) after the exposure period and 10^6 cells per sample were seeded to assess mutagenicity.

Expression period:
After treatment, 10^6 cells per culture were plated in 10 mL growth medium and incubated for an expression period of 7 - 9 days. The cells were sub-cultured every 2 - 3 days to maintain exponential growth during the expression time. After the expression period, each culture was divided. One aliquot of each culture was plated to determine the viability (cloning efficiency 2) of the cultures in the absence of selection medium, the other one was used for the selection of mutants.

Selection period:
For the selection of mutants, 10^6 cells of each post-expression culture were seeded into 100 mm plates (5 plates with 2 x 10^5 cells per culture) containing 8 mL of 6-thioguanine enriched selection medium. After an incubation period of 8 - 12 days, the developed colonies were fixed, stained and counted.

4. Cytotoxicity:

Cloning efficiency CE_1 (survival)
The survival (cloning efficiency 1) of glyphosate treated cells relative to solvent controls was determined in parallel to the mutagenicity test. At the end of the exposure period, a sample of each cell culture was collected to assess survival of the cells. 200 cells per culture were plated and re-incubated for 7 - 9 days. After the incubation period, the colonies developed were fixed, stained and counted.

Cloning efficiency CE_2 (viability)
The viability (cloning efficiency 2) was determined in parallel to the selection of mutants. After the expression period, 200 cells of each culture were plated in triplicates in selection medium without 6-thioguanine to assess cell viability. After an incubation period of 8 - 12 days, the developed colonies were fixed, stained and counted.

5. Evaluation:

Cytotoxicity (cloning efficiency CE)
The number of colonies divided by the number of cells plated was calculated for each sample. The absolute cloning efficiency was determined for each test group, as well as the relative cloning efficiency in comparison to the solvent control group.

CE_1 (survival)
The cytotoxicity of the test substance after the exposure period was determined for each test group and is indicated as absolute and relative cloning efficiency (CE_1 and RCE_1, respectively).

CE_2 (viability)
The cytotoxicity of the test substance at the end of the expression period was determined for each test group and is given as absolute and relative cloning efficiency (CE_2 and RCE_2, respectively).

The cloning efficiency (CE, %) was calculated for each test group as follows:

$$CE_{absolute} = \frac{\text{Total number of colonies}}{\text{Total number of cells plated}} \times 100$$

$$RCE_x = \frac{CE_{absolute} \text{ of the test group}}{CE_{absolute} \text{ of the vehicle control group}} \times 100$$

Mutant frequency (MF)
The cloning efficiency of mutant colonies in selective medium divided by the cloning efficiency in non-selective medium measured for the same culture at the time of selection was calculated for each sample.

Uncorrected mutant frequency:
The uncorrected mutant frequency (MF_{uncorr}) was calculated for each test group as follows:

$$\text{MF uncorrected} = \frac{\text{Total number of mutant colonies}}{\text{Number of seeded cells}} \times 10^6$$

Corrected mutant frequency
The corrected mutation frequency (MF_{corr}) was calculated regarding the values of CE_2:

$$\text{MF corrected} = \frac{\text{MF uncorrected}}{CE_2} \times 100$$

6. Statistics:
Mutagenicity data were analysed according to the statistical method of Snee and Irr (1981). Mutant frequency values were transformed according to the equation $Y = (X+1)^{0.15}$, with Y = transformed mutant frequency and X = observed mutant frequency. Student's t-test was then used to compare treatment data to solvent control data.

7. Acceptance criteria:
Acceptance criteria were not specified in the study report.

8. Evaluation criteria:
Evaluation criteria were not specified in the study report.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS
Analytical determinations were not performed in the study, as not required by the test guideline.

B. CYTOTOXICITY
In the preliminary cytotoxicity and mutagenicity test, glyphosate-related cytotoxicity was observed at all S9 concentrations tested. In both the preliminary and in the main mutagenicity experiment, the test material was shown to be cytotoxic at concentrations of 10 mg/mL and above.

C. SOLUBILITY
Information on precipitation or pH changes of glyphosate in the medium have not been provided in the study report.

D. MUTANT FREQUENCY
In the preliminary experiment, none of the samples treated with glyphosate exhibited a statistically significantly increased mutant frequency compared to the control cultures, neither in the presence nor in the absence of metabolic activation. In addition, mutant frequencies of test item-treated samples were comparable to those of control cultures at all S9 mix concentrations tested. Therefore, an S9 concentration of 5 % was chosen as representative S9 concentration in the main mutagenicity assay.

In the main mutagenicity experiment, there was as well no statistically significant increase in the number of mutant colonies upon treatment with glyphosate, neither in the presence nor in the absence of metabolic activation. In addition, there was no statistically significant dose-response relationship.

Mutant frequencies of the medium control cultures remained low as expected, whereas the positive control mutagens ethylmethane sulfonate and benzo(a)pyrene yielded large increases in mutant frequencies, demonstrating the sensitivity of the test and the functionality of the S9 mix.

Table 5.4.1-48: CHO/HGPRT Gene Mutation Assay with Glyphosate ([redacted] 1983), preliminary test

Test group	Mutant frequency (per 10^6 cells)		Cloning efficiency			
			CE_1 (survival, %)		CE_2 (viability, %)	
	uncorr.	corr.[#]	abs.	rel.	abs.	rel.
Without metabolic activation; 3-hour exposure period						
Medium control	5.00	7.40	63.50	100.00	69.92	100.00
Test item [mg/mL]						
5.0	5.00	7.10	70.58	111.15	71.58	102.38
17.5	9.50	15.40	42.00	66.14	61.67	88.20
22.5	3.00	5.30	6.60	10.39	59.83	85.58
Positive control [µg/mL]						
(EMS) 200.0	85.50	**163.70**	58.20	91.65	52.42	74.97
With metabolic activation; 3-hour exposure period						
Medium control	4.50	4.40	78.25	100.00	106.08	100.00
Test item [mg/mL]						
5.0	7.50	6.40	84.58	108.09	117.00	110.29
17.5	4.00	6.90	65.33	83.49	52.67	49.65
22.5	5.50	8.50	51.83	66.24	65.83	62.06
Positive control [µg/mL]						
(B(a)P) 2.0	73.00	**121.20**	36.25	46.33	60.92	57.42

[#] Correction on the basis of the absolute cloning efficiency 2 at the end of the expression period

Data shown for samples with 5 % S9 mix only

Values were re-calculated based on raw data given in the study report

Table 5.4.1-49: CHO/HGPRT Gene Mutation Assay with Glyphosate ([redacted] 1983), main mutagenicity test

Test group	Mutant frequency (per 10^6 cells)		Cloning efficiency			
			CE_1 (survival, %)		CE_2 (viability, %)	
	uncorr.	corr.[#]	abs.	rel.	abs.	rel.
Without metabolic activation; 3-hour exposure period						
Medium control	8.30	11.30	51.06	100.00	74.94	100.00
Test item [mg/mL]						
2.0	2.30	3.50	50.39	98.69	67.61	90.21

Table 5.4.1-49: CHO/HGPRT Gene Mutation Assay with Glyphosate (, 1983), main mutagenicity test

Test group	Mutant frequency (per 10^6 cells)		Cloning efficiency			
			CE_1 (survival, %)		CE_2 (viability, %)	
	uncorr.	corr.#	abs.	rel.	abs.	rel.
5.0	7.30	11.30	47.39	92.82	66.83	89.18
10.0	8.30	10.80	46.06	90.21	74.44	99.33
15.0	14.70	20.80	52.89	103.59	68.56	91.48
20.0	7.00	10.10	19.56	38.30	69.12	92.29
Positive control [µg/mL]						
(EMS) 200.0	83.00	**135.40**	46.94	91.95	61.28	81.76
With metabolic activation; 3-hour exposure period						
Medium control	5.70	7.70	49.22	100.00	75.72	100.00
Test item [mg/mL]						
5.0	4.00	5.70	55.64	113.04	69.75	92.11
10.0	9.30	13.10	49.29	100.13	68.56	90.54
15.0	6.00	9.90	55.44	112.64	60.13	79.40
20.0	9.00	14.90	47.50	96.50	56.86	75.09
25.0	9.30	13.10	22.11	44.92	68.44	90.39
Positive control [µg/mL]						
(B(a)P) 2.0	17.70	**76.80**	23.17	47.07	67.22	88.77

Correction on the basis of the absolute cloning efficiency 2 at the end of the expression period
Values were re-calculated based on raw data given in the study report

III. CONCLUSION:

Based on the experimental findings and under the conditions of the test, glyphosate did not induce gene mutations in the HGPRT locus, neither in the presence nor in the absence of metabolic activation and is therefore considered negative for mutagenicity in mammalian cells *in vitro*.

3. **Assessment and conclusion**

Assessment and conclusion by applicant:

Negative for mutagenicity at the HGPRT locus in CHO cells with and without metabolic activation. This non-GLP study was performed equivalent to OECD 476 (2016). Although a number of deviations became evident when compared with the currently valid guideline, these were considered to be of minor degree and to not compromise the validity of the study. Therefore, the study is considered valid and acceptable.

Assessment and conclusion by RMS:

DNA damage and repair

Seven studies investigating glyphosate in *in vitro* DNA repair assays are available. Two of these studies (CA 5.4.1/033 and CA 5.4.1/035) are considered as supportive data, the remaining studies are considered invalid for use in the evaluation of glyphosate genotoxicity. These assays are no longer a data requirement and either have no OECD guideline or the OECD guideline was deleted in 2014. However, none of these studies show any adverse activity associated with glyphosate and therefore provide supportive evidence assessment that glyphosate is not genotoxic.

Table 5.4.1-50: Summary of *in vitro* genotoxicity testing with glyphosate acid: DNA damage and repair

Annex Point	Study	Study type Test system	Substance Dose levels Exposure Metabolic activation Purity	Status	Result
CA 5.4.1/033	[redacted], 1994	UDS assay Primary rat hepatocytes (Sprague-Dawley)	Glyphosate 0.20 - 48.98 mM (1st exp.) and 1.14 - 111.69 mM (2nd exp.) Exposure: 18 h Purity: > 98 %	Supportive, Category 2a	negative
CA 5.4.1/034	[redacted], 1983	UDS assay Primary rat hepatocytes (Fischer 344)	Glyphosate 0.0125 - 125 µg/mL Exposure: 18 - 20 h Purity: not reported	Invalid, Category 3b	negative
CA 5.4.1/035	[redacted], 1995	Rec assay *B. subtillis* strains H17 and M45	Glyphosate 7.5 - 240 µg/disk ± S9 Exposure: 24 h Purity: 95.68 %	Supportive, Category 2a	negative
CA 5.4.1/036	[redacted] 1978	Rec assay *B. subtillis* strains H17 and M45	Glyphosate 20 - 2000 µg/disk - S9 Exposure: over night Purity: 98.4 %	Invalid, Category 3b	negative
CA 5.4.1/037	[redacted] 1993	Poly $A^{+/-}$ assay *E. coli* strains W3110 ($polA^+$) and p3478 ($polA^-$)	Glyphosate isopropylamine salt 0.1 - 10000 µg/mL - S9 Exposure: 48 h Purity: 64 %	Invalid, Category 3b	negative
CA 5.4.1/038	[redacted] 1993	SCE assay Chinese hamster ovary cells	Glyphosate isopropylamine salt 0.1 - 100 µg/mL ± S9 Exposure: 24 h Purity: 64 %	Invalid, Category 3b	negative
CA 5.4.1/039	[redacted] 1990	SCE assay Human peripheral lymphocytes	Glyphosate active 78.125 - 312.5 µg/mL ± S9 Exposure + S9: 2h Exposure – S9: 24 h Purity: not reported	Invalid, Category 3b	negative

UDS: Unscheduled DNA synthesis
SCE: Sister chromatid exchange

1. Information on the study

Data point	CA 5.4.1/033
Report author	[redacted]
Report year	1994
Report title	DNA repair test with primary rat hepatocytes
Report No	931564
Document No	Not reported
Guidelines followed in study	OECD 482 (1986)
Deviations from current test guideline	Not applicable. OECD 482 was deleted in 2014. When compared to the previous OECD 482 (1986), several deviations became evident. Instead of autoradiography or liquid scintillation counting procedures, incorporation of radioactivity into the DNA was determined based on UV absorbance and mathematical calculations. According to OECD 482 (1986) at least two cell cultures per condition are required. In the current study only one culture per condition was tested. However, a second experiment at concentrations in a similar range was conducted. As no individual value of glyphosate treated cultures at all showed an increase in ^{3}H-dCyd, this deviation seems to not compromise the validity of the study.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Glyphosate (batch: F/93/032, purity > 98 %) was tested in primary rat hepatocytes *in vitro* for its ability to induce unscheduled DNA synthesis (UDS) according to the bromodeoxyuridine density shift method. Hepatocytes were isolated from the livers of adult male Sprague-Dawley rats and used for two separate experiments. Based on the results of a preliminary solubility and cytotoxicity test (data not provided in the study report), appropriate glyphosate concentrations were selected for the UDS assay.

In both experiments, freshly isolated hepatocytes were exposed to test item concentrations in the range of 0.20 – 48.98 mM (first experiment) and 1.14 – 111.69 mM (second experiment) in the presence of tritiated deoxycytidine (^{3}H-dCyd) and bromodeoxyuridine (BrdUrd). Medium and positive controls (dimethylnitrosamine (DNM) and 2-acetamidofluorene (2-AAF)) were included in each experiment. After 18 hours of incubation, the cells were lysed, nucleated DNA was extracted and the replicated, BrdUrd containing DNA as well as the repaired ^{3}H-dCyd and BrdUrd containing DNA were separated by centrifugation on an alkaline cesium salt gradient. Repaired and replicated DNA was quantified by measuring the incorporated radioactivity. Genotoxicity was evaluated based on the induction of DNA repair and expressed as incorporated ^{3}H-dCyd per µg DNA, whereas cytotoxicity was assessed based on the degree of inhibition of replicative DNA synthesis (incorporated BrdUrd per µgDNA).

Precipitation of the test substance in medium, as well as cytotoxic effects were observed at 111.69 mM in the second experiment only. Treatment with glyphosate did not induce a significant increase in DNA repair above those of control conditions in none of the experiments at any tested concentration. Incorporation of

^{3}H-dCyd of the medium control was within the normal range of control data, while the two positive controls DNM and 2-AAF showed a significant increase in ^{3}H-dCyd incorporation, confirming the responsiveness and metabolic activity of the test system.

Under the conditions of the test, glyphosate did not induce unscheduled DNA synthesis in primary rat hepatocytes *in vitro*.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material: Glyphosate

Identification:	Not specified
Description:	Not specified
Lot/Batch number:	F/93/032
Purity:	> 98 %
Stability of test compound:	The stability of the test item at storage conditions (light protected, at 20 – 25 °C) or in solvent was not specified.

2. Control material:

Negative control:	Untreated cell cultures which were cultivated in cultivation medium only were included in each experiment.
Solvent (vehicle) control:	As culture medium was used as solvent for the test item, the solvent control represents actually the negative control.
Positive controls:	Dimethylnitrosamine (DMN, 10 mM) and 2-acetamidofluorene (2-AAF, 0.2 mM)

3. Hepatocyte isolation:

Primary rat hepatocytes were obtained by *in situ* collagenase perfusion from the livers of a single adult male Sprague-Dawley rat weighing approximately 200 – 350 g. The liver was perfused with a Ca^{2+}-free modified Hank's solution containing 500 µM EGTA, continued by the same Ca^{2+}-free solution without EGTA. Finally, the liver was perfused with Dulbecco's modified Eagle's medium (DMEM) including Ca^{2+} and collagenase (0.13 U/mL) and harvested. After filtration through a nylon mesh gauze, the cells were washed in DMEM three times and the viability was determined by trypan blue exclusion. Only hepatocyte preparations with a viability > 80 % were used.

4. Cell culture:

Cell culture establishment:	Freshly isolated hepatocytes were seeded in plating medium at a density of 6 x 10^6 cells in 75 cm^2 flasks, incubated and allowed to attach for 2 hours. Afterwards, the cells were washed twice with pre-warmed salt solution prior to treatment.
Plating medium:	William's medium E, supplemented with 10 % fetal calf serum, 100 U/mL penicillin and 100 µg/mL streptomycin
Pre-treatment medium:	*William's medium E, supplemented with 40 µM fluorodeoxyuridine (FdUrD) and 200 µM Bromodeoxyuridine (BrdUrd)*
Treatment medium:	*William's medium E, supplemented with 40 µM FdUrD, 200 µM BrdUrd and 10 µCi/mL ^{3}H-*

	deoxycytidine (^{3}H-dCyd)
Incubation:	*Not specified*

5. Test concentrations:

Experiment	Concentrations
First experiment:	0.20, 0.61, 1.81, 5.44, 16.32 and 48.98 mM
Second experiment:	1.14, 3.41, 10.23, 30.69, 92.08 and 111.69 mM

B: STUDY DESIGN AND METHODS

1. Dates of experimental work: 01 Feb – 18 Mar 1994
Finalisation date: 28 Mar 1994

2. Cytotoxicity

A preliminary dose-range finding test was not conducted as part of the described study. However, solubility and cytotoxicity have been examined in a pre-test (data not provided in study report), which enabled the selection of the test item concentrations for the UDS assay. In the present study, cytotoxicity was estimated based on the degree of inhibition of replicative DNA synthesis.
The degree of cytotoxicity was therefore determined as part of the genotoxicity test (described below), indicated by a reduction in the incorporation of radioactivity in semiconservatively replicated (dense) DNA strands.

3. Unscheduled DNA synthesis

Cell treatment and harvest:
After cell culture establishment, the cells were incubated in pre-treatment medium containing fluorodeoxyuridine(FdUrD) and BrdUrd for one hour. Following pre-incubation, the medium was exchanged to treatment medium containing additionally ^{3}H-dCyd.

Two independent experiments were performed in parallel, using hepatocytes from the same animal. The cells were exposed to test item concentrations in the range of 0.20 – 48.98 mM (first experiment) and 1.14 – 111.69 mM (second experiment). Medium and positive control cultures (10 mM dimethylnitrosamine and 0.2 mM 2-acetamidofluorene) were included in each experiment.

After 18 hours of incubation, the cells were washed with phosphate buffered saline, lysed with sodium dodecylsarcosine and digested with proteinase K.

Preparation of DNA and determination of radioactivity:
The DNA was precipitated with ethanol, dried and stored at -80 °C. DNA pellets were dissolved and centrifuged to equilibrium in alkaline $CsCl/Cs_2So_4$ gradients at 20 °C for 16 hours. Following centrifugation, the lower half of each gradient was removed. The remaining part containing parental DNA strands was mixed with $CsCl/Cs_2SO_4$ rebanded by centrifugation. After fractionation of the gradients, the UV absorbance and the acid precipitable incorporated radioactivity were determined for each fraction.

Evaluation:
Repair synthesis (cpm/µg DNA) was estimated from the gradient profiles by calculating the radioactivity incorporated into light (parental) DNA, i. e. by integrating the radioactivity binding

exactly coincident with the UV-absorbance peak, and dividing it by the amount of parental DNA. DNA was quantitated by integrating the UV-absorbance peak and converting it into DNA by means of a calibration curve. Normal (semiconservative) DNA synthesis (cpm/µg DNA) was determined from the gradient profiles as radioactivity incorporated into dense DNA.

4. Statistics:
Statistical analysis was not performed in the study.

5. Acceptance criteria:
Acceptance criteria were not defined in the study report.

6. Evaluation criteria:
A substance was considered genotoxic if it produced a reproducible and significant dose-related increase in radiolabel incorporation. A substance was considered non-genotoxic if it produced neither a significant, dose-related increase in ^{3}H incorporation, nor a reproducible positive response at any test point.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS
Analytical determinations were not performed in the study, as not required by the test guideline.

B. CYTOTOXICITY
Cytotoxicity, indicated by inhibition of replicative DNA synthesis, was observed in the second experiment only at a glyphosate concentration of 111.69 mM (refer to Table 5.4.1-51).

C. SOLUBILITY
Precipitation of the test item in culture medium was observed at the highest concentration of 111.69 mM.

D. UNSCHEDULED DNA SYNTHESIS
There was no significant increase in ^{3}H-dCyd incorporation for glyphosate treated hepatocytes when compared to control levels observed, in any experiment and at any tested concentration.

Radiolabel incorporations of ^{3}H-dCyd in the untreated medium control cultures were within the variability of the control values of individual experiments. The two positive compounds DMN and 2-AAF markedly enhanced DNA repair in the hepatocytes, confirming the responsiveness and metabolic activity of the test system.

Table 5.4.1-51: DNA repair test with primary rat hepatocytes (UDS assay) ([redacted], 1994)

	Genotoxicity	Cytotoxicity
	Repair synthesis [cpm/µg DNA]	Replicative synthesis [cpm/µg DNA]
First experiment		
Medium control#	112.7	2442.2
Test item [mM]		
0.20	114.8	1998.4
0.61	109.4	2363.0
1.81	115.1	2641.0

Table 5.4.1-51: DNA repair test with primary rat hepatocytes (UDS assay) ([redacted], 1994)

	Genotoxicity	Cytotoxicity
	Repair synthesis [cpm/µg DNA]	**Replicative synthesis [cpm/µg DNA]**
5.44	102.4	2509.1
16.32	109.9	2356.6
48.98	98.2	1729.1
Positive controls		
DMN [10 mM]	973.7	1794.9
2-AAF [0.2 mM]	817.4	306.2
Second experiment		
Medium control[#]	95.5	2573.7
Test item [mM]		
1.14	96.2	2784.2
3.41	88.5	2514.7
10.23	95.7	2902.6
30.69	87.8	3175.0
92.08	71.6	2664.6
111.63	66.9	737.2
Positive controls		
DMN [10 mM]	928.5	1521.8
2-AAF [0.2 mM]	728.5	829.9

2-AAF: 2-acetamidofluorene; DMN: Dimethylnitrosamine
[#] Mean value of 2 replicates, calculated based on raw data given in study report

III. CONCLUSION:

Under the conditions of the test, glyphosate did not induce any increase in tritiated cytidine incorporation more than 10 % when compared to control values. Thus, glyphosate did not induce DNA damage leading to unscheduled DNA synthesis in primary rat hepatocytes *in vitro*.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Negative for unscheduled DNA synthesis in primary rat hepatocytes *in vitro*.

The study was performed under GLP conditions and in accordance with OECD guideline 482 (1986), which was deleted in 2014. As the Unscheduled DNA Synthesis (UDS) assay is no longer a standard method described by current guidelines, the study was considered to provide supporting information. When compared to OECD guideline 482 (1986), a number of deviations became evident, all of them of minor degree and not compromising the validity of the study.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.4.1/034
Report author	[redacted]
Report year	1983
Report title	The hepatocyte primary culture / DNA repair assay on compound JJN-1020 using rat hepatocytes in culture
Report No	AH-83-181
Document No	M-645649-01-1
Guidelines followed in study	Similar to OECD 482 (1986)
GLP	No, not conducted under GLP/ Officially recognised testing facilities. When the study was conducted, GLP was not compulsory.
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	Glyphosate (batch: XHJ-64, purity not specified) was tested for unscheduled DNA synthesis (UDS) in primary rat hepatocytes *in vitro*. Hepatocytes were isolated from the livers of adult male F344 rats and triplicate cultures per condition were exposed to test item concentrations in the range of 0.0125 to 125 µg/mL in medium supplemented with radiolabeled tritiated thymidine (^{3}H-TdR). Medium, solvent (DMSO and 0.1 N NaOH), negative (50 µM pyrene) and positive controls (50 µM benzo(a)pyrene) were tested in parallel. After 18 - 20 hours of incubation at 37 °C, the cells were processed for slide preparation and autoradiographs developed. Cytotoxicity was assessed by the absence of S-phase cells in the autoradiographs and by morphology. Unscheduled DNA synthesis was quantified by determining the net increase in nuclear grain counts induced by the test item or corresponding controls in a total of 20 - 80 cells per culture.
Short description of results:	Precipitation of the test item in culture medium was not reported and there was no cytotoxicity observed up to the highest tested concentration of 125 µg/mL. Glyphosate did not induce a significant increase in the mean number of net nuclear grain counts when compared to solvent controls at any of the tested concentrations. The values obtained with the solvent and negative controls were in the expected range, whereas the positive control B(a)P significantly increased the number of mean net nuclear grain counts, thus demonstrating the sensitivity of the test system towards DNA damaging substances. Based on the experimental findings and under the conditions of the test, glyphosate did not induce unscheduled DNA synthesis indicated by DNA repair activity in primary rat hepatocytes *in vitro*.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	The study was not conducted under GLP. The corresponding OECD 482 (1986) was deleted in 2014. When compared to OECD 482 (1986), a number of deviations became evident. The selection of test concentrations was not justified in the study report and the highest concentration tested did not cause cytotoxicity. In addition, there is no information on the test items analytical purity. According to OECD 482 (1986) at least 50 cells per culture should be counted and evaluated for nuclear grain counts. In the present

	study, 20 -80 cells per culture were evaluated. A confirmatory experiment was performed, but raw data of the repeat assay were not included in the study report. The study is therefore considered not acceptable.
Reasons why the study report is not available for submission	
Category study in AIR5 dossier (L-docs)	Category 3b

1. Information on the study

Data point	CA 5.4.1/035
Report author	[redacted]
Report year	1995
Report title	HR-001: DNA Repair Test (Rec-Assay)
Report No	IET 94-0141
Document No	Not reported
Guidelines followed in study	U.S. EPA FIFRA Guidelines, Subdivision F
Deviations from current test guideline	Not applicable.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The DNA-damaging activity of glyphosate (HR-001, batch: 940908-1, purity: 95.68 %) was investigated in an DNA repair test with *Bacillus subtilis* strains H17 and M45. In a well diffusion assay, the bacteria were exposed to test item concentrations in the range of 7.5 to 240 µg/disk in the presence and absence of metabolic activation (phenobarbital and 5,6 benzoflavone-induced rat liver S9 fraction). Each concentration was tested in duplicates.

Vehicle (sterile water), negative (kanamycin) and positive controls (mitomycin c without S9 mix and 3-amino-1,4-dimethyl-5H-pyrido [4,3-b] indole (Trp-p-1) in the presence of S9 mix) were included. After an incubation time of 24 h at 37 °C the diameter of the growth inhibition zone was measured for each strain.

Precipitation of the test item was not reported.

A relevant growth inhibition of bacteria strains H17 (rec^+) or M45 ($recE^-$) was not observed at any tested condition, neither in the presence, nor in the absence of metabolic activation. Although a growth inhibition zone of 1 mm diameter was noted at the highest test concentration of 240 µg/disk in strain M45 in the absence of metabolic activation, the differences of growth inhibitory zones between the strains H17 and M45 were 1 mm or less. There was no growth inhibition in strain M45 in the presence of S9 mix nor in strain H17 in the presence or absence of S9 mix.

Positive, negative and vehicle controls showed the expected results and demonstrated the validity of the test system and the functionality of the S9 mix.

Based on the results of the present study, glyphosate technical has no DNA-damaging activity in bacteria.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material: Glyphosate

Identification:	HR-001
Description:	Solid crystals
Lot/Batch #:	940908-1
Purity:	95.68 %
Stability of test compound:	The stability of the test item at storage conditions (in a dark cold room at approx. 5 °C) or in the solvent (vehicle) were not specified.
Solvent (vehicle) used:	Sterile water

2. Control materials:

Negative control:	Kanamycin, 0.2 µg/disk
Solvent (vehicle) control:	Sterile water
Solvent (vehicle) /final concentration:	20 µL/disk
Positive controls:	- S9 mix: Mitomycin C, 0.01 µg/disk + S9 mix: 3-amino-1,4-dimethyl-5H-pyrido [4,3-b] indole (Trp-p-1), 5 µg/disk

3. Metabolic activation:

S9 mix was purchased from [redacted] ([redacted]; Lot no. RAA-314). The homogenate was produced from the livers of 7 weeks old male Sprague-Dawley rats, weighing 188 – 238 g, that received intraperitoneal injections of phenobarbital (30 mg/kg bw on Day 1, each 60 mg/kg bw on Days 2, 3 and 4) and 80 mg/kg bw 5,6 benzoflavone on Day 3. The S9 mix was prepared immediately before the experiment by mixing S9 fraction and co-factor.

S9 mix component	Concentration	Unit
Sodium phosphate buffer (pH 7.4)	100	mM
KCl	33	mM
NADPH-generating system		
Glucose 6-phosphate	5	mM
NADH	4	mM
NADP	4	mM
$MgCl_2$	8	mM
S9	1	% (v/v)

4. Test organisms:

Tester strains			
B. subtilis		**Bacteria batch checked for**	
Recombination wild (rec^+) H17	✓	UV-light sensitivity (recE)	✓
Recombination deficient ($recE^-$) M45	✓	Response to negative and positive control chemicals	✓

5. Test concentrations:

Diffusion assay ± S9 mix:		
Concentrations:	*7.5, 15, 30, 60, 120 and 240 µg/disk*	
Tester strains:	H17, M45	
Replicates:	*Duplicates in a single experiment*	

B: STUDY DESIGN AND METHODS

1. **Dates of experimental work:** 14 – 15 Feb 1995
   Finalisation date: 14 Mar 1995

2. **Diffusion assay**

0.1 mL pre-cultured bacterial suspension (3 x 10^7 cells/mL), 5 mL molten B2 top agar and, in experiments with metabolic activation, 0.05 mL of S9 mix were mixed uniformly in an 90 mm petri dish and the agar plates were left at room temperature for solidification. Paper discs (8 mm diameter) impregnated with 20 µL test solution, positive, negative or vehicle control were placed on the prepared spore agar plate. Each concentration was tested in duplicates. After an incubation time of 24 h at 37 °C the diameter of the growth inhibition zone was measured for each strain.

3. **Statistics**

Results were judged without statistical analysis.

4. **Acceptance criteria**

The test was valid if

- Growth inhibition was not observed in solvent controls of either strain.
- For the positive control, the growth inhibitory zone in strain M45 was larger than the zone in strain H17 and the difference in diameter was ≥ 5 mm.
- For the negative control, the difference in diameter of growth inhibitory zone between the strains was ≤ 4 mm.

5. **Evaluation criteria**

In case the test item caused growth inhibition in at least one strain, results were judged positive when the following criteria were met:

- The growth inhibitory zone of M45 was larger than that of H17 and
- The difference in diameter was 5 mm or more at one or more dose levels that caused growth inhibitory zones with diameters of 4 mm or less in the H17 strain.

If a positive result was obtained, a re-test was conducted to confirm the reproducibility of the positive result.

Results were judged negative when the test substance causes no growth inhibition in either strain.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Analytical determinations were not performed in the study, as not required for this type of *in vitro* study.

B. SOLUBILITY

Precipitation of the test substance was not reported.

C. MUTATION ASSAY

A relevant growth inhibition of bacteria strains H17 (rec$^+$) or M45 (recE$^-$) was not observed at any tested condition, neither in the presence, nor in the absence of metabolic activation. Although a growth inhibition zone of 1 mm diameter was noted at the highest test concentration of 240 µg/disk in strain M45 in the absence of metabolic activation, the differences of growth inhibitory zones between the strains H17 and M45 were 1 mm or less. There was no growth inhibition in strain M45 in the presence of S9 mix or in strain H17 in the presence nor absence of S9 mix.

Negative controls treated with kanamycin induced growth inhibition zones with differences between 2 - 3 mm between M45 and H17, while positive controls treated with mitomycin C (-S9 mix) or Trp-p-1 (+S9 mix) caused large growth inhibitory zones with differences in diameter of 19 and 11 - 12 mm.

Table 5.4.1-52: DNA Repair Test (Rec-Assay) ([redacted], 1995)

Compound	Dose (µg/disk)	S9 fraction (-)			S9 fraction (+)		
		Inhibitory zone* (mm)		Difference** (mm)	Inhibitory zone* (mm)		Difference** (mm)
		M45	H17		M45	H17	
Solvent control (H_2O)		0	0	0	0	0	0
		0	0	0	0	0	0
Test item	7.5	0	0	0	0	0	0
		0	0	0	0	0	0
	15	0	0	0	0	0	0
		0	0	0	0	0	0
	30	0	0	0	0	0	0
		0	0	0	0	0	0
	60	0	0	0	0	0	0
		0	0	0	0	0	0
	120	0	0	0	0	0	0
		0	0	0	0	0	0
	240	1	0	1	0	0	0
		0	0	0	0	0	0
Negative control (Kanamycin)	0.2	8	6	2			
		9	6	3			
Positive control (Mitomycin C)	0.01	20	1	19			
		20	1	19			
Positive control (Trp-p-1)	5				11	0	11
					12	0	12

Trp-p-1: 3-amino-1,4-dimethyl-5H-pyrido [4,3-b] indole, positive control in the presence of S9 mix

III. CONCLUSION

According to the results of the present study and under the experimental conditions chosen, the test item does not show DNA-damaging activity in the DNA repair test (Rec-Assay) in the presence or absence of metabolic activation.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Negative for genotoxicity in bacteria (*B. subtilis* H17 and M45) with and without metabolic activation. The study was conducted under GLP conditions and according to US EPA FIFRA guidelines subdivision F. It was considered to provide supplementary information only because the Rec assay is not a standard method for this endpoint (DNA damage and repair). Furthermore, the dose selection was not explained and viability data were not included in the study report.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.4.1/036
Report author	[redacted]
Report year	1978
Report title	The report of mutagenic study with bacteria for CP67573
Report No	ET-78-241
Document No	Not reported
Guidelines followed in study	No guideline followed, the study was conducted similar to U.S. EPA FIFRA Guidelines, Subdivision F
GLP	No, not conducted under GLP/ Officially recognised testing facilities. When the study was conducted, GLP was not compulsory.
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	The DNA-damaging activity of glyphosate (CP67573, batch: XHJ-46, purity: 98.4 %) was investigated in a DNA repair test with *Bacillus subtilis* strains H17 and M45. In a single experiment, the two bacterial strains were exposed to the test item at concentrations in the range of 20 – 2000 µg/disk. Solvent (water), negative (kanamycin, 10 µg/disk) and positive controls (mitomycin C, 0.1 µg/plate were included in the experiment. After overnight incubation at 37 °C, the growth inhibitory zones were measured for both strains.
Short description of results:	Precipitation of the test item was not investigated. Treatment with CP67573 did not induce any inhibitory zone in any of the two tester strains at any tested concentration. Solvent controls showed the expected results and negative controls treated with kanamycin induced growth inhibition zones of similar lengths in both strains. The positive control mitomycin C caused a marked difference in the length of the inhibitory zones, demonstrating the functionality of the test. Based on the results of the present study, glyphosate was negative for DNA-damaging activity in bacteria.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	The study was not conducted under GLP and not according to current testing guidelines. The test was performed in the absence of S9 mix only and no viability data (actual plate count) were provided. In addition, there were some reporting deficiencies. The study is therefore considered not acceptable.
Reasons why the study report is not available for submission	
Category study in AIR5 dossier (L-docs)	Category 3b

1. Information on the study

Data point:	CA 5.4.1/037
Report author	[redacted]
Report year	1993 (Finalisation of the English translation of report 87BME014

	in Chinese language dated 03 Mar 1987)
Report title	Mutagenicity evaluation of glyphosate in *Escherichia coli.* DNA repair (poly A^+/A^-) assay.
Report No	87BME014-E
Document No	Not reported
Guidelines followed in study	No guideline followed. The study was conducted according to the main criteria of U.S. EPA FIFRA Guidelines, Subdivision F.
GLP	No, not conducted under GLP/ Officially recognised testing facilities. When the study was conducted, GLP was not compulsory.
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	A DNA damage repair ($polA^+$/ $polA^-$) assay in *E. coli* strains W3110 ($polA^+$) and p3478 ($polA^-$) was performed to detect the mutagenic potential of glyphosate isopropylamine salt (code: SN-750721, purity: 64 %) on DNA damage level. In a single experiment with five replicates per condition, both bacterial strains were exposed to test item concentrations in the range of 0.1 – 10000 µg/mL. Solvent and positive controls (methyl methanesulfonate, 10 µL/disk) were included. After 48 hours of incubation at 37 °C, the length of inhibition zones around the disk was determined for both strains.
Short description of results:	Precipitation was not investigated in the study report. At the highest concentration of 10000 µg/mL, there was a strong growth inhibition observed in both strains, with a statistically significantly increased inhibition zone in strain p3478 ($polA^-$) when compared to strain W3110 ($polA^+$). There was no growth inhibition observed at lower test item concentrations. The solvent and positive control showed the expected results, demonstrating the sensitivity of the test. As growth inhibition induced by the test item was observed at one concentration only, the test result did not match the evaluation criteria for a positive result. Although growth inhibition was observed at the top dose level, the result was not confirmed in an independent experiment. Therefore, the test substance was considered equivocal for mutagenicity on DNA-damage level.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	The study was not conducted under GLP and not according to current testing guidelines. The test was performed in the absence of metabolic activation only and no viability data (actual plate count) were provided. In addition, the title is misleading because not glyphosate acid but the isopropylamine salt has been tested. It is not clear whether the given purity refers to the contents of glyphosate in the formulation or the salt. A confirmatory experiment was not conducted even though there was some inhibition of cell growth at the top dose level. The study is therefore considered not acceptable.
Reasons why the study report is not available for submission	
Category study in AIR5 dossier (L-docs)	Category 3b

1. Information on the study

Data point:	CA 5.4.1/038

Report author	[redacted]
Report year	1993 (Finalisation of the English translation of report 87BMS013-E in Chinese language dated 03 Mar 1987)
Report title	Mutagenicity Evaluation of Glyphosate in Sister Chromatid Exchange Assay (SCE Test)
Report No	87BMS013-E
Document No	Not reported
Guidelines followed in study	No guideline followed. The study was conducted similarly to OECD 479 (1986)
GLP	No, not conducted under GLP/ Officially recognised testing facilities. When the study was conducted, GLP was not compulsory.
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	Glyphosate isopropylamine salt (code: SN-750721, purity: 64 %) was tested in a sister chromatid exchange assay in Chinese hamster ovary (CHO) cells in the presence and absence of metabolic activation (Aroclor 1254-induced rat liver S9 fraction). Untreated, solvent (DMSO) and positive controls (3.14 mM ethylmethane sulfonate without S9 mix and 0.01 mM cyclophosphamide with S9 mix) were included. A single experiment was performed. Duplicate cultures were exposed to test item concentrations in the range of 0.1 – 100 µg/mL for 1 hour, followed by 22 hours of incubation in the presence of 5-bromo-2-deoxyuridine. After 24 hours of incubation, the cells were harvested and a total of 30 metaphase cells per culture was scored for sister chromatid exchanges (SCEs) per chromosome.
Short description of results:	Cytotoxicity and solubility / precipitation were not investigated in the present study. Treatment with glyphosate isopropylamine salt did not induce a statistically significant increase in the frequency of SCEs per chromosome up to the highest tested concentration, neither in the presence nor in the absence of S9 mix. SCE frequencies of the untreated and solvent controls matched the acceptability criteria. A strong increase in the frequency of SCEs was noted for the positive control compounds, showing the activity of the S9 mix and demonstrating the sensitivity of the test. Under the experimental conditions reported, the test item was considered negative for sister chromatid exchange in CHO cells *in vitro*, with and without metabolic activation.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	The study was not conducted under GLP and not according to current testing guidelines. In addition, it is not clear whether the given purity refers to the contents of glyphosate in the formulation or the salt. The study is therefore considered not acceptable.
Reasons why the study report is not available for submission	
Category study in AIR5 dossier (L-docs)	Category 3b

1. Information on the study

Data point:	CA 5.4.1/039

Report author	[redacted]
Report year	1990
Report title	Agrichem glyphosate active: OECD 479 sister chromatid exchange in human lymphocytes *in vitro*
Report No	300/2
Document No	Not reported
Guidelines followed in study	OECD TG 479 (1986)
GLP	Yes
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	Glyphosate active (batch: 0190A, purity: not reported) was tested in a sister chromatid exchange assay in human lymphocytes (lymphocyte preparation not further specified). The cells were exposed to the test item, solvent (medium) or positive controls (ethyl methane sulfonate, 500 µg/mL without S9 mix and cyclophosphamide, 20 µg/mL with S9 mix) in the presence and absence of metabolic activation (Aroclor 1254-induced rat liver fraction). A single experiment was performed. Duplicate cultures were exposed to test item concentrations in the range of 78.125 – 2500 µg/mL in medium containing bromodeoxyuridine (BrdU). In the presence of S9 mix, the cells were exposed for 2 hours, followed by re-incubation in test substance-free medium for further 22 hours. In the absence of S9 mix, the cells were exposed for 24 hours. Afterwards, the mitotic cells were collected and prepared for staining. A total of 25 metaphase cells per culture (50 per condition) were scored for sister chromatid exchanges (SCE) per cell and per chromosome.
Short description of results:	Precipitation of the test substance in medium was not reported. Cytotoxicity (absence of metaphases) was observed at 1250 and 2500 µg/mL in the presence and absence of metabolic activation. Based on these findings, 625 µg/mL was selected as highest concentration for the evaluation of SCE. Treatment with glyphosate active did not induce a statistically significant, dose-related increase in the frequency of SCE per cell or per chromosome, neither in the presence, nor in the absence of metabolic activation. SCE values for the solvent and positive controls showed the expected results, indicating that the metabolic activation system was functional and demonstrating the sensitivity and validity of the test system. Under the conditions of the test, glyphosate active did not induce SCE in human lymphocytes *in vitro*, neither with nor without S9 mix.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	The study was considered not acceptable as only a single experiment was performed and the negative test result was not confirmed in an independent experiment. In addition, the purity of the test material was not stated. Furthermore, there were some reporting deficiencies, as lymphocyte preparation was not described in detail (remaining uncertainty about exposure of isolated lymphocytes or whole blood cell culture). The study is therefore considered invalid.
Reasons why the study report is not available for submission	
Category study in AIR5 dossier (L-	Category 3b

docs)	

CA 5.4.2 *In vivo* studies in somatic cells

Thirteen *in vivo* micronucleus studies have been conducted with glyphosate, one conducted in the rat (CA 5.4.2/014) and the remaining studies conducted in the mouse (Table 5.4.2-1). Although three of the mouse studies are considered invalid and a further two as supportive only, the remaining studies (including the study in rats) are considered to provide a robust assessment of glyphosate mutagenicity *in vivo*. Seven of the nine valid studies (and also the five invalid or supportive only studies) summarized below are clearly negative; administration of glyphosate to rats or mice did not induce any increases in micronuclei. Very weak increases in micronuclei were reported in only two studies: CA 5.4.2/010 ([redacted], 1993) and CA 5.4.2/007 [redacted], 2006). CA 5.4.2/010 ([redacted], 1993) was reviewed in the 2001 EU glyphosate evaluation and it was concluded that the result of this study was unlikely to be relevant because the effect was only seen in females (usually the less sensitive sex in a micronucleus test) and the variation in the % of polychromatic erythrocytes with micronuclei was considerably high among female dose groups compared to controls, whereas the results in the male groups were much more homogeneous. In addition, in a chromosome aberration study conducted in the same laboratory, under nearly identical conditions using the same doses and test material, did not provide any evidence of chromosome aberrations but did indicate a certain degree of cytoxicity at the highest dose level to bone marrow cells. In CA 5.4.2/007 ([redacted], 2006), a small but statistically significant increase in micronuclei was observed at the highest dose but only at the 24 hour sample (48 hour data were clearly negative). The increase in micronuclei was within the range of the laboratory's historical control data and was accompanied by a reduction in the target cells (polychromatic erythrocytes), thus indicating that the increase in micronuclei was the consequence of a haematopoetic response to bone marrow toxicity rather than to a specific genotoxic effect.

Two *in vivo* chromosome aberration studies have been conducted with glyphosate, one was performed in mice by oral gavage and one performed in rats by intraperitoneal (IP) injection. Although both studies are considered as supportive only, due to deficiencies when compared with OECD 475 (2016) they provide further evidence that glyphosate is not clastogenic *in vivo*.

The overwhelming weight of evidence from this large database of *in vivo* somatic cell mutagenicity studies is that glyphosate is not genotoxic to rodents. These studies were conducted in rats and mice using two different administration routes (oral gavage and IP injection) and there are several lines of evidence that demonstrate exposure to the bone marrow (thus validating the negative conclusions).

1. Section 5.1 of MCA 5 confirms that following oral administration of glyphosate to rats, there is rapid absorption from the gastrointestinal tract and subsequently approximately 20 % of administered glyphosate is systemically available. Furthermore, radiolabel studies confirmed the presence of glyphosate in the bone and bone marrow following oral administration (CA 5.1.1/011 and CA 5.1.1/006)
2. Evidence of bone marrow toxicity was observed in three mouse studies (CA 5.4.2/004: IP injection, CA 5.4.2/007: IP injection; CA 5.4.2/015: oral gavage)
3. Several studies were conducted using IP injection, thus ensuring high systemic exposure, and the doses tested caused clinical signs of toxicity consistent with systemic exposure.

Furthermore, the lack of any convincing evidence of glyphosate genotoxicity *in vivo* is consistent with the clear negative conclusions for *in vitro* genotoxicity. It can be concluded that the glyphosate active ingredient does not pose any mutagenic, clastogenic, or aneuploidy risk to humans.

Table 5.4.2-1: Summary of *in vivo* cytogenicity testing with glyphosate acid

Annex Point	Study	Study type Test system, Dosing and Sampling	Substance Dose levels Purity	Reference list-related category§	Result
CA 5.4.2/001	[redacted] 2012	Micronucleus test in bone marrow NMRI mice, 6 ♂/group Oral gavage on 2 consecutive days Sampling: 24 h after 2nd dose	Glyphosate TGAI 2000 mg/kg bw/day Purity: 98.9 %	Valid Category 2a	negative
CA 5.4.2/002	[redacted], 2012	Micronucleus test in bone marrow NMRI mice, 7 ♂/group Oral gavage (single treatment) Sampling: 24 and 48 h	Glyphosate technical 2000 mg/kg bw Purity: 96.3 %	Valid Category 2a	negative
CA 5.4.2/003 CA 5.4.2/004	[redacted] 2008 and 2010	Micronucleus test in bone marrow Swiss albino mice, 5/sex/group IP injection on 2 consecutive days Sampling: 24 h after 2nd dose	Glyphosate technical 15.62, 31.25, 62.5, 125, 250 and 375 mg/kg bw/day Purity: 98.0 %	Supportive Category 3a	negative
CA 5.4.2/005	[redacted] 2008	Micronucleus test in bone marrow NMRI mice, 6 ♂/group Oral gavage (single treatment) Sampling: 24 h (all groups) and 48 h (control and high-dose group)	Glyphosate technical 500, 1000 and 2000 mg/kg bw Purity: 99.1 %	Valid Category 2a	negative
CA 5.4.2/006	[redacted] 2007	Micronucleus test in bone marrow Swiss mice, 6 ♂/group Oral gavage on 2 consecutive days Sampling: 24 h after 2nd dose	Glyphosate technical 8, 15 and 30 mg/kg bw/day Purity: 98.01 %	Invalid, Category 3b	negative
CA 5.4.2/007	[redacted] 2006	Micronucleus test in bone marrow CD-1 mice, 7 ♂/group IP injection (single treatment) Sampling: 24 h (all groups) and 48 h (control and high-dose group)	Glyphosate Technical 150, 300 and 600 mg/kg bw Purity: 95.7 %	Valid Category 2a	negative
CA 5.4.2/008	[redacted] 1999	Micronucleus test in bone marrow Swiss albino mice, 5/sex/group IP injection on 2 consecutive days Sampling: 24 h after 2nd dose	Glifosate Técnico Nufarm 187.5, 375 and 562.5 mg/kg bw/day Purity: 95 %	Valid Category 2a	negative
CA 5.4.2/009	[redacted] 1996	Micronucleus test in bone marrow CD-1 mice, 5/sex/group Oral gavage (single treatment) Sampling: 24 and 48 h	Glyphosate acid 5000 mg/kg bw Purity: 95.6 %	Valid Category 2a	negative
CA 5.4.2/010	[redacted], 1993	Micronucleus test in bone marrow Swiss albino mice, 5/sex/group Oral gavage on 2 consecutive days Sampling: 24 h after 2nd dose	Glyphosate 50, 500 and 5000 mg/kg bw/day Purity: 96.8 %	Supportive, Category 2a	negative in ♂ weakly positive in ♀
CA	[redacted],	Micronucleus test in bone	Glyphosate	Invalid,	negative

5.4.2/0011	1990	marrow Albino BKW mice, 5/sex/group Oral gavage (single treatment) Sampling: 24, 48 and 72 h	technical 4000 mg/kg bw Purity: not reported	Category 3b	
CA 5.4.2/012	[redacted] 1991	Micronucleus test in bone marrow Bom:NMRI mice, 5/sex/group Oral gavage (single treatment) Sampling: 24, 48 and 72 h	Glyphosate technical 5000 mg/kg bw Purity: 98.6 %	Valid Category 2a	negative
CA 5.4.2/013	[redacted] 1989	Micronucleus test in bone marrow NMRI mice, 7/sex/group Oral gavage (single treatment) Sampling: 24, 48 and 72 h	Glyphosate active ingredient 2000 mg/kg bw Purity: not reported	Invalid, Category 3b	negative
CA 5.4.2/014	[redacted] 2009#	Micronucleus test in bone marrow CD rats, 5/sex/group Oral gavage (single treatment) Sampling: 24 h (all groups) and 48 h (control and high-dose group)	Glyphosate technical 500, 1000 and 2000 mg/kg bw Purity: 98.8 %	Valid Category 2a	negative
CA 5.4.2/015	[redacted] 1994	Chromosome aberration in bone marrow Swiss albino mice, 5/sex/group Oral gavage on 2 consecutive days Sampling: 25.5 h after 2nd dose	Glyphosate 50, 500 and 5000 mg/kg bw/day Purity: 96.8 %	Supportive, Category 2a	negative
CA 5.4.2/016	[redacted], 1983	Chromosome aberration in bone marrow Sprague-Dawley rats, 3/sex/sampling time IP injection (single treatment) Sampling: 6, 12 and 24 h	Glyphosate 1000 mg/kg bw Purity: 98.7 %	Supportive, Category 2a	negative

IP Intraperitoneal injection
Testing was performed at the [redacted]
§ The category describes the acceptability/reliability of the study within the AIR 5 submission

Micronucleus tests in the bone marrow of mice *in vivo*:

1. Information on the study

Data point	CA 5.4.2/001
Report author	[redacted]
Report year	2012
Report title	Glyphosate TGAI: Micronucleus test of glyphosate TGAI in mice.
Report No	485-1-06-4696
Document No	DR-0112-6927-003
Guidelines followed in study	OECD 474 (1997), US EPA OPPTS 870.5395 (1998), EC 440/2008 B.12 (2008)
Deviations from current test guideline OECD 474 (2016)	According to the current guideline OECD TG 474 (2016), at least 4000 polychromatic erythrocytes per animal should be evaluated for the presence of micronuclei. In the present study only 2000 polychromatic erythrocytes were evaluated, since that was required in the previous OECD guideline (1997). In addition, the percentage of polychromatic erythrocytes among total erythrocytes was determined for 200 erythrocytes instead of 500

	erythrocytes. Bone marrow exposure, indicated by a reduced polychromatic to normochromatic erythrocyte ratio, was not confirmed and there was no systemic toxicity observed, but dose levels included limit concentrations specified in the current guideline. In addition, the evaluation criteria specified in the study report did not consider historical control data of solvent controls.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Glyphosate TGAI (TSN105914, batch: 20061109, purity: 98.9 %) was assessed for its genotoxic potential in a micronucleus test. Based on the results of a preliminary toxicity study, in which no systemic toxicity was observed at 2000 mg/kg bw/day, groups of six male mice were treated with a limit dose of 2000 mg/kg bw/day for two consecutive days. The test item was dissolved in vegetable oil and administered by oral gavage at a constant dosage volume of 10 mL/kg bw. In addition, 6 male mice were treated with the solvent under the same experimental conditions or received a single intraperitoneal injection of the positive control (1.0 mg/kg bw/day mitomycin C).

Mortality, clinical signs of toxicity and individual body weights were monitored. 24 hours following the last dose, the animals were sacrificed and bone marrow smears were prepared. For each animal, a minimum of 2000 polychromatic erythrocytes (PCE) was scored for the presence of micronuclei. In addition, the ratio of PCE to normochromatic erythrocytes (NCE) was recorded from a minimum of 200 erythrocytes.

Treatment with 2000 mg/kg bw/day glyphosate TGAI did not induce any clinical signs of systemic toxicity and no changes in body weight. Furthermore, there was no statistically significant difference in the ratio of PCE/NCE, confirming that the test item did not induce bone marrow toxicity in the animals.
In addition, there was no statistically significant increase in the frequency of micronucleated PCE in glyphosate TGAI-treated mice when compared with the solvent control group.

Incidences of micronucleated PCE in the solvent and positive control groups were within the range of the laboratory's historical control data, demonstrating the capability of the test animals to respond to mutagenic substances and confirming the sensitivity of the test.

Based on the experimental findings and under the conditions of the test, glyphosate TGAI has no potential to induce micronuclei in the bone marrow of Swiss albino mice *in vivo*.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material: Glyphosate TGAI

Identification:	TSN105914
Description:	White to off-white crystals
Lot/Batch #:	20061109
Purity:	98.9 %
Stability of test compound:	The stability of the test item at storage conditions was guaranteed until the expiry date 20 Apr 2014. The stability of the test item in solvent was not specified.
Solvent (vehicle) used:	Vegetable oil

2. Control materials

Solvent (vehicle) control:	Vegetable oil
Positive control:	Mitomycin C, 1 mg/kg bw/day

3. Test animals:

Species:	Mouse
Strain:	NMRI
Sex:	Males
Source:	[redacted]
Age at dosing:	8 - 9 weeks
Mean weight at dosing:	35 – 50 g
Acclimation period:	6 days
Diet/Food:	Teklad Certified Global 16 % Protein Rodent Diet, *ad libitum*
Water:	UV sterilized drinking water, filtered through a reverse osmosis water filtration system, *ad libitum*
Housing:	In groups of 6/cage polypropylene mouse cages measuring 29 x 37.5 x 14 cm with stainless steel grid top and clean rice husk bedding

4. Environmental conditions:

Temperature:	19 - 22 °C
Humidity:	64 – 65 %
Air changes:	Minimum 15/hour
Photoperiod:	12-hour light and dark cycle

5. Test concentrations and treatment groups:

a) Preliminary toxicity study

Dose levels:	2000 mg/kg bw
Concentrations:	Not specified
Dose volume:	Not specified
Number of animals:	3/sex
Route of administration:	Oral gavage

b) Main micronucleus test

Dose levels:	2000 mg/kg bw/day
Concentrations:	200 mg/mL
Dose volume:	10 mL/kg bw
Number of animals:	6 males/group
Route of administration:	Oral gavage

B: STUDY DESIGN AND METHODS

1. Dates of experimental work: 28 May – 07 Aug 2012
Finalisation date: 13 Sep 2012

2. Animal assignment and treatment:

Preliminary toxicity study:
A dose range finding study was conducted to determine the maximum tolerated dose (MTD). Three male and three female mice were treated at the dose level of 2000 mg/kg bw/day for two consecutive days.
Mortality, clinical symptoms and change in body temperature were monitored up to 72 hours after the initial dose. The rectal temperature was measured prior to dosing, approx. 2, 5 and 24 hours after dosing and before sacrifice.
Based on the results of the preliminary toxicity study, 2000 mg/kg bw/day were selected as dose level for the micronucleus test.

Main micronucleus test:
Groups of 6 male mice received a dose of 2000 mg/kg bw/day for two consecutive days. The test item was dissolved in vegetable oil and administered by oral gavage at a constant dosage volume of 10 mL/kg bw. Similar constituted groups of mice were treated twice with the vehicle following the same route or received a single intraperitoneal injection of the positive control (1.0 mg/kg bw/day mitomycin C).
The animals were observed for clinical signs of toxicity post-dosing and pre-sacrifice. In addition, individual body weights were recorded prior to dosing and before sacrifice. The animals were sacrificed by CO_2 asphyxiation 24 hours after the second dose.

3. Slide preparation:

Femur bones from the sacrificed animals were excised and the epicondyle tips were removed. Bone marrow was flushed out with foetal bovine serum and the collected cells were pelleted by centrifugation. After re-suspension in 0.2 – 0.3 mL medium, the pellet was dissociated with a pipette and a drop of cell suspension was smeared on a clean slide and allowed to air-dry. For each animal two slides were prepared. The cells were fixed with absolute methanol and air-dried for 15 – 20 minutes, followed by 5 % Giemsa staining for 25 minutes. Subsequently, the slides were rinsed with distilled water, air-dried and mounted.

4. Slide evaluation:

Slides were randomly coded and evaluated by microscopical analysis. A minimum of 2000

polychromatic erythrocytes (PCE) per animal were scored for the presence of micronuclei. In addition, the ratio of PCE to normochromatic erythrocytes (NCE) was recorded from a minimum of 200 erythrocytes.

5. Statistics:

The percentage of micronucleated polychromatic erythrocytes (PCE) and the ratio of PCE to normochromatic erythrocytes (NCE) was statistically analysed using Bartlett's test and Analysis of Variance (ANOVA), followed by Dunnett's test to determine the level of significant differences between the vehicle control and the treatment group. Where data did not meet the homogeneity of variance, Student's t-test was performed to determine the level of significant difference between the vehicle control, treatment group and the positive control group.

6. Acceptance criteria:

The study was considered valid if the following criteria were met:

- The prepared slides had uniform staining properties and a sufficient number of polychromatic erythrocytes (PCE) to allow accurate micronucleus determination.
- The solvent controls were in the range of the laboratories historical control data.
- The positive controls were in the range of the laboratories historical control data.
- At least 5 animals per group and sex were evaluated.
- The PCE to erythrocyte ratio was not less than 20 % of the solvent control.

7. Evaluation criteria:

A test substance was considered positive for induction of micronuclei when the following criteria were met:

- There was a dose-dependent, statistically significant increase in the incidence of micronuclei or increase in a single dose group.
- The result was of biological relevance.

A test item was considered negative for induction of micronuclei if no evident statistically significant increase in the numbers of micronucleated polychromatic erythrocytes (mPCE) was observed, relative to the concurrent and established historical control frequencies for PCE induction.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Analytical determinations were not performed in this study, as not compulsory by the test guideline.

B. PRELIMINARY TOXICITY STUDY

There was no mortality and no adverse effects of systemic toxicity observed upon treatment with glyphosate TGAI. In addition, there was no impact on body temperature 24 and 48 hours after treatment.

Based on these findings, 2000 mg/kg bw/day were selected as the limit dose level for the micronucleus test.

C. MAIN MICRONUCLEUS TEST

Systemic toxicity:

Mortality:

No mortality occurred.

Clinical signs of toxicity:

Clinical signs of toxicity were not observed.

Body weight:
Body weights were comparable among all groups during the experimental period.

Evaluation of bone marrow slides:
Upon treatment with glyphosate TGAI, there was no statistically significant change in the ratio of polychromatic (PCE) to normochromatic erythrocytes (NCE), indicating that there was no evidence of cell proliferation or bone marrow cytotoxicity.
In addition, there was no statistically significant increase in the frequency of micronucleated PCE (mPCE) in glyphosate TGAI-treated mice when compared with the solvent control group.
Incidences of mPCE in the solvent and positive control groups were within the range of the laboratory's historical control data, demonstrating the capability of the test animals to respond to mutagenic substances and confirming the sensitivity of the test.

Table 5.4.2-2: Glyphosate TGAI: Micronucleus test of glyphosate TGAI in mice ([redacted], 2012), summary of genotoxicity data

Treatment	Dose (mg/kg bw/day)	Number of animals	Total number of PCE	Total number of mPCE	Mean mPCE	Mean% PCE	Mean PCE/NCE ratio
Vehicle	-	6	12014	4	0.667	0.033	0.525
HCD vehicle#							
mean ± SD						0.02 ± 0.02	0.58 ± 0.04
range						0.00 - 0.07	0.48 - 0.65
Test item	2000	6	12019	0	0.000	0.000	0.531
MMC	1.0	6	12030	300	50.000**	2.492**	0.687*
HCD positive control#							
mean ± SD						1.40 ± 0.36	0.57 ± 0.04
range						0.81 - 2.52	0.45 - 0.69

mPCE: micronucleated polychromatic erythrocytes

MMC: Mitomycin C, positive control

* and **: statistically significant at $p < 0.05$ and $p < 0.011$, respectively

Historical control data, generated in the laboratory from Sep 2009 - Feb 2012 (data from male animals only)

III. CONCLUSION:

Based on the experimental findings glyphosate TGAI, administered at a limit dose of 2000 mg/kg bw/day, did not induce micronuclei in bone marrow in mice *in vivo*.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Negative for clastogenic/aneugenic effects in the bone marrow of male Swiss albino mice i*n vivo*.
The study was performed in compliance with GLP and according to OECD guideline 474 (1997). There

were some deviations when compared to the current OECD guideline 474 (2016), which were considered to be of minor degree. The number of polychromatic erythrocytes investigated was 2000, corresponding to the number required by the previous guideline (1997). In addition, the percentage of polychromatic erythrocytes among total erythrocytes was determined for 200 erythrocytes instead of 500 erythrocytes. Evaluation criteria specified in the study report did not consider historical control data, but the values obtained in the present study were in line with historical controls. Bone marrow toxicity, indicated by a reduced polychromatic to normochromatic erythrocyte ratio or signs of systemic toxicity, was not evident, but the test was performed at limit dose levels in line with the current guideline. Therefore, the deviations were considered to not compromise the scientific validity of the study. The study is therefore considered valid and acceptable.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.4.2/002
Report author	[redacted]
Report year	2012
Report title	Glyphosate Technical – Micronucleus Assay in Bone Marrow Cells of the Mouse.
Report No	1479200
Document No	Not reported
Guidelines followed in study	OECD 474 (1997), US EPA OPPTS 870.5395 (1998), EC 440/2008 B.12 (2008)
Deviations from current test guideline OECD 474 (2016)	According to the current guideline OECD TG 474 (2016), at least 4000 polychromatic erythrocytes per animal should be evaluated for the presence of micronuclei. In the present study only 2000 polychromatic erythrocytes were evaluated, since that was required in the previous OECD guideline (1997). Bone marrow exposure, indicated by a reduced polychromatic to normochromatic erythrocyte ratio, was not confirmed and there was no systemic toxicity observed, but dose levels included limit concentrations specified in the current guideline. In addition, the acceptance and evaluation criteria specified in the study report did not consider historical control data.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Glyphosate technical (BX20070911, batch: 569753, purity: 96.3 %) was tested for its genotoxic potential in mice using the micronucleus test. Based on the results of a preliminary toxicity study, in which no toxicity was observed at 2000 mg/kg bw, groups of seven male mice each received a single oral dose of 2000 mg/kg bw. The test item was dissolved in 1 % (w/w) carboxymethyl cellulose and administered at a constant dosage volume of 20 mL/kg bw. Groups of 5 male control animals received the vehicle or the positive

control (40 mg/kg bw cyclophosphamide in sterile water).

About 1, 2-4, 6, 24 and 48 hours after dosing, the animals were examined for signs of systemic toxicity. Bone marrow was sampled 24 and 48 hours after dosing and for each animal 2000 polychromatic erythrocytes (PCE) were scored for the presence of micronuclei. In addition, the ratio of PCE to normochromatic erythrocytes (NCE) was determined per 2000 erythrocytes.

Treatment with glyphosate technical did not induce signs of systemic toxicity or mortality in the animals. Further, based on the ratio of PCE among the total number of erythrocytes, there was no evidence for bone marrow toxicity.

In addition, there was no biologically relevant and no statistically significant increase in the frequency of micronucleated PCE of test item-treated animals at any sampling time point when compared to vehicle controls.

Incidences of micronuclei in the solvent and positive control groups were within the range of the laboratory's historical control data, confirming the sensitivity of the test and demonstrating the capability of the test animals to respond to mutagenic substances.

Based on the experimental findings, the test item did not induce clastogenic/aneugenic effects in mice *in vivo*.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material: Glyphosate Technical

Identification:	BX20070911
Description:	White solid
Lot/Batch #:	569753
Purity:	96.3 %
Stability of test compound:	The stability of the test item under storage conditions (< 30° C) was guaranteed until March 2015. The stability of the test item in solvent (vehicle) was not indicated by the sponsor.
Solvent (vehicle) used:	1 % carboxymethyl cellulose (CMC)

2. Control materials:

Solvent (vehicle) control:	1 % carboxymethyl cellulose (CMC)
Positive control:	Cyclophosphamide, 40 mg/kg bw in sterile water

3. Test animals:

Species:	Mouse
Strain:	NMRI
Sex:	Males
Source:	[redacted]
Age at study initiation:	8 - 9 weeks
Mean weight at dosing:	35.5 ± 1.8 g (range: 32.4 – 38 g)
Acclimation period:	Minimum 5 days

Diet/Food:	Pelleted standard diet (Harlan laboratories B.V., Horst, The Netherlands), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in Macrolon type II/III cages with wire mesh top and granulated soft wood bedding

4. Environmental conditions:

Temperature:	22 ± 2 °C
Humidity:	45 – 65 %
Air changes:	Not specified
Photoperiod:	12-hour light and dark cycle

5. Test concentrations and treatment groups:

a) Preliminary toxicity study

Dose levels:	2000 mg/kg bw
Concentrations:	Not reported (calculated: 100 mg/mL)
Dose volume:	20 mL/kg bw
Number of animals:	2 males and 2 females
Route of administration:	Oral gavage

b) Main micronucleus test

Dose levels:	2000 mg/kg bw
Concentrations:	Not reported (calculated: 100 mg/mL)
Dose volume:	20 mL/kg bw
Number of animals:	7 males/group
Route of administration:	Oral gavage

B: STUDY DESIGN AND METHODS

1. Dates of experimental work: 04 – 25 Apr 2012
Finalisation date: 28 Sep 2012

2. Animal assignment and treatment:

Preliminary toxicity study:
The test item was dissolved in 1 % carboxymethyl cellulose (CMC) and administered by oral gavage at a constant dosage volume of 20 mL/kg bw to groups of 2 male and 2 female mice at a single dose of 2000 mg/kg bw.
The animals were examined for acute toxic symptoms about 1, 2-4, 6, 24, 30 and 48 hours after administration of the test substance. Since no signs of toxicity were evident at 2000 mg/kg bw, testing of lower doses was not regarded to be required.
Based on the results of the preliminary toxicity study, 2000 mg/kg bw were selected as dose level for the micronucleus test.

Main micronucleus test:
Groups of 7 male mice per dose level were administered a single dose of 2000 mg/kg bw by oral gavage at a constant dosage volume of 20 mL/kg bw. Similar constituted groups of 5 mice/dose level received the vehicle (1 % carboxymethyl cellulose) or the positive control (40 mg/kg bw cyclophosphamide).
Except for the positive control group, all mice were examined for acute toxic symptoms about 1, 2-4, 6, 24 and 48 hours after dosing. The animals were sacrificed 24 and 48 hours after test substance

administration by CO_2 asphyxiation followed by bleeding.

3. Slide preparation:

After sacrifice the femurs of the animals were removed, the epiphysis were cut off and the marrow was flushed out with foetal calf serum. After centrifugation and re-suspension, a small drop of re-suspended cell pellet was spread on a slide. The smear was air-dried, stained with May-Grünwald/Giemsa and mounted with coverslips. At least one slide was made from each bone marrow sample.

4. Slide evaluation:

Slides were coded and evaluated by microscopical analysis at 100 x magnification. For each animal, 2000 polychromatic erythrocytes (PCE) were scored for the presence of micronuclei. In addition, the ratio of PCE to normochromatic erythrocytes (NCE) was determined from the same slide and expressed in PCE per 2000 erythrocytes.

5. Statistics:

The results were evaluated using the non-parametric Mann-Whitney U-test.

6. Acceptance criteria:

The study was considered valid if the following criteria were met:

- At least 5 animals per group were evaluated.
- The PCE to erythrocyte ratio was not less than 20 % of the negative control.
- The positive control showed a statistically significant and biologically relevant increase of micronucleated PCE (mPCE) compared to the negative control.

7. Evaluation criteria:

The test item was considered mutagenic if the following criteria were met:

- It induced either a dose-related or a clear increase in the number of micronucleated polychromatic erythrocytes in a single dose group.
- There was a biological relevance of the result.

A test item that failed to produce a biologically relevant increase in the number of micronucleated polychromatic erythrocytes was considered non-mutagenic in this system.
A test item failing to meet the criteria for a positive or negative response was judged equivocal in this assay and considered for further investigation.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Analytical determinations were not performed in this study, as not compulsory by the test guideline.

B. PRELIMINARY TOXICITY STUDY

There were no signs of systemic toxicity observed. Based on these findings, 2000 mg/kg bw were selected as dose level for the micronucleus test.

C. MAIN MICRONUCLEUS TEST

Systemic toxicity:

Mortality:

No mortality occurred.

Clinical signs of toxicity:

Clinical signs of toxicity were not observed.

Evaluation of bone marrow slides:

Treatment with glyphosate technical did not affect the mean number of polychromatic erythrocytes (PCE) when compared to the mean value of PCE in the solvent control, indicating that the test item did not induce bone marrow toxicity.
In addition, there was no biologically relevant and no statistically significant increase in the frequency of micronucleated PCE of test item-treated animals at any sampling time point when compared to vehicle controls.
Incidences of micronuclei in the solvent and positive control groups were within the range of the laboratory's historical control data, confirming the sensitivity of the test and demonstrating the capability of the test animals to respond to mutagenic substances.

Table 5.4.2-3: Micronucleus Assay in Bone Marrow Cells of the Mouse [redacted], 2012), summary of genotoxicity data

Treatment	Dose (mg/kg bw)	Sampling time	mPCE ± SD /2000 PCE	% mPCE	PCE/2000 erythrocytes mean ± SD
DMSO	-	24 h	3.2 ± 3.6	0.160	1245
	-	48 h	1.4 ± 1.1	0.070	1197
HCD solvent control#					
mean ± SD			0.108 ± 0.039		
range			0 - 9	0.010 - 0.250	
Test item					
	2000	24 h	2.3 ± 0.5	0.114	1247
	2000	48 h	1.1 ± 1.3	0.057	1092
Positive control					
CPA	40	24 h	40.2 ± 18.2	2.010	1149
HCD positive control#					
mean ± SD			2.533 ± 0.632		
range			8 - 139	0.858 - 4.370	

mPCE: micronucleated polychromatic erythrocytes

CPA: cyclophosphamide

HCD#: Historical control data from 2006 - 2011

III. CONCLUSION:

Based on the experimental findings glyphosate technical did not induce micronuclei in bone marrow in mice and is therefore considered negative for clastogenicity/aneuploidy *in vivo*.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Negative for clastogenic/aneugenic effects in the bone marrow of male NMRI mice i*n vivo*.

The study was performed under GLP conditions and in accordance with OECD guideline 474 (1997). There were only minor deviations when compared to the current OECD 474 (2016). The number of polychromatic erythrocytes investigated was 2000, corresponding to the number required by the previous guideline (1997). In addition, the evaluation criteria specified in the study report did not

consider historical control data, but the values obtained in the present study were in line with historical controls. Bone marrow toxicity, indicated by a reduced polychromatic to normochromatic erythrocyte ratio, or signs of systemic toxicity were not evident, but the test was performed at limit dose levels in line with the current guideline. The deviations were considered to not compromise the validity of the study. The study is therefore considered valid and acceptable

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.4.2/003
Report author	[redacted]
Report year	2008
Report title	Evaluation of the mutagenic potential of Glyphosate Technical Micronucleus assay in mice
Report No	[redacted]-3996.402.395.07 (Helm AG)
Document No	Not reported
Guidelines followed in study	OECD 474 (1997)
Deviations from current test guideline OECD 474 (2016)	According to the current guideline OECD 474 (2016), at least 4000 polychromatic erythrocytes per animal should be evaluated for the presence of micronuclei. In the present study, 2000 polychromatic erythrocytes were evaluated (requirement of previous OECD guideline (1997)). In addition, the test item was administered via intraperitoneal injection, which is not representative for a human route of exposure. Historical control data on the mean number of micronucleated polychromatic erythrocytes / 2000 polychromatic erythrocytes were reported, but no standard deviations were given. Acceptance criteria were not reported and evaluation criteria specified in the study report were inconsistent with the historical control data provided.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 3a

Data point	CA 5.4.2/004
Report author	[redacted]
Report year	2010
Report title	First amendment to the report: Evaluation of the mutagenic potential of Glyphosate Technical Micronucleus assay in mice
Report No	[redacted]-3996.402.395.07
Document No	Not reported
Guidelines followed in study	OECD 474 (1997)
Deviations from current test guideline OECD 474 (2016)	According to the current guideline OECD 474 (2016), at least 4000 polychromatic erythrocytes per animal should be evaluated for the presence of micronuclei. In the present study, 2000 polychromatic erythrocytes were

	evaluated (requirement of previous OECD guideline (1997)). In addition, the test item was administered via intraperitoneal injection, which is not representative for a human route of exposure. Historical control data on the mean number of micronucleated polychromatic erythrocytes / 2000 polychromatic erythrocytes were reported, but no standard deviations were given. In addition, evaluation criteria specified in the study report were inconsistent with the historical control data provided.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

Glyphosate technical (batch: 20070606, purity 98.0 %) was tested for its genotoxic potential in a micronucleus test conducted in male and female Swiss mice (main study and amendment).

Based on the results of a preliminary toxicity study, in which mortality was observed at ≥ 500 mg/kg bw/day and bone marrow toxicity was evident at ≥ 250 mg/kg bw, dose levels for the main micronucleus test were selected. 5 mice per sex and dose received on two consecutive days (24 hours apart) doses of 15.62, 31.25, 62.5, 125, 250 and 375 mg/kg bw/day by intraperitoneal injection. Similar constituted groups of mice received the vehicle (corn oil) or the positive control (25 mg/kg bw/day cyclophosphamide). Bone marrow sampling was performed 24 hours after the second dose administration, followed by preparation and staining of smears. For each animal, 2000 polychromatic erythrocytes (PCE) were scored for the presence of micronuclei. At the same time, normochromatic erythrocytes (NCE) with micronuclei were recorded. The ratios of PCE/NCE were determined among the first 2000 PCE.

Intraperitoneal injection of glyphosate technical was associated with clinical signs of toxicity in males and females at ≥ 125 mg/kg bw/day. Common signs of toxicity were bristling and tachypnea, which were observed in both sexes at 125, 250 and 375 mg/kg bw/day throughout the whole study period. After the second dose, lethargy was observed in the animals, which was reversible in the 125 mg/kg bw/day dose group but remained until study termination in the animals of the 250 and 375 mg/kg bw/day dose groups. The animals of the high dose group (375 mg/kg bw/day) further showed loss of motor coordination 30 minutes after the second dose, which was reversible up to 4 hours post dosing. These signs of systemic toxicity were clearly attributed to treatment with glyphosate technical and considered to be of toxicological relevance.

A statistically significant increase in the ratio of PCE/NCE when compared to solvent control animals was observed at ≥ 250 mg/kg bw/day in males and at 375 mg/kg bw/day in females. The observation indicated that strong bone marrow toxicity was evident in the animals.
There was no statistically significant increase in the incidence of micronucleated PCE (mPCE) when compared to solvent control animals, up to the highest dose tested.

The frequency of mPCE of solvent and positive control animals were within the range of the laboratories historical control data, demonstrating the validity and the sensitivity of the test.

Based on the experimental findings and under the conditions of the test, glyphosate technical did not induce micronuclei in bone marrow in male and female mice and was therefore considered negative for clastogenicity/aneugenicity *in vivo*.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material: Glyphosate Technical

Identification:	AGR-0790/07
Description:	Not specified
Lot/Batch #:	20070606
Purity:	98.0 %
Stability of test compound:	As given in the study report, the substance was described[15] to not photochemically degrade in buffered water and to be stable in air. It was further stated to be stable to hydrolysis at pH 3, 6 and 9 at 5 – 35 °C.
Solvent (vehicle) used:	Corn oil

2. Control materials

Solvent (vehicle) control:	Corn oil
Positive control:	Cyclophosphamide monohydrate, 25 mg/kg bw/day in physiological solution

3. Test animals:

Species:	Mouse
Strain:	Swiss albino
Sex:	Male and female
Source:	[redacted]
Age at study initiation:	7 - 12 weeks
Mean weight at dosing:	Approximately 30 g
Acclimation period:	5 days
Diet/Food:	Purina Labina (Purina do Brasil Ltda.), *ad libitum*
Water:	*ad libitum*
Housing:	5/sex in polypropylene cages closed with a metallic grid and padded out with sterile sawdust bedding.

4. Environmental conditions:

Temperature:	20 - 24 °C
Humidity:	50 - 70 %
Air changes:	10 – 15/h
Photoperiod:	12-hour light and dark cycle

[15] Tomlin, C.D.S. (2006-2007): The e-Pesticide Manual (14th Edition) Version 4.0; Software engineered by P.J. Mann – Web Design & Consultancy. BCPC (British Crop Protection Council) ISBN 1 901396 42 8

5. Test concentrations and treatment groups:

a) Preliminary toxicity study

Dose levels:	62.5, 125, 250, 500 and 1000 mg/kg bw/day
Concentrations:	Not specified
Dose volume:	Not specified
Number of animals:	3/sex/group
Route of administration:	Intraperitoneal injection

b) Main micronucleus test

Dose levels:	15.62, 31.25, 62.5, 125*, 250* and 375* mg/kg bw/day [16] *
Concentrations:	1.04, 2.08, 4.16, 8.33*, 16.66*, 25.00* mg/mL
Dose volume:	15 mL/kg bw
Number of animals:	5/sex/group
Route of administration:	Intraperitoneal injection

B: STUDY DESIGN AND METHODS

1. Dates of experimental work: 29 May – 13 Aug 2008
Finalisation date: 29 Sep 2008 (study report) and 31 Aug 2010 (first amendment to study report)

2. Animal assignment and treatment:

Preliminary toxicity study:
The test item was dissolved in corn oil and administered via intraperitoneal injection to groups of six mice per dose level (3/sex/group). The animals received two injections 24 hours apart at doses of 62.5, 125, 500 and 1000 mg/kg bw/day. Following administration, the animals were observed for signs of mortality. Surviving animals were sacrificed 24 hours following the second dose administration.

Bone marrow toxicity was assessed in surviving animals by determining the ratio of polychromatic (PCE) to normochromatic erythrocytes (NCE) among 200 PCE per animal.
Based on the results of the preliminary toxicity study, the dose levels for the main micronucleus test were chosen. In the original study report, 62.5 mg/kg bw/day was selected as highest dose level. In the first amendment to the study report, 3 higher dose levels were tested with a maximum dose level of 375 mg/kg bw/day.

Main micronucleus test:
Groups of 5 mice/sex received two separate intraperitoneal injections of 15.62, 31.25, 62.5, 125*, 250* and 375* mg/kg bw/day 24 hours apart[16]. Similar constituted groups received the vehicle (corn oil) or positive control (25 mg/kg bw/day cyclophosphamide in physiological solution).
About 24 hours after the second injection, the animals were sacrificed by CO_2 asphyxiation and bone marrow slides were prepared from each animal.

3. Slide preparation:

After sacrifice, the femurs of the animals were excised, dissected and the marrow was flushed out with fetal calf serum. After centrifugation and re-suspension in fetal calf serum, a drop of re-suspended cells was smeared on microscope slides. Two slides were prepared for each animal. The smears were air-dried, fixed in 70 % ethanol for 10 minutes, stained with Wright's concentrated solution for 3 minutes, placed in Wright's phosphate buffer solution and rinsed. Thereafter, the slides were immersed for 10 minutes in Giemsa buffer – deionised water solution, washed in streamed water, dried and assembled in Permount.

* tested in amendment to study report.

4. Slide evaluation:

Slides were coded and evaluated by microscopical analysis at 1000 x magnification. For each animal, 2000 polychromatic erythrocytes (PCE) were scored for the presence of micronuclei. At the same time, normochromatic erythrocytes (NCE) were screened for micronuclei. The ratios of PCE/NCE were determined among the first 2000 PCE.

5. Statistics:

Differences in the incidence of micronucleated polychromatic (PCE) and normochromatic erythrocytes (NCE) per 2000 cells and the relation of PCE/NCE were compared using the Mann-Whitney U-test and the K'test for independent variable in accordance with Kruskal & Wallis Test.

6. Acceptance criteria:

Acceptance criteria were not specified in the study report.

7. Evaluation criteria:

The test item was considered positive for induction of micronuclei if the following criteria were met:

- There was a statistically significant, dose-related increase in the number of micronucleated polychromatic erythrocytes when compared to the solvent control.
- There was a reproducible and statistically significant positive response for at least one of the dose levels tested.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Analytical determinations were not performed. The substance was described to not photochemically degrade in buffered water and to be stable in air. It was further stated to be stable to hydrolysis at pH 3, 6 and 9 at 5 – 35 °C.

B. PRELIMINARY TOXICITY STUDY

Mortality was observed at 500 mg/kg bw/day in 1/3 males and 2/3 females and at 1000 mg/kg bw/day in 3/3 males and 3/3 females.

Bone marrow cytotoxicity, evident as a ratio of polychromatic (PCE) to normochromatic erythrocytes (NCE) lower than 20 % when compared to solvent control animals, was observed at 250 and 500 mg/kg bw/day.

Based on the results of the preliminary toxicity study, the dose levels for the main micronucleus assay were selected. In the original study report, 62.5 mg/kg bw/day was chosen as highest dose level. In the first amendment to the study report, 3 higher dose levels were included with a maximum dose level of 375 mg/kg bw/day.

C. MAIN MICRONUCLEUS TEST

Systemic toxicity:

Mortality:

No mortality occurred.

Clinical signs of toxicity:

Clinical signs of toxicity were observed at 125, 250 and 375 mg/kg bw/day.

At 125 mg/kg bw/day, bristling was observed in 5/5 males and 5/5 females and tachypnea in 1/5 males and 1/5 females starting 30 minutes after the first dose. After the second dose, all males and females of this dose group showed bristling, tachypnea and lethargy starting 30 minutes post administration. Bristling and tachypnea were observed in all animals until study termination.

At 250 mg/kg bw/day, bristling and tachypnea was observed in 5/5 males and 5/5 females after the first and second dose until study termination. About 30 minutes after the second dose, all animals showed lethargy, which was still present in 1/5 males 4 hours after dosing and remained in 2/5 females until study termination.

At 375 mg/kg bw/day, 5/5 males and 5/5 females showed bristling and tachypnea immediately following test item administration (first and second dosing), which was observed in all animals until bone marrow sampling. 24 hours after the first dose, 5/5 males and 2/5 females showed agitation. After the second dose, 5/5 males and 5/5 females showed lethargy and loss of motor coordination within the first 30 minutes post dosing. The animals recovered from the loss of motor coordination within 4 hours post dosing, while lethargy was observed in 4/5 males and 3/5 females until study termination.

The signs of systemic toxicity were attributed to treatment and considered to be of toxicological relevance.

Evaluation of bone marrow slides:
Treatment with glyphosate technical at concentrations of 250 mg/kg bw/day and above caused a statistically significant change in the PCE/NCE ratio, indicating that bone marrow toxicity was evident at these concentrations. Bone marrow toxicity was observed in males at ≥ 250 mg/kg bw/day and in females at 375 mg/kg bw/day.

There was no statistically significant increase in the incidence of mPCE when compared to solvent control animals up to the highest dose tested.

The frequency of mPCE of solvent and positive control animals were within the range of the laboratories historical control data, demonstrating the validity and the sensitivity of the test.

Table 5.4.2-4: Evaluation of the mutagenic potential of Glyphosate Technical Micronucleus assay in mice ([redacted], 2008), summary of genotoxicity data for males and females

Treatment	Dose (mg/kg bw/day)	Sampling time	Males		Females	
			mPCE/2000 PCE mean ± SD	PCE/(PCE + NCE) mean	mPCE/2000 PCE mean ± SD	PCE/(PCE + NCE) mean
Corn oil	15 mL/kg bw[a]	24 h	0.0 ± 0.0	1.781	0.0 ± 0.0	1.772
	15 mL/kg bw[b]	24 h	0.4 ± 0.89	0.5147	0.4 ± 0.55	0.523
	HCD[#] mean	24 h	0.87	1.43 ± 0.72[$]	0.76	1.54 ± 0.68[$]
	HCD[##] mean	24 h	0.51	0.54 ± 0.08[$]	0.41	0.60 ± 0.36[$]
Test item	15.62[(a)]	24 h	0.0 ± 0.0	1.768	0.0 ± 0.0	1.791
	31.25[(a)]	24 h	0.2 ± 0.45	1.744	0.0 ± 0.0	1.761
	62.5[(a)]	24 h	0.6 ± 1.34	1.711	0.0 ± 0.0	1.787
	125[(b)]	24 h	0.2 ± 0.45	0.527	0.0 ± 0.0	0.528
	250[(b)]	24 h	0.0 ± 0.0	0.534*	0.0 ± 0.0	0.528
	375[(b)]	24 h	0.2 ± 0.45	0.556**	0.0 ± 0.0	0.555*
CPA	25[(a)]	24 h	23.0 ± 8.94**	1.549	12.2 ± 7.92**	1.728
	25[(b)]	24 h	8.0 ± 2.12**	0.535*	6.4 ± 1.67**	0.539*
	HCD[#] mean	24 h	9.30	1.31 ± 0.69[$]	9.20	1.35 ± 0.65[$]
	HCD[##] mean	24 h	10.02	0.59 ± 0.34[$]	9.45	0.54 ± 0.06[$]

Table 5.4.2-4: Evaluation of the mutagenic potential of Glyphosate Technical Micronucleus assay in mice [redacted], 2008), summary of genotoxicity data for males and females

Treatment	Dose (mg/kg bw/day)	Sampling time	Males		Females	
			mPCE/2000 PCE mean ± SD	PCE/(PCE + NCE) mean	mPCE/2000 PCE mean ± SD	PCE/(PCE + NCE) mean

mPCE: micronucleated polychromatic erythrocytes

CPA: cyclophosphamide, positive control

$: ± average amplitude

[a]: data from study report (2008), standard deviations were not included in study report but calculated from raw data

[b]: data from amendment to study report (2010)

HCD[#]: historical control data from the testing laboratory generated in Jan - Dec 2007

HCD[##]: historical control data from the testing laboratory generated in Jan - Dec 2009

* $p \leq 0.05$ and ** $p \leq 0.01$ difference statistically significant from solvent control by Mann-Whitney (Kruskal Wallis) test

Table 5.4.2-5: Evaluation of the mutagenic potential of Glyphosate Technical Micronucleus assay in mice ([redacted], 2008), summary of genotoxicity data for males and females combined

Treatment	Dose (mg/kg bw/day)	Sampling time	Both sexes combined	
			mPCE/2000 PCE mean ± SD	PCE/(PCE + NCE) mean
Corn oil	15 mL/kg bw[(a)]	24 h	0.0 ± 0.0	1.777
	15 mL/kg bw[(b)]	24 h	0.4 ± 0.70	0.519
Test item	15.62[(a)]	24 h	0.0 ± 0.0	1.780
	31.25[(a)]	24 h	0.1 ± 0.32	1.752
	62.5[(a)]	24 h	0.3 ± 0.95	1.749
	125[(b)]	24 h	0.1 ± 0.32	0.527
	250[(b)]	24 h	0.0 ± 0.0	0.531*
	375[(b)]	24 h	0.1 ± 0.32	0.556**
CPA	25[(a)]	24 h	17.6 ± 9.79**	1.639
	25[(b)]	24 h	7.2 ± 1.99**	0.537**

mPCE: micronucleated polychromatic erythrocytes

CPA: cyclophosphamide, positive control

$: ± average amplitude

[a]: data from study report (2008), standard deviations were not included in study report but calculated from raw data

[b]: data from amendment to study report (2010)

HCD[#]: historical control data from the testing laboratory generated in Jan - Dec 2007

HCD[##]: historical control data from the testing laboratory generated in Jan - Dec 2009

* $p \leq 0.05$ and ** $p \leq 0.01$ difference statistically significant from solvent control by Mann-Whitney (Kruskal Wallis) test

III. CONCLUSION

Based on the experimental findings and under the conditions of the test, glyphosate technical did not induce micronuclei in bone marrow in male and female mice *in vivo*.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Negative for clastogenic/aneugenic effects in the bone marrow of male and female Swiss albino mice i*n vivo*.

The study was performed under GLP conditions and according to OECD guideline 474 (1997). There were only minor deviations when compared to the current OECD guideline 474 (2016). The number of polychromatic erythrocytes investigated per animal was 2000 (instead of 4000), which was the number recommended by the previous OECD guideline (1997). In addition, the test item was administered via intraperitoneal injection, which does not represent a typical human route of exposure. There was clear evidence of systemic toxicity and bone marrow toxicity in animals of both sexes, but there was no evidence for induction of micronuclei at any dose level.

The study is considered to provide supporting information, because two different batches of the test material were used in the original study and in the amendment to the study. In addition, the treatment of the animals used in the amendment was not described and the amendment does not replace a full study report. However, it can be assumed that the treatment was the same as in the original study.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.4.2/005
Report author	[redacted]
Report year	2008
Report title	Glyphosate Technical – Micronucleus Assay in Bone Marrow Cells of the Mouse
Report No	1158500
Document No	Not reported
Guidelines followed in study	OECD 474 (1997), EPA OPPTS 870.5395 (1998), 2000/32/EEC B.12 (2000)
Deviations from current test guideline OECD 474 (2016)	Only 2000 polychromatic erythrocytes were evaluated per animal instead of 4000 polychromatic erythrocytes as recommended by OECD guideline 474 (2016). Bone marrow exposure, indicated by a reduced polychromatic to normochromatic erythrocyte ratio, was not confirmed and there was no systemic toxicity observed, but dose levels included limit concentrations specified in the current guideline. Acceptance criteria were not specified in the study report. In addition, historical controls were not considered in the evaluation criteria.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Glyphosate technical (batch: 20070545, purity: 99.1 %) was tested for its genotoxic potential in mice using the micronucleus test. Based on the results of a preliminary toxicity study, groups of six mice each received a single oral dose of 500, 1000 and 2000 mg/kg bw glyphosate technical. The test item was dissolved in 0.5 % (w/w) carboxymethyl cellulose and administered at a constant dosage volume of 20 mL/kg bw. Similar constituted groups of control animals received the vehicle (0.5 % carboxymethyl cellulose) or the positive control (40 mg/kg bw cyclophosphamide in deionised water). All animals were observed for clinical signs of toxicity at regular intervals. 24 and 48 hours after administration, the animals were sacrificed and bone marrow smears were prepared. For the incidence of micronucleated cells, 2000 polychromatic erythrocytes (PCE) per animal were scored. To describe a cytotoxic effect, the ratio between PCE and normochromatic erythrocytes (NCE) was determined and expressed in PCE per 2000 erythrocytes.

Oral administration of glyphosate technical did not induce any clinical signs of toxicity or mortality in the animals. In addition, there was no statistically significant decrease in the ratio of PCE to the total amount of erythrocytes in any dose group or at any preparation interval, indicating that glyphosate technical did not induce cytotoxicity in the bone marrow.

A biologically relevant or statistically significant increase in the frequency of micronucleated polychromatic erythrocytes was not observed for the test item at any preparation interval and any dose level.

The positive control cyclophosphamide showed a marked increase in the frequency of induced micronuclei, demonstrating the sensitivity of the test system.
Based on the results of the test and under the experimental conditions chosen, the test item was considered to be negative for clastogenicity/aneugenicity in mice *in vivo*.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material: Glyphosate Technical

Identification:	S846011
Description:	White solid
Lot/Batch #:	20070545
Purity:	99.1 % (w/w)
Stability of test compound:	The stability of the test item at storage conditions (< 10° C) was guaranteed until the expiry date Oct 2009. The stability of the test item in solvent was not specified.
Solvent (vehicle) used:	0.5 % carboxymethyl cellulose (CMC)

2. Control materials

Solvent (vehicle) control:	0.5 % carboxymethyl cellulose (CMC), 20 mL/kg bw
Positive control:	Cyclophosphamide, 40 mg/kg bw in deionised water, 10 mL/kg bw

3. Test animals:

Species:	Mouse

Strain:	NMRI
Sex:	Males
Source:	[redacted]
Age at study initiation:	7 - 8 weeks
Mean weight at dosing:	39.0 ± 2.6 g
Acclimation period:	Minimum 5 days
Diet/Food:	Pelleted standard diet (Harlan Winkelmann GmbH, Borchen, Germany), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually, in Makrolon type I cages with wire mesh top

4. Environmental conditions:

Temperature:	22 ± 3 °C
Humidity:	30 - 70 %
Photoperiod:	12-hour light and dark cycle

5. Test concentrations and treatment groups:

a) Preliminary toxicity study

Dose levels:	2000 mg/kg bw
Dose volume:	20 mL/kg bw
Number of animals:	2 males and 2 females
Route of administration:	Oral gavage

b) Main micronucleus test

Dose levels:	500, 1000 and 2000 mg/kg bw
Dose volume:	20 mL/kg bw
Number of animals:	500 and 1000 mg/kg bw: 6 males 2000 mg/kg bw: 12 males
Route of administration:	Oral gavage

B: STUDY DESIGN AND METHODS

1. **Dates of experimental work:** 25 Feb – 13 Mar 2008
   Finalisation date: 09 Jun 2008

2. **Animal assignment and treatment:**

Preliminary toxicity study:
A preliminary study on acute oral toxicity was performed in male and female mice. Two animals per sex received a single dose of 2000 mg/kg bw by oral gavage. Formulations were applied at a constant dosage volume of 20 mL/kg bw. The animals were examined for acute toxic symptoms at intervals of around 1 hour, 2-4 hours, 6 hours, 24, 30 and 48 hours after administration of the test item.

Main micronucleus test:
Based on the results of the preliminary toxicity study, groups of six mice each received a single oral dose of 500, 1000 and 2000 mg/kg bw glyphosate technical, which was administered at a constant dosage volume of 20 mL/kg bw. Similar constituted groups of control animals received the vehicle (0.5 % carboxymethyl cellulose) or the positive control (40 mg/kg bw cyclophosphamide in deionised

water). All animals were observed for acute toxic symptoms at intervals of around 1 hour, 2-4 hours, 6 hours (except for high dose group animals), 24 and 48 hours after test item administration.

Bone marrow sampling was performed 24 and 48 hours after treatment; the latter sampling time point was performed for the control and high dose group only. The animals were sacrificed by CO_2 asphyxiation followed by bleeding.

1. Slide preparation:

After sacrifice the femurs of the animals were removed, dissected and the marrow was flushed out with foetal calf serum. After centrifugation and re-suspension, a small drop of re-suspended cell pellet was spread on a glass slide. The smear was air-dried, stained with May-Grünwald/Giemsa and mounted with coverslips. At least one slide was made from each bone marrow sample.

2. Slide evaluation:

Slides were coded and examined using a NIKON microscope with 100x oil immersion objectives. For the incidence of micronucleated cells, 2000 polychromatic erythrocytes (PCE) per animal were scored. To evaluate a potential cytotoxic effect, the ratio between PCE and normochromatic erythrocytes (NCE) was determined and expressed in PCE per 2000 erythrocytes.

The presence of micronuclei was scored for 5 males per group. The remaining 6th animal in the respective test group was kept as backup in case of spontaneous death.

3. Statistics:

All data were statistically analysed using the nonparametric Mann-Whitney U-test.

4. Acceptance criteria:

The test was considered valid when the following criteria were met:

- The negative controls were in the range of the laboratory's historical control data.
- The positive controls were in the range of the laboratory's historical control data.
- At least 4 animals per group were evaluated.
- The PCE to erythrocyte ratio was not less than 20 % to those of the negative control value.

5. Evaluation criteria:

The test substance was considered to be mutagenic if it induced either a statistically significant, dose-related increase or a clear statistically significant increase in the number of micronucleated PCE in a single dose group.

A test item was considered to be non-clastogenic if it failed to produce a biologically relevant increase in the number of micronucleated PCE.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Analytical determinations have not been performed in this study, as not compulsory by the test guideline.

B. PRELIMINARY TOXICITY STUDY

Animals treated at the limit dose of 2000 mg/kg bw did not express any toxic reactions. Based on the results of the preliminary toxicity study, 2000 mg/kg bw was selected as high dose level for the main micronucleus test. Since gender-specific differences in the sensitivity towards glyphosate technical were not observed, the main experiment was performed using only males.

C. MAIN MICRONUCLEUS TEST

Systemic toxicity:

Mortality:
No mortality occurred.

Clinical signs of toxicity:
There were no clinical signs of toxicity observed.

Evaluation of bone marrow slides:
There was no decrease in the mean number of polychromatic erythrocytes among total erythrocytes in test item treated groups when compared to vehicle controls, indicating that glyphosate technical did not induce cytotoxicity in the bone marrow.

In addition, there was no biologically relevant or statistically significant increase in the frequency of micronucleated polychromatic erythrocytes at any preparation interval and any dose level.

A dose of the positive substance cyclophosphamide showed a marked increase in the frequency of induced micronuclei, demonstrating the capability of the test animals to respond to mutagens.

Table 5.4.2-6: Micronucleus Assay in Bone Marrow Cells of the Mouse ([redacted], 2006) summary of genotoxicity data

Treatment	Dose (mg/kg bw)	Sampling time	mPCE ± SD /2000 PCE	% cells with micronuclei	PCE/2000 erythrocytes (mean ± SD)
Saline	20 mL/kg	24 h	1.40 ± 1.34	0.07	1202 ± 144
	20 mL/kg	48 h	1.40 ± 1.14	0.07	1153 ± 40
HCD vehicle[#] mean ± SD			0.093 ± 0.040		
range			0.01 - 0.20		
Test item	500	24 h	1.60 ± 0.55	0.08	1147 ± 70
	1000	24 h	1.60 ± 0.55	0.08	1162 ± 186
	2000	24 h	1.40 ± 0.89	0.07	1173 ± 23
	2000	48 h	1.60 ± 1.14	0.08	1188 ± 78
CPA	40	24 h	63.0 ± 17.78	3.15	1030 ± 76
HCD positive control[#] mean ± SD			2.202 ± 0.705		
range			0.70 - 4.52		

mPCE: micronucleated polychromatic erythrocytes

CPA: cyclophosphamide, positive control

[#] HCD Historical control data from the laboratory generated in 2002 - 2007 (vehicle control: 293 experiments in males, 275 experiments in females; positive control: 292 experiments in males, 274 experiments in females)

III. CONCLUSION

Based on the results of the present study, glyphosate technical did not induce micronuclei in bone marrow of male mice *in vivo*. Thus, the test item was considered to be negative for clastogenicity/aneugenicity under the conditions of the test.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Negative for clastogenic/aneugenic effects in the bone marrow of male mice *in vivo*.
The study was performed under GLP conditions and in accordance with OECD guideline 474 (1997). There were only minor deviations when compared to the current OECD 474 (2016), which were considered to not compromise the validity of the study. The number of polychromatic erythrocytes investigated was 2000, corresponding to the number required by the previous guideline (1997). Bone marrow toxicity, indicated by a reduced polychromatic to normochromatic erythrocyte ratio, or systemic toxicity were not observed. However, the test was performed at limit dose levels in line with the current guideline, therefore the deviation is considered negligible. The study is considered valid and acceptable.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.4.2/006
Report author	[redacted]
Report year	2007
Report title	Mammalian Erythrocyte Micronucleus Test for GLIFOSATO TÈCHNICO HELM
Report No	RL33393/2007-3.0MN-B
Document No	Not reported
Guidelines followed in study	OECD 474 (1997), Commission Directive 2000/32/EC B.12
GLP	Yes
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	Glyphosate technical (batch: 2007091801, purity: 98.01 %) was assessed for genotoxic effects in a micronucleus test in male Swiss mice *in vivo*. The dose levels for the micronucleus assay were selected based on the results of a preliminary toxicity study, in which 30 mg/kg bw/day turned out to be the maximum tolerated dose. Groups of six mice received on two consecutive days doses of 8.0, 15.0 and 30.0 mg/kg bw/day by oral gavage. Concurrent control animals received the vehicle (deionized water) or 75 mg/kg bw/day of the well-known mutagen cyclophosphamide (positive control). Bone marrow sampling was performed 24 hours after administration of the second dose. Smears were prepared from the femoral bone marrow of each animal and 3000 polychromatic erythrocytes (PCE) were scored for the presence of micronuclei. In addition, the ratio of PCE to normochromatic erythrocytes (NCE) was determined for each animal by counting a total of 2000 erythrocytes.
Short description of results:	Oral treatment with glyphosate technical did not induce any mortality in the animals and signs of systemic toxicity were as well not reported. In addition, the ratio of PCE to NCE was not affected upon treatment with the test item up to the highest dose level. When compared to solvent control animals, treatment with

	glyphosate doses of 8.0 and 15.0 mg/kg bw/day did not lead to a statistically significant increase the frequency of micronucleated PCE. At 30 mg/kg bw/day, a statistically significant increase in micronucleated PCE was observed, but the values remained within the range of historical control data and were therefore considered to be without biological relevance. Micronuclei formation in the solvent controls was consistent to the historical control data. The positive control cyclophosphamide showed the expected result and demonstrated the functionality of the test system. Under the conditions of the test, glyphosate technical was considered negative for clastogenic/aneugenic activity in male mice *in vivo*.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	The study is considered to be not acceptable since the dose levels were far too low for any meaningful conclusion with regard to micronucleus formation. In the original report, some justification for dose selection is given, based on a range-finding test suggesting effects at even lower dose levels. These findings were obviously in contradiction to more reliable acute toxicity tests with glyphosate in the mouse. In other micronucleus assays described in this section, much higher dose levels were assessed.
Reasons why the study report is not available for submission	Not applicable
Category study in AIR5 dossier (L-docs)	Category 3b

1. Information on the study

Data point	CA 5.4.2/007
Report author	[redacted]
Report year	2006
Report title	Glyphosate Technical: Micronucleus Test In The Mouse
Report No	2060/014
Document No	Not reported
Guidelines followed in study	OECD 474 (1997); Commission Directive 2000/32/EC B.12 (2000), USA EPA, JMAFF
Deviations from current test guideline OECD 474 (2016)	According to the current guideline OECD 474 (2016), at least 4000 polychromatic erythrocytes per animal should be evaluated for the presence of micronuclei. In the present study 2000 polychromatic erythrocytes were evaluated (requirement of previous OECD guideline (1997)). In addition, the animals were treated by intraperitoneal injection, which does not represent a route relevant for human exposure. Data on historical controls obtained in the testing laboratory were not provided. Acceptance criteria were not mentioned and evaluation criteria specified in the study report were inconsistent with those specified in the guideline.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Glyphosate technical (batch: H05H016A, purity: 95.7 %) was tested for its genotoxic potential in mice using the micronucleus test. Prior to the micronucleus test, a preliminary toxicity study was performed. Due to severe clinical signs of toxicity and mortality at 800 and 1000 mg/kg bw, a maximum tolerated dose of 600 mg/kg bw was established and selected for the main study. No marked difference in toxicity was observed between male and female mice.

Based on these findings, the main micronucleus test was performed in male mice with dose levels of 150, 300 and 600 mg/kg bw. Seven mice per group received a single intraperitoneal injection at a constant dosage volume of 10 mL/kg bw. Similarly constituted groups received the vehicle (phosphate buffered saline, 7 mice/group) or the positive control (cyclophosphamide, 5 mice/group). Animals were sacrificed 24 (all treatment groups, vehicle and positive control animals) and 48 hours (vehicle control and high dose group) followed by preparation of bone marrow smears from the femoral bone marrow from each animal. After staining of the preparations, 2000 erythrocytes per animal were scored for the presence of micronucleated polychromatic and normochromatic cells. In addition, 1000 erythrocytes were counted to determine the percentage of polychromatic to normochromatic erythrocytes.

Intraperitoneal injection of the test item induced clinical signs of toxicity at ≥ 150 mg/kg bw in all animals of the 24 and 48 hour sacrifice. The observations comprised hunched posture, ptosis, ataxia and lethargy.

A statistically significant decrease in the ratio of polychromatic cells per 1000 erythrocytes was observed compared to the vehicle control in the 24-hour 600 mg/kg bw group, as well as a statistically non-significant decrease in the 48-hour 600 mg/kg bw group. This observation, as well as the occurrence of clinical signs demonstrated systemic absorption and confirmed exposure of the bone marrow.

There was a small but statistically significant increase in the incidence of micronucleated polychromatic erythrocytes in animals dosed with 600 mg/kg bw in the 24-hour sampling group when compared to the concurrent vehicle control group. However, the increase was very modest, within the range of the laboratory's historical control data and did not include any individual value that would not be acceptable for vehicle control animals. The observed response was attributed to a haematopoietic effect induced by the cytotoxic effect of the test material on the bone marrow rather than to any specific genotoxic effect and was therefore not considered to be of toxicological relevance. No statistically significant increase in the incidence of micronucleated polychromatic erythrocytes was observed after 48 hours in animals dosed at 600 mg/kg bw.

The positive control group showed a marked increase in the incidence of micronucleated PCE hence demonstrating capability of the test animals to show mutagenic effects in response to known mutagenic agents and confirming the sensitivity of the test.

Based on the results of the test and under the experimental conditions chosen, the test item was considered to be negative for clastogenicity/ aneugenicity in mice *in vivo*.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material:

Identification:	Glyphosate Technical
Description:	White crystalline solid
Lot/Batch #:	H05H016A
Purity:	95.7 %
Stability of test compound:	At room temperature stable until March 2008

Solvent (vehicle) used: Phosphate buffered saline (PBS)

2. Control materials

Solvent (vehicle) control: Phosphate buffered saline (PBS)
Positive control: Cyclophosphamide, 50 mg/kg bw in PBS

3. Test animals:

Species: Mouse
Strain: CD-1
Sex: Males
Source: [redacted]
Age at study initiation: Approx. 5 - 8 weeks
Weight at dosing: 21 - 29 g
Acclimation period: At least 7 days
Diet/Food: Certified Rat and Mouse Diet Code 5LF2, BCM (IPS Ldt., London UK), *ad libitum*
Water: Tap water, *ad libitum*
Housing: In groups up to seven in solid-floor polypropylene cages with wood flake bedding.

4. Environmental conditions:

Temperature: 19 - 25°C
Humidity: 30 - 70 %
Air changes: Approximately 15/hour
Photoperiod: 12-hour light and dark cycle

5. Test concentrations and treatment groups:

a) Preliminary toxicity study

Dose levels: 600, 800 and 1000 mg/kg bw
Concentrations: 60, 80 and 100 mg/mL
Dose volume: 10 mL/kg bw
Number of animals: 600 and 800 mg/kg bw: 1 male and 1 female
1000 mg/kg bw: 2 males and 2 females
Route of administration: Intraperitoneal injection

b) Main micronucleus test

Dose levels: 150, 300 and 600 mg/kg bw
Concentrations: 15, 30 and 60 mg/mL
Dose volume: 10
Number of animals: 7 males/group
Route of administration: Intraperitoneal injection

B: STUDY DESIGN AND METHODS

1. Dates of experimental work: 07 Jun – 20 Jul 2005

Finalisation date: 13 Feb 2006

2. Animal assignment and treatment:

Preliminary toxicity study:
To examine the toxicity caused by glyphosate technical, single doses of 600, 800 and 1000 mg/kg bw were administered by intraperitoneal injection. One male and one female were used for the low and mid dose group whereas 2 males and females were used for the high dose group. Formulations were applied at a constant dosage volume of 10 mL/kg bw. The animals were observed for one hour following dosing and subsequently once daily for two consecutive days. Clinical signs of toxicity and mortality were recorded.

Main micronucleus test:
Groups of seven mice each received an intraperitoneal injection of 150, 300 and 600 mg/kg bw glyphosate technical, which was administered at a constant dosage volume of 10 mL/kg bw. Similar constituted groups of control animals received the vehicle (PBS, 7 mice) or the positive control (50 mg/kg bw cyclophosphamide in PBS, 5 mice/group). All animals were observed for clinical signs of toxicity and mortality one hour post dosing and once daily thereafter and immediately prior to termination.

About 24 hours post-injection, the animals were sacrificed by cervical dislocation. For the high dose (600 mg/kg bw) as well as for the vehicle control group, a second group of 7 mice was sacrificed 48 hours post-injection.

3. Slide preparation:
Immediately following termination both femurs were dissected from each animal, aspirated with foetal calf serum and bone marrow smears prepared following centrifugation and re-suspension. The smears were air-dried, fixed in absolute methanol, stained in May-Grünwald/Giemsa, allowed to air-dry and coverslipped using mounting medium.

4. Slide evaluation:
Slides were randomly coded and examined under a light microscope at 1000x magnification. For the incidence of micronucleated cells, 2000 polychromatic erythrocytes (PCE) per animal were scored. In addition, the number of normochromatic erythrocytes (NCE) associated with 1000 erythrocytes was counted and also scored for incidences of micronuclei. After decoding the slide number, the percentage of PCE per 1000 erythrocytes was calculated.

5. Statistics:
The number of micronucleated PCE occurring in each test item treated group was compared to the number occurring in the corresponding solvent control. All data were statistically analysed following a √(x 1) transformation using Student's t-test (two tailed) and any significant results were confirmed using the one-way analysis of variance.

6. Acceptance criteria:
Acceptance criteria were not specified in the study report.

7. Evaluation criteria:
The test substance was judged positive for genotoxicity in vivo if the there was a dose-related, toxicologically relevant increase in the number of micronucleated PCE observed for either the 24 or 48 hour sacrifice time point when compared to solvent controls.
If these criteria were not fulfilled, then the test material was considered to be non-genotoxic under the conditions of the test.

A positive response for bone marrow toxicity was demonstrated when the dose group mean percentage

of PCE per 1000 erythrocytes was shown to be statistically significantly lower than the concurrent vehicle control group.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Analytical determinations were not performed, as not compulsory by the test guideline.

B. PRELIMINARY TOXICITY STUDY

Severe clinical signs of toxicity were observed at 800 mg/kg bw and above. The animals showed hunched posture, lethargy, ataxia, ptosis, piloerection, tip toe gait, distended abdomen and hypothermia. At 600 mg/kg bw, clinical signs comprised hunched posture, ptosis, piloerection and ataxia of moderate severity. 2/2 and 2/4 animals at 800 and 1000 mg/kg bw, respectively, were killed in extremis due to the severity of clinical signs.

The test material showed no marked difference in its toxicity to male or female mice, it was therefore considered to be acceptable to use males only for the main test.

Based on the results of the preliminary toxicity study, 600 mg/kg bw was identified as maximum tolerated dose and chosen as highest dose level for the main micronucleus test.

C. MAIN MICRONUCLEUS TEST

Systemic toxicity:

Mortality:

No mortality occurred.

Clinical signs of toxicity:

Clinical signs of toxicity were observed at 150 mg/kg bw and above in all animals. The observations comprised hunched posture, ptosis, ataxia and lethargy.

Evaluation of bone marrow slides:

A statistically significant decrease in the ratio of PCE (immature) to total erythrocytes compared to the vehicle control was observed in the 24-hour 600 mg/kg bw group, as well as a statistically non-significant decrease in the 48-hour 600 mg/kg bw group. The decreased ratio of PCE accompanied by the presence of clinical signs indicated systemic absorption and confirmed exposure of the bone marrow. Compared to solvent controls, a small but statistically significant increase in the incidence of micronucleated PCE was evident in the 24 hour 600 mg/kg bw group, but the values remained within the range of the laboratory's historical control data. The response was attributed to a haematopoetic effect due to bone marrow toxicity rather than to a specific genotoxic effect. The authors suggested that the increased erythropoiesis caused by test material toxicity might cause some cells to cycle more quickly than in the vehicle control animals and, therefore, there may also be less opportunity to repair spontaneously-occurring DNA damage before the final mitosis and enucleation, resulting in small increases in micronucleated cells. Therefore, the response was considered to have no toxicological significance. No statistically significant increase in the incidence of micronucleated PCE was observed in the remaining dose groups.

The positive control group showed a marked increase in the incidence of micronucleated PCE hence demonstrating validity and sensitivity of the conducted test to identify mutagenic effects in response to known mutagenic agents.

Table 5.4.2-7: Mammalian Erythrocyte Micronucleus Test [redacted], 2006), summary of genotoxicity data in mice

Treatment	Dose (mg/kg bw)	Sampling time	mPCE ± SD/2000 PCE	%PCE with micronuclei	%PCE/1000 Erythrocytes
PBS		24 h	1.3 ± 1.1	0.06 ± 0.06	38.46 ± 4.58
		48 h	2.0 ± 2.4	0.1 ± 0.12	36.01 ± 4.39
Test item	150	24 h	1.4 ± 0.8	0.07 ± 0.04	45.23 ± 6.12
	300	24 h	1.1 ± 1.1	0.06 ± 0.05	38.57 ± 8.69
	600	24 h	3.9 ± 1.5 *	0.19 ± 0.07*	27.71 ± 4.95
	600	48 h	1.9 ± 2.1	0.09 ± 0.1	28.16 ± 14.23
CPA	50	24 h	60.6 ± 9.7 ***	3.03 ± 0.49 ***	51.46 ± 4.45

mPCE: micronucleated polychromatic erythrocytes

SD: Standard deviation

PCE: polychromatic erythrocytes

* Statistical significance at $p < 0.05$ (*), at $p < 0.01$ (**) and at $p < 0.01$ (***) in the Student's t-test on transformed data

PBS: phosphate buffered saline, solvent control

CPA: cyclophosphamide, positive control

Table 5.4.2-8: Historical control data for relative frequency categories of micronuclei per 1000 PCE*

24-h sampling			48-h sampling		
Frequency categories	Groups	%	Frequency categories	Groups	%
0.0 – 0.4	15	25	0.0 – 0.4	21	35
0.5 – 0.9	25	42	0.5 – 0.9	18	30
1.0 – 1.4	14	23	1.0 – 1.4	14	23
1.5 – 2.0	3	5	1.5 – 2.0	7	12
2.1 – 2.5	3	5	2.1 – 2.5	0	0

* Data from 60 studies

III. CONCLUSION:

Based on the results of the present study, glyphosate technical did not lead to clastogenic/aneugenic effects in bone marrow of male mice *in vivo*. Therefore, the test item was considered to be non-genotoxic under the conditions of the test.

3. Assessment and conclusion

<u>Assessment and conclusion by applicant</u>:

The study was performed under GLP conditions and in accordance with OECD guideline 474 (1997). Under the conditions of the test, the test material was considered negative for clastogenic/aneugenic effects in the bone marrow of male mice *in vivo*. The small but statistically significant increase in the

incidence of micronucleated PCE in the 24 hour 600 mg/kg bw group remained within the range of the laboratory's historical control data. Moreover, no increase was seen after 48 hours. Thus, the response is interpreted as a haematopoetic effect due to bone marrow toxicity rather than to a specific genotoxic effect.

There were only minor deviations when compared to the currently valid OECD guideline 474 (2016), which were considered to be of minor degree. The number of polychromatic erythrocytes investigated per animal was 2000, which was the number recommended by the previous OECD guideline (1997). Further deviations were considered to not compromise the validity of the study. Therefore, the study was considered valid and acceptable.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.4.2/008
Report author	[redacted]
Report year	1999
Report title	A micronucleus study in mice for glifosate técnico Nufarm
Report No	[redacted]-G12.79/99
Document No	Not reported
Guidelines followed in study	Not specified. The test was conducted similary to OECD 474 (2016).
Deviations from current test guideline OECD 474 (2016)	Only 1000 polychromatic erythrocytes (PCE) per animal were evaluated for the presence of micronuclei, whereas at least 4000 PCE should be evaluated according to the current guideline OEC 474 (2016). The animals were injected intraperitonealy for test substance administration, which is not represetative for a human route of exposure. Bone marrow exposure, indicated by a reduced polychromatic to normochromatic erythrocyte ratio, was not confirmed and there was no systemic toxicity reported. However, due to the route of exposure it can be assumed that bone marrow exposure was achieved. Historical control data for the positive or vehicle controls were not provided. In addition, acceptance and evaluation criteria specified in the study report were inconsistent with those specified by the guideline.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Glyphosate technical (batch: 3578/99, purity: 95 %) was tested in a mouse bone marrow micronucleus assay. Prior to the micronucleus test, a preliminary toxicity study was performed. Based on the results of the toxicity study, the dose levels for the main mutagenicity study were selected to represent 25, 50 and 75 % of the LD_{50} in mice. The test item was dissolved in water and two intraperitoneal injections at dose levels of 187.5, 375 and 562.5 mg/kg bw/day were administered to two groups of 5 male and 5 female mice. The injections were performed on two consecutive days at an interval of 24 hours. A group of vehicle (water) and positive control animals (25 mg/kg bw/day cyclophosphamide in physiological solution) were

treated in an identical manner.

All animals were sacrificed 24 hours after the second injection, followed by preparation and staining of bone marrow smears for all animals.

A total of 1000 polychromatic erythrocytes (PCE) and 1000 normochromatic erythrocytes (NCEs) were scored for the presence of micronuclei and the percentage of PCE to NCE was determined among 1000 erythrocytes.

Intraperitoneal injection of the test material did not induce clinical signs of toxicity in the mice. In addition, there was no increase in the number of micronucleated polychromatic or normochromatic erythrocytes when compared to the vehicle control and the ratio of PCE to NCEs was unaffected by treatment.

Treatment with the positive control cyclophosphamide revealed a marked increase in the incidence of micronucleated PCE, hence demonstrating capability of the test animals to show mutagenic effects in response to known mutagenic agents and confirming the sensitivity of the test.

Based on the results of the test and under the experimental conditions chosen, the test item was considered to be negative for induction of micronuclei in mice *in vivo*.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material: GLIFOSATE TECNICO NUFARM

Identification:	3578/99
Description:	White powder
Lot/Batch #:	037-919-113
Purity:	95 %
Stability of test compound:	The stability of the test item, storage conditions or expiry date were not reported. The stability of the test item in solvent was not specified.
Solvent (vehicle) used:	Water

2. Control materials:

Solvent (vehicle) control:	Water
Positive control:	Cyclophosphamide, 25 mg/kg bw/day in physiological solution

3. Test animals:

Species:	Mouse
Strain:	Swiss albino
Sex:	Male and female
Source:	[redacted]
Age at study initiation:	7 - 12 weeks
Mean weight at dosing:	30.22 g
Acclimation period:	At least 7 days
Diet/Food:	commercial pelleted diet (Labina, Purina), *ad libitum*
Water:	Tap water, *ad libitum*

Housing: In groups of 5/sex on wood shavings in propylene rodent cages with stainless mesh lids

4. Environmental conditions:

Temperature: 20 – 24 °C
Humidity: 50 – 60 %
Air changes: Not specified
Photoperiod: 12-hour light and dark cycle

5. Test concentrations and treatment groups:

a) Preliminary toxicity study

Dose levels: 250, 500, 1000 and 2000 mg/kg bw/day
Number of animals: 5/group
Route of administration: Intraperitoneal injection

b) Main micronucleus test

Dose levels: 187.5, 375 and 562.5 mg/kg bw/day
Concentrations: 12.5, 25 and 37.5 mg/mL
Dose volume: 0.45 mL/30 g bw
Number of animals: 5/sex/group
Route of administration: Intraperitoneal injection

B: STUDY DESIGN AND METHODS

1. Dates of experimental work: 28 Oct – 10 Dec 1999
Finalisation date: 27 Dec 1999

2. Animal assignment and treatment:

Preliminary toxicity study:
The LD_{50} of the test material was determined in a preliminary toxicity study. Groups of five animals received two intraperitoneal injections of 250, 500, 1000 or 2000 mg/kg bw/day on two consecutive days. The animals were observed for four days and occurrence of mortality was recorded. Based on the findings of the study, the dose levels for the main micronucleus test were selected as 25, 50 and 75 % of the LD_{50}.

Main micronucleus test:
Groups of ten mice (5 per sex) received on two consecutive days (24 hours apart) two intraperitoneal injections of 187.5, 375 and 562.5 mg/kg bw/day glyphosate technical. Similar constituted groups of control animals were treated in an identical manner with the vehicle (water) or the positive control (25 mg/kg bw/day cyclophosphamide in physiological solution).

About 24 hours after the second injection, the animals were sacrificed by cervical dislocation.

3. Slide preparation:

Immediately after sacrifice both femurs were dissected from each animal, flushed with foetal calf serum and bone marrow smears prepared following centrifugation and re-suspension. The following day the smears were fixed in 70 % ethanol, air-dried and stained with Eosin methylene blue solution.

4. Slide evaluation:

Slides were randomly coded and examined under a light microscope at 1000x magnification. For

each animal 1,000 polychromatic erythrocytes (PCE) and 1,000 normochromatic erythrocytes (NCE) were examined for the presence of micronuclei (MN). The relation PCE to NCEwere determined in the first 1,000 PCE or NCE enumerated.

5. Statistics:

Differences in the ratio of PCE to NCE and the incidence of micronucleated PCE and NCE per 1000 cells scored were compared using the Kruskal Wallis test for independent variables.

6. Acceptance criteria:

The test was considered valid only if the number of micronuclei in the vehicle control was within the range of the laboratory's historical control data.

7. Evaluation criteria:

The test substance was judged positive for genotoxicity in vivo if the following criteria were met:

- There was a reproducible and statistically significant positive response for at least one dose level and
- The increase in the number of micronuclei was at least twice the vehicle control.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Information on the stability of the test substance in the vehicle was not given in the study report.

B. PRELIMINARY TOXICITY STUDY

Based on the occurrence of mortality within 4 days after treatment, the acute lethal dose for intraperitoneal toxicity (LD_{50}) was determined to be 750 mg/kg bw/day. Dose levels for the main micronucleus test were chosen to represent 25, 50 and 75 % of the LD_{50} obtained in the preliminary toxicity study.

C. MAIN MICRONUCLEUS TEST

Systemic toxicity:

Mortality:

No mortality occurred.

Clinical signs of toxicity:

Clinical signs of toxicity were not reported.

Evaluation of bone marrow slides:

No statistically or biologically significant increases in the incidence of micronucleated polychromatic erythrocytes or in the percentage of polychromatic erythrocytes were observed for the test substance either in male or female mice when compared to vehicle controls (water).

The positive control group revealed a statistically significant increase in the incidence of micronucleated PCE, demonstrating the sensitivity of the test.

Table 5.4.2-9: A micronucleus study in mice ([redacted], 1999), Summary of genotoxicity data

Treatment	Dose (mg/kg bw/day)	Sampling time	mPCE ± SD /1000 PCE	PCE#	mNCE ± SD /1000 NCE	NCE#	PCE/NCE
Water		24 h	0.6	879	0	997.7	0.915
Test item	187.5	24 h	0.3	779.2	0.1	978.1	0.813

Table 5.4.2-9: A micronucleus study in mice ([redacted], 1999), Summary of genotoxicity data

Treatment	Dose (mg/kg bw/day)	Sampling time	mPCE ± SD /1000 PCE	PCE#	mNCE ± SD /1000 NCE	NCE#	PCE/NCE
Test item	375.0	24 h	0.6	871.7	0.3	948.4	0.935
Test item	562.5	24 h	0.5	832.8	0.3	987.8	0.851
CPA	25.0	24 h	4.8*	648.9	2.0*	1029.5	0.63

mPCE: micronucleated polychromatic erythrocytes, mNCE: micronucleated normochromatic erythrocytes

* Statistical significance at $p < 0.05$ (*), at $p < 0.01$ (**) and at $p < 0.01$ (***) using the Kruskal Wallis test for independent variables

Mean number of PCE or NCE scored per 10 animals

CPA: cyclophosphamide, positive control

III. CONCLUSION:

Based on the results of the present study, glyphosate technical did not increase the frequency of micronuclei in the bone marrow of mice in vivo. Thus, the test item was considered to be non-genotoxic under the conditions of the test.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Negative for clastogenic/aneugenic effects in the bone marrow of male and female mice *in vivo*.

The study was performed under GLP conditions and the experimental procedure was similar to the main criteria of OECD guideline 474 (2016), except for some deviations of minor degree. The number of polychromatic erythrocytes (PCE) evaluated was less than the 2000 PCE which are recommended by the current guideline (2016). However, there was no positive effect and no trend at all for induction of micronuclei, therefore it can be assumed that the outcome of the study would be the same if more cells were evaluated.

Dose levels were selected based on LD_{50} values which were determined in a preliminary toxicity study. Although clinical signs of systemic toxicity were not reported and there was no bone marrow toxicity evident, it can be assumed that due to the route of exposure (intraperitoneal injection) bone marrow exposure was achieved. The study is therefore considered valid and acceptable.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.4.2/009
Report author	[redacted]
Report year	1996
Report title	Glyphosate Acid: Mouse Bone Marrow Micronucleus Test
Report No	[redacted]/P/4954
Document No	Not reported
Guidelines followed in	OECD 474 (1997); US EPA (1991) and EEC Annex V B.12 (1992)

study	
Deviations from current test guideline OECD 474 (2016)	The currently defined highest dose for an administration of less than 14 days is exceeded (5000 mg/kg bw/day instead of 2000 mg/kg bw/day), however, no signs indicative of severe toxicity are reported. Only 2000 polychromatic erythrocytes were screened for the presence of micronuclei, instead of 4000, which are recommended by OECD 474 (2016). Bone marrow exposure, indicated by a reduced polychromatic to normochromatic erythrocyte ratio, was not confirmed and there was no systemic toxicity observed, but dose levels exceeded limit concentrations specified in the current guideline. Historical control data established in the testing laboratory were not provided. In addition, acceptance criteria differed from those specified in the current guideline and evaluation criteria were not specified in the study report.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Glyphosate acid (batch: P24, purity: 95.6 %) was tested for its genotoxic potential in mice using the micronucleus test. Prior to the micronucleus test, a preliminary toxicity study was conducted in order to identify the maximum tolerated dose in the animals.

Based on the results of the toxicity study, the test item was dissolved in physiological saline and groups of 5 male and 5 female mice received a single oral dose of 5000 mg/kg bw. Similarly constituted groups received the vehicle (physiological saline) or positive control (65 mg/kg bw cyclophosphamide in physiological saline) and were treated in an identical manner. The animals were sacrificed 24 or 48 hours after dosing, followed by preparation and staining of bone marrow smears.

A total of 2000 polychromatic erythrocytes (PCE) was evaluated for the presence of micronuclei. In addition, 1000 erythrocytes were counted to determine the percentage of PCE in the total erythrocyte population.

Oral administration of 5000 mg/kg bw did not induce any adverse reactions to treatment in any animal. In addition, there was no statistically significant change in the ratio of PCE among the total fraction of erythrocytes when compared to control animals, indicating that no relevant cytotoxicity occurred up to the maximum tolerated dose of 5000 mg/kg bw.

There was no statistically or biologically significant increases in the incidence of micronucleated PCE over the vehicle control values observed in either males or females at any sampling time investigated.

The positive control cyclophosphamide induced a statistically and biologically significant increase in the frequency of micronucleated polychromatic erythrocytes in both male and female mice, demonstrating the sensitivity of the test system and the capability of the test animals to respond to known mutagens.

Based on the results of the test and under the experimental conditions chosen, the test item was considered to be negative for induction of micronuclei in mice *in vivo*.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material: Glyphosate acid

Identification:	17984
Description:	White solid
Lot/Batch #:	P24
Purity:	95.6 %
Stability of test compound:	The stability of the test item under storage conditions (at ambient temperature in the dark) was guaranteed when used within the stated expiry date. The stability of the test item in solvent (vehicle) was not specified.
Solvent (vehicle) used:	Physiological saline

2. Control materials

Solvent (vehicle) control:	Physiological saline
Positive control:	Cyclophosphamide, 65 mg/kg bw in physiological saline

3. Test animals:

Species:	Mouse
Strain:	CD-1
Sex:	Male and female
Source:	[redacted]
Age at study initiation:	6-7 weeks
Weight at dosing:	22.8 – 37.6 g
Acclimation period:	At least 5 days
Diet/Food:	CT1 (supplied by Special Diets Services, Stepfield, Witham, Essex, UK, *ad libitum*
Water:	Water, supplied by an automated watering system, *ad libitum*
Housing:	In groups of 5/sex per cage on mobile mouse cage racks

4. Environmental conditions:

Temperature:	19-23 °C
Humidity:	40-70 %
Air changes:	15/hour
Photoperiod:	12-hour light and dark cycle

5. Test concentrations and treatment groups:

a) Preliminary toxicity study

Dose levels: 5000 mg/kg bw
Number of animals: 5 /sex / group
Route of administration: Oral gavage

b) Main micronucleus test

Dose levels: 5000 mg/kg bw
Dose volume: 20 mL/kg bw
Number of animals: 5 /sex / group
Route of administration: Oral gavage

B: STUDY DESIGN AND METHODS

1. Dates of experimental work: 11 Dec 1995 – 30 Jan 1996
Finalisation date: 21 Mar 1996

2. Animal assignment and treatment:

Preliminary toxicity study:
The maximum tolerated dose (MTD), was determined in a preliminary toxicity study (termed as "Phase 1" in the study report). 5 male and 5 female mice received a single dose of 5000 mg/kg bw by oral gavage. Based on the occurrence of mortality and clinical signs of toxicity within 4 days after treatment, the maximum tolerated dose was determined.

Main micronucleus test:
Groups of ten mice (5 per sex) were given a single oral dose of 5000 mg/kg bw by oral gavage, representing the maximum tolerated dose. Similar constituted groups of control animals were treated in an identical manner with the vehicle (physiological saline) or the positive control (65 mg/kg bw cyclophosphamide in physiological saline).

The animals were observed for adverse reactions to treatment and sacrificed 24 and 48 hours after dosing by asphyxiation of halothane followed by cervical dislocation.

3. Slide preparation:

Immediately after sacrifice both femurs were dissected from each animal. The iliac end of the femur was removed and a fine paint brush wetted in a solution of 6 % (w/v) albumin in saline was dipped into the marrow canal. From each marrow, four smears in total were painted on appropriately labelled, clean and dry microscope slides. The slides were allowed to air-dry and stained with polychrome methylene blue and eosin.

4. Slide evaluation:

Slides were coded and scored by microscopy. For each animal 2000 polychromatic erythrocytes (PCE) were examined for the presence of micronuclei. In addition, 1000 erythrocytes were counted to determine the percentage of PCE in the total erythrocyte population.

5. Statistics:

The incidence of micronucleated PCE and the percentage of PCE in the erythrocyte sample were considered by analysis of variance at 24 and 48 hours, separately for males and females. Prior to analysis, the data for the incidence of micronucleated PEC was transformed using a square root transformation and the data for the ratio of PCE among the total number of erythrocytes was

transformed using the double arcsin transformation of Freeman and Tukey (1950)[17]. Each treatment group mean was compared with the control group mean at the corresponding sample time using a one-sided Student's t-test based on the error mean square in the analysis.

6. Acceptance criteria:

The test was considered valid if the following criteria were met:

- The positive control substance induced a significant elevation in micronucleated polychromatic erythrocytes when compared to vehicle controls.
- The test material was tested at a level that caused a decrease in the percentage of erythrocytes (indicating a cytotoxic effect on the bone marrow) or at the maximum tolerated dose level.

7. Evaluation criteria:

Evaluation criteria were not specified in the study report.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Analytical determinations were not performed, as not compulsory by the test guideline.

B. PRELIMINARY TOXICITY STUDY

During the four days observation period, no patterns of lethality or severe toxicity were observed. As no clinical signs and no lethality was evident at the limit dose level, 5000 mg/kg bw were taken to represent the maximum tolerated dose for males and females.

C. MAIN MICRONUCLEUS TEST

Systemic toxicity:

Mortality:

No mortality occurred.

Clinical signs of toxicity:

No adverse reactions to treatment were observed for either males or females.

Evaluation of bone marrow slides:

No statistically significant differences in the percentage of polychromatic erythrocytes was observed for glyphosate acid treated animals when compared to control animals, indicating that no relevant cytotoxicity occurred up to the maximum tolerated dose of 5000 mg/kg bw.

In addition, no statistically or biologically significant increases in the incidence of micronucleated polychromatic erythrocytes over the vehicle control values were observed in either males or females at either sampling time investigated.

The positive control cyclophosphamide induced a statistically and biologically significant increase in the frequency of micronucleated polychromatic erythrocytes in both male and female mice, demonstrating the sensitivity of the test system.

Table 5.4.2-10: Mouse Bone Marrow Micronucleus Test ([redacted], 1996), genotoxicity results

Treatment	Dose (mg/kg bw)	Sampling time	Males		Females	
			mPCE ± SD	mean%	mPCE ± SD	mean%

[17] Freeman M.F. and Tukey *J.* W. (1950). Transformations related to the angular and the square root. Annals of Maths Stats 21, 607

			/1000 PCE	PCE ± SD	/1000 PCE	PCE ± SD
Saline	20 mL/kg	24 h	1.6 ± 0.8	46.0 ± 4.1	1.4 ± 0.7	46.6 ± 1.9
Saline	20 mL/kg	48 h	1.7 ± 1.3	49.8 ± 4.8	0.7 ± 0.6	46.4 ± 4.4
Test item	5000	24 h	2.1 ± 1.6	46.4 ± 4.3	2.1 ± 2.5	42.3 ± 6.2
Test item	5000	48 h	2.1 ± 1.9	48.4 ± 4.0	0.8 ± 0.8	45.8 ± 2.9
CPA	65	24 h	22.2 ± 6.1 **	46.5 ± 2.0	23.3 ± 4.9 **	46.0 ± 4.1

mPCE: micronucleated polychromatic erythrocytes

* Statistical significance at $p < 0.05$ (*), at $p < 0.01$ (**) and at $p < 0.01$ (***) in the Student's t-test (one sided) in transformed data

CPA: cyclophosphamide, positive control

III. CONCLUSION:

Based on the results of the present study, glyphosate acid has no clastogenic/aneugenic activity in bone marrow of male and female mice when tested up to the limit dose of 5000 mg/kg bw. Thus, the test item was considered to be non-genotoxic under the conditions of the test.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Negative for clastogenic/aneugenic effects in the bone marrow of male and female mice *in vivo*.

The study was conducted under GLP conditions and in line with OECD guideline 474 (1997). There were a number of deviations when compared to the currently valid OECD guideline 474 (2016), all of them considered to be of minor degree. Only 2000 polychromatic erythrocytes were investigated, which was the number required according to the previous guideline (1997) that was in force when the test was conducted. Bone marrow toxicity, indicated by a shifted polychromatic to normochromatic erythrocyte ratio or systemic toxicity were not observed. However, test item doses included the dose level of 5000 mg/kg bw, which exceeds the limit value specified in the current guideline (2016). The study is therefore considered valid and acceptable.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.4.2/010
Report author	[redacted]
Report year	1993
Report title	Glyphosate technical: Mutagenicity - Micronucleus Test in Swiss Albino Mice
Report No	889-MUT.MN
Document No	Not reported
Guidelines followed in study	OECD 474 (1983)
Deviations from current test guideline	The currently defined highest dose for an administration of less than 14 days is exceeded (5000 mg/kg bw/day instead of 2000 mg/kg bw/day), however,

OECD 474 (2016)	no signs indicative of severe toxicity are reported. According to the current guideline OECD 474 (2016), at least 4000 polychromatic erythrocytes per animal should be evaluated for the presence of micronuclei. In the present study 2000 erythrocytes (RBC) were evaluated, including approx. 800 – 1100 polychromatic erythrocytes per animal. In addition, no data on proficiency and/or historical control data were provided. Acceptance and evaluation criteria were not specified in the study report.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Glyphosate technical (batch: FSG 03090, purity: 96.8 %) was assessed for its ability to induce cytogenetic effects in Swiss albino mice. Based on the results of a preliminary range-finding study, dose levels for the micronucleus test were selected. Groups of 5 mice/sex were treated by oral gavage at dose levels of 50, 500 and 5000 mg/kg bw/day at a constant dosage volume of 10 mL/kg bw for two consecutive days. Similar constituted groups received the vehicle (refined groundnut oil, 10 mice/sex) or the positive control cyclophosphamide (100 mg/kg bw/day, 5 mice/sex).

The animals were observed for clinical signs of toxicity and mortality twice daily and the time of onset, duration and severity were recorded. In addition, the body weight of the individual animals was recorded on study Days 1, 2 and at sacrifice. 24 hours after the second dose administration the animals were sacrificed and femoral bone marrow smears were prepared for each animal. At least 2000 erythrocytes per animal were scored for the presence of micronuclei and the ratio of polychromatic to normochromatic erythrocytes was determined.

Oral administration of glyphosate technical did not induce any clinical signs of toxicity or mortality. However, at sacrifice, animals of the 500 and 5000 mg/kg bw/day group had lost body weight. The ratio of polychromatic to normochromatic erythrocytes (NCEs) of glyphosate treated mice was unaffected and comparable to those of mice treated with the vehicle, indicating that no bone marrow toxicity had occurred.

In female mice, there was a slight but statistically significant increase in the frequency of micronucleated polychromatic erythrocytes (PCEs) at the highest dose level of 5000 mg/kg bw/day when compared to control animals. There was no increase in the frequency of micronucleated PCEs at the low and the mid dose level and no effect in male mice at any dose level.

The frequency of micronucleated PCE, NCE and total erythrocytes in the solvent control animals were comparably low for all animals. The positive control cyclophosphamide showed the expected statistically significant increase in the incidence of micronucleated PCE and NCE in both sexes and a high degree of bone marrow cytotoxicity evident as markedly affected PCE/NCE ratio, demonstrating the sensitivity of the test system.

Under the conditions of the test and based on the experimental findings, glyphosate technical was considered weakly positive for induction of micronuclei in female mice *in vivo*.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material: Glyphosate (N-(Phosphonomethyl) glycine)

Identification:	C140500
Description:	Odorless white crystals
Code:	FSG 03090 h/05, March 1990
Purity:	96.8 %
Stability of test compound:	The stability of the test item at storage conditions (at ambient temperature) was guaranteed until the expiry date Jul 1992. The stability of the test item in solvent was not specified. Dosing formulations were prepared fresh prior to treatment. Homogeneity of the test compound suspension was maintained by constant stirring/mixing in mortar during treatment.
Solvent (vehicle) used:	Refined groundnut (peanut) oil

2. Control materials

Solvent (vehicle) control:	Refined groundnut (peanut) oil
Positive control:	Cyclophosphamide, 100 mg/kg bw/day

3. Test animals:

Species:	Mouse
Strain:	Swiss albino
Sex:	Male and female
Source:	[redacted]
Age at study initiation:	8 - 10 weeks
Weight at dosing:	25 - 35 g
Acclimation period:	At least one week
Diet/Food:	Gold Mohur pelleted mice feed (Lipton India Ltd., Bangalore, India), *ad libitum*
Water:	Protected, deep borewell water, passed through activated charcoal filter and exposed to UV rays in Aquaguard on-line water filter-cum-purifier, *ad libitum*
Housing:	In groups of 5/sex in sterilised standard polypropylene cages measuring 290 x 220 x 140 mm with paddy husk bedding

4. Environmental conditions:

Temperature:	22 ± 3 °C
Humidity:	40 - 70 %
Air changes:	Approximately 12-15/hour
Photoperiod:	12-hour light and dark cycle

5. Test concentrations and treatment groups:

Dose levels:	50, 500 and 5000 mg/kg bw/day
Concentrations:	5, 50, 500 mg/mL
Dose volume:	10 mL/kg bw
Number of animals:	5/sex/group (test item and positive control) and 10/sex/group (vehicle control)
Route of administration:	Oral gavage

B: STUDY DESIGN AND METHODS

1. Dates of experimental work: 10 Aug – 10 Dec 1991
Finalisation date: 06 May 1993

2. Animal assignment and treatment:

Groups of five mice per sex received two doses of 50, 500 and 5000 mg/kg bw/day on two consecutive days. The test item was dissolved in refined groundnut (peanut) oil and administered via oral gavage at a constant dosage volume of 10 mL/kg bw. Groups of 10 mice/sex received the vehicle. Groups of 5 mice/sex were treated with the positive control (100 mg/kg bw/day cyclophosphamide).

The animals were observed for clinical signs of toxicity and mortality twice daily and the time of onset, duration and severity were recorded. In addition, the body weight of the individual animals was recorded on study Days 1, 2 and at sacrifice.

24 hours after the last treatment, the animals were sacrificed by cervical dislocation and bone marrow smears were prepared.

3. Slide preparation:

After sacrifice, the femur bones from both sides were removed, dissected and marrow cells were flushed out with sterile physiological saline. The cells were centrifuged, re-suspended in physiological saline, smeared evenly on microscopic glass slides and air-dried. 4-6 slides were prepared for each animal. Finally, the slides were fixed in methanol for 30 minutes by immersing them in a coplin jar and stained with May-Grünwald/Giemsa. Afterwards, the slides were blow-dried, immersed in xylene and coverslip mounted with DPX.

4. Slide evaluation:

For each animal a minimum of 2000 erythrocytes was scored for the incidence of polychromatic (PCE) and normochromatic (NCE) erythrocytes and for the presence of micronuclei. The following data were recorded:

- Total red blood cells /erythrocytes scored
- Number of PCEs differentiated
- Number and percentage of PCE with micronuclei
- Number of NCE differentiated
- Number and percentage of NCE with micronuclei
- Number and percentage of total red blood cells with micronuclei
- Ratio of PCE/NCE

5. Statistics:

The data was analysed group-wise and sex-wise using one way Anova and t-test for unequal number of observations for the percentage of micronucleated cells in the treated and control groups. The t-test was further used for dose-effect relationship.

6. Acceptance criteria:
Acceptance criteria were not specified in the study report.

7. Evaluation criteria:
Evaluation criteria were not specified in the study report.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS
Analytical determinations were not performed in this study, as not compulsory by the test guideline.

B. MICRONUCLEUS TEST
Systemic toxicity:
Mortality:
No mortality occurred.

Clinical signs of toxicity:
There were no compound- and dose-related signs of systemic toxicity observed.

Body weight:
At sacrifice, marginal body weight loss was noted in the animals of the mid (500 mg/kg bw/day, -1.9 % in males and -2.0 % in females) and high dose group (5000 mg/kg bw/day, -4.3 % in males and -1.0 % in females), respectively.

Evaluation of bone marrow slides:
There was no difference in the ratio of polychromatic (PCE) to normochromatic erythrocytes (NCE) for any of the glyphosate treated groups when compared to solvent control animals, indicating that no bone marrow toxicity had occurred.

In female mice, there was a slight but statistically significant increase in the frequency of micronucleated polychromatic erythrocytes (PCEs) at the highest dose level of 5000 mg/kg bw/day when compared to control animals. A dose effect relationship was evident in those animals.

There was no increase in the frequency of micronucleated PCEs at the low, mid and high dose level in males or as combined sex data.

The toxicological significance of the glyphosate-related increase of micronucleated PCEs in one sex was considered doubtful, as the variation in the percentage of micronucleated PCEs was considerably high among female dose groups as compared to the control.

The frequency of micronucleated PCE, NCE and total erythrocytes in the solvent control animals were comparably low for all animals. The positive control cyclophosphamide showed the expected statistically significant increase in the incidence of micronucleated PCE and NCE in both sexes and a high degree of bone marrow cytotoxicity evident as markedly affected PCE/NCE ratio, demonstrating the sensitivity of the test system.

Table 5.4.2-11: Mutagenicity – Micronucleus test in Swiss Albino Mice ([redacted] 1993), summary of genotoxicity data, male mice

Treatment	Dose (mg/kg bw/day)	Number of animals	Sampling time	Males				
				mPCE ± SD /2000 RBC [X]	mean% PCE ± SD [X]	mNCE ± SD /2000 RBC [X]	mean% NCE ± SD [X]	PCE/NCE ratio
Vehicle		10	24 h	6.40 ± 5.56	0.69	6.60 ± 5.70	0.62	1:1.1
Test item	50	5	24 h	7.60 ± 4.72	0.84	6.60 ± 3.29	0.64	1:1.1
	500	5	24 h	7.20 ± 3.83	0.73	2.80 ± 2.77	0.22	1:1.2
	5000	5	24 h	9.60 ± 2.41	0.89	6.40 ± 1.82	0.47	1:1.3
CPA	100	5	24 h	21.10 ± 6.42	2.33[$]	20.20 ± 5.89	1.18[$]	1:1.9

mPCE: micronucleated polychromatic erythrocytes; mNCE: micronucleated normochromatic erythrocytes
RBC: red blood cells, 2000 RBCs were scored for mPCE and mNCE; based on the values reported in the study report approx. 800 – 1100 PCE /animal were scored for the presence of micronuclei

[$]: significantly higher than control by contingency test; *: t-test for dose response relationship significant at $p < 0.1$

[X]: mPCE and mNCE values calculated based on number of PCE and NCE, raw data provided in study report

Table 5.4.2-12: Mutagenicity – Micronucleus test in Swiss Albino Mice ([redacted] 1993), summary of genotoxicity data, female mice

Treatment	Dose (mg/kg bw/day)	Number of animals	Sampling time	Females				
				mPCE ± SD /2000 RBC [X]	mean % PCE ± SD [X]	mNCE ± SD /2000 RBC [X]	mean% NCE ± SD [X]	PCE/NCE ratio
Vehicle		10	24 h	5.10 ± 3.35	0.51	4.70 ± 2.11	0.39	1:1.2
Test item	50	5	24 h	2.60 ± 1.67	0.28	1.80 ± 0.84	0.15	1:1.3
	500	5	24 h	5.40 ± 4.51	0.52	2.80 ± 1.48	0.23	1:1.2
	5000	5	24 h	10.60 ± 5.08	1.05[$*]	6.00 ± 2.00	0.46	1:1.3
CPA	100	5	24 h	17.60 ± 5.81	2.39[$]	23.40 ± 2.70	1.65[$]	1:1.9

mPCE: micronucleated polychromatic erythrocytes; mNCE: micronucleated normochromatic erythrocytes
RBC: red blood cells, 2000 RBCs were scored for mPCE and mNCE; based on the values reported in the study report approx. 800 – 1100 PCE/ animal were scored for the presence of micronuclei

[$]: significantly higher than control by contingency test; *: t-test for dose response relationship significant at $p < 0.1$

[X]: mPCE and mNCE values calculated based on number of PCE and NCE, raw data provided in study report

Table 5.4.2-13: Mutagenicity – Micronucleus test in Swiss Albino Mice ([redacted] 1993), summary of genotoxicity data, both sexes combined

Treatment	Dose (mg/kg bw/day)	Number of animals	Sampling time	Combined sex				
				mPCE ± SD /2000 RBC [X]	mean% PCE ± SD [X]	mNCE ± SD /2000 RBC [X]	mean% NCE ± SD [X]	PCE/NCE ratio
Vehicle		20	24 h	5.75 ± 4.52	0.6	5.65 ± 4.30	0.5	1:1.2
Test item	50	10	24 h	5.10 ± 4.25	0.55	4.20 ± 3.39	0.38	1:1.2
	500	10	24 h	6.30 ± 4.06	0.62	2.80 ± 2.10	0.23	1:1.2
	5000	10	24 h	10.10 ± 3.78	0.96*	6.20 ± 1.81	0.47	1:1.3
CPA	100	10	24 h	19.40 ± 6.08	2.36[$]	21.80 ± 4.64	1.39[$]	1:1.9

mPCE: micronucleated polychromatic erythrocytes; mNCE: micronucleated normochromatic erythrocytes
RBC: red blood cells, 2000 RBCs were scored for mPCE and mNCE; based on the values reported in the study report approx. 800 – 1100 PCE/ animal were scored for the presence of micronuclei

[$]: significantly higher than control by contingency test; *: t-test for dose response relationship significant at $p < 0.1$

[X]: mPCE and mNCE values calculated based on number of PCE and NCE, raw data provided in study report

III. CONCLUSION:

Based on the experimental findings glyphosate technical induced micronuclei in female mice at a dose level of 5000 mg/kg bw/day. No increase in micronuclei formation was observed at lower dose levels in females or in males at any dose level. In conclusion, the test substance was considered weakly positive for clastogenic/aneugenic effects in female mice and negative in male mice *in vivo*.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Based on the experimental findings the test item was considered weakly positive for clastogenic/aneugenic effects in the bone marrow of female mice *in vivo*. The study was performed according to OECD guideline 474 (1983) and under GLP conditions. The number of evaluated polychromatic erythrocytes was far below the recommendation of the current OECD guideline (2016), which resulted in rather high standard deviations. Due to missing historical control data, the biological significance of the weak positive result observed in females is unclear. Besides this, only minor deviations to the currently valid OECD guideline are present. Therefore, the study is considered to provide supporting information. Further deviations were considered to not compromise the outcome of the study.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.4.2/011
Report author	[redacted]
Report year	1990
Report title	Agrichem glyphosate active: OECD 474 micronucleus test in the

	mouse
Report No	300/3
Document No	Not reported
Guidelines followed in study	OECD TG 474 (1981), EEC Commission Directive 84/449 Method B12 (1984)
GLP	Yes
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	Glyphosate technical (batch: 0190A, purity: not reported) was tested for its genotoxic potential in BKW mice in a micronucleus test. Based on the results of a preliminary toxicity study, in which no mortality was observed up to an oral dose level of 5000 mg/kg bw, a dose of 4000 mg/kg bw was selected for the main mutagenicity test. The test item was dissolved in water and administered by oral gavage to three groups of mice for 3 sampling time points (24, 48 and 72 h after administration). Similar constituted groups received the solvent (distilled water) or the positive control (50 mg/kg bw cyclophosphamide). The animals were observed for clinical signs of toxicity and death one hour after dosing and once daily thereafter. Sampling of the vehicle control occurred at 24, 48 and 72 hours after treatment. For the positive control, bone marrow was sampled 24 hours following treatment. Smears were prepared from the femoral bone marrow of each animal and 1000 polychromatic erythrocytes (PCE) per animal were scored for the presence of micronuclei. In addition, the number of micronucleated normochromatic erythrocytes (NCE) associated with 1000 PCE were counted and scored for the incidence of micronuclei.
Short description of results:	Upon treatment with glyphosate technical, 4/10, 2/10 and 1/10 animals died prematurely of the 24 hour, 48 hour and 72 hour sampling group, respectively. Substitute animals were included for the respective dose groups. Except one substitute animal of the 48 hour sampling group, all animals survived until scheduled sacrifice. Thus, the toxicological relevance of the observed mortality is unclear taking into consideration the survival rate of the substitute animals and the test animals in the preliminary study. Clinical signs of toxicity were observed in one animal and comprised hunched posture, lethargy, piloerection, decreased respiratory rate, ptosis and ataxia. The majority of substitute animals showed minor clinical signs of toxicity. When compared to solvent controls, the ratio of PCE/NCE was not affected by treatment, indicating that glyphosate did not induce bone marrow toxicity. There was no statistically significant increase in the incidence of micronucleated PCE or NCE for any sampling time point when compared to their concurrent vehicle controls. However, the respective standard deviations indicate a rather high heterogeneity in results. The positive control cyclophosphamide showed a marked increase in the incidence of micronucleated PCE and a small elevation of the PCE/NCE ratio, hence confirming the sensitivity of the test. Under the conditions of the test, the results indicate that glyphosate technical is negative for cytogenetic effects in bone marrow in mice *in vivo*.

Reasons for why the study is not considered relevant/reliable or not considered as key study:	The study was considered not acceptable due to a large number of guideline deviations. According to the current guideline OECD 474 (2016), at least 4000 polychromatic erythrocytes per animal should be evaluated for the presence of micronuclei. In the present study only 1000 polychromatic erythrocytes were evaluated. No data on proficiency and/or historical control data were provided and the purity of the test material was not reported. The highest tested dose level of 4000 mg/kg bw exceeds the currently defined maximum dose of 2000 mg/kg bw for administration periods < 14 days. Strong mortality was noted in the main mutagenicity study among various test groups, although a dose-response relationship was not evident and the deaths were not clearly attributable to treatment. The mortality observed was inconsistent with observations in other studies. In addition, evaluation criteria were inconsistent with those specified in the current guideline (2016).
Reasons why the study report is not available for submission	Not applicable
Category study in AIR5 dossier (L-docs)	Category 3b

1. Information on the study

Data point	CA 5.4.2/012
Report author	[redacted]
Report year	1991
Report title	Mutagenicity test: Micronucleus test with Glyphosate, batch 206-JaK-25-1
Report No	12324
Document No	Not reported
Guidelines followed in study	OECD 474 (1983), US EPA FIFRA 84-2
Deviations from current test guideline OECD 474 (2016)	The currently defined highest dose for an administration of less than 14 days is exceeded (5000 mg/kg bw/day instead of 2000 mg/kg bw/day), however, no signs indicative of severe toxicity are reported. According to the current guideline OECD 474 (2016), at least 4000 polychromatic erythrocytes per animal should be evaluated for the presence of micronuclei. In the present study 2000 polychromatic erythrocytes were evaluated (requirement of previous OECD guideline (1983)). In addition, the percentage of polychromatic erythrocytes among total erythrocytes was determined for 200 erythrocytes instead of 500 erythrocytes. Bone marrow exposure, indicated by a reduced polychromatic to normochromatic erythrocyte ratio or systemic toxicity were not observed. In addition, no 24-hour control animals were included (first sampling time point). No data on proficiency and/or historical control data were provided. Acceptance and evaluation criteria were not specified in the study report.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Glyphosate technical (batch: 206-JaK-25-1, purity: 98.6 %) was tested for its clastogenic/aneugenic potential in NMRI mice in a micronucleus test. Based on the results of an initial toxicity study in which no signs of toxicity became evident, the main micronucleus test was performed at a single dose level of 5000 mg/kg bw.

Three groups of 5 animals/sex received a single dose of the test item, dissolved in 0.5 % carboxymethyl cellulose and administered at a constant dosage volume of 10 mL/kg bw. Similar constituted groups of 5 mice/sex received the positive (cyclophosphamide, 30 mg/kg bw) or vehicle control (0.5 % carboxymethyl cellulose in distilled water).

Bone marrow sampling of the test item treated groups was performed 24, 48 and 72 hours after treatment. Positive and vehicle control animals were sacrificed 24 and 48 hours after dosing, respectively. Smears were prepared from the femoral bone marrow of each animal and 2000 immature erythrocytes (PCE) per animal were scored for the presence of micronuclei. The percentage of PCE in 200 erythrocytes was calculated. In addition, the number of micronucleated normochromatic erythrocytes (NCEs) was determined.

Oral administration of glyphosate technical did not induce any clinical signs of systemic toxicity. In addition, the ratio of PCE to NCEs indicates that no bone marrow toxicity was evident in any of the tested conditions.

The incidence of micronucleated polychromatic erythrocytes after treatment with glyphosate was comparable between control and treatment groups for any sampling time point. The frequency of micronuclei in the solvent and positive control groups were in accordance with the laboratory's historical control data, confirming validity and sensitivity of the test. Based on the experimental findings, glyphosate technical is negative for cytogenetic effects in bone marrow in mice *in vivo*.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material:

Identification:	Glyphosate Technical
Description:	White powder
Lot/Batch #:	206-JaK-25-1
Purity:	98.6 %
Stability of test compound:	The stability of the test item, storage conditions or expiry date were not reported. The stability of the test item in solvent was not specified.
Solvent (vehicle) used:	0.5 % carboxymethyl cellulose in distilled water

2. Control materials

Solvent (vehicle) control:	0.5 % carboxymethyl cellulose in distilled water
Positive control:	Cyclophosphamide, 30 mg/kg bw

3. Test animals:

Species:	Mouse

Strain:	Bom:NMRI (NMRI SPF mice)
Sex:	Male and female
Source:	[redacted]
Age at study initiation:	7 - 10 weeks
Weight at dosing:	26 - 31 g
Acclimation period:	5 days
Diet/Food:	Altromin 1314 (Chr. Petersen Ltd., Ringsted, Denmark), *ad libitum*
Water:	Tap water acidified with hydrochloric acid to pH 2.5, *ad libitum*
Housing:	In groups of 5/sex in Macrolon type III cages measuring 420 x 260 x 150 mm with softwood sawdust bedding.

4. Environmental conditions:

Temperature:	21 ± 3 °C
Humidity:	55 ± 15 %
Air changes:	Approximately 10/hour
Photoperiod:	12-hour light and dark cycle

5. Test concentrations and treatment groups:

a) Preliminary toxicity study

Dose levels:	5000 mg/kg bw
Concentrations:	Not specified
Dose volume:	10 mL/kg bw
Number of animals:	5/sex/group
Route of administration:	Oral gavage

b) Main micronucleus test

Dose levels:	5000 mg/kg bw
Concentrations:	500 mg/mL
Dose volume:	10 mL/kg bw
Number of animals:	5/sex/group
Route of administration:	Oral gavage

B: STUDY DESIGN AND METHODS

1. Dates of experimental work: 24 Jan – 01 Feb 1991
Finalisation date: 12 Sep 1991

2. Animal assignment and treatment:

Preliminary toxicity study:
Glyphosate was expected to be of low toxicity. To determine the maximum tolerated dose, a single dose of 5000 mg/kg bw was administered by oral gavage to 4 male and 5 female mice. A group of 3 mice further received the vehicle and served as control. The test substance or the vehicle was applied at a constant dosage volume of 10 mL/kg bw. Bone marrow samples were prepared 24, 48 and 72 hours after dosing from 3 mice per sampling time point and 48 hours after dosing for the control group. To assess bone marrow toxicity upon treatment with the test item, the percentage of polychromatic erythrocytes among the total of erythrocytes was determined for 200 erythrocytes for each animal. The

amount of PCE was comparable between the control and test group.

Based on these results, 5000 mg/kg bw was selected as dose level for the main micronucleus test.

Main micronucleus test:
Groups of five mice per sex were administered a single dose of 5000 mg/kg bw by oral gavage at a constant dosage volume of 10 mL/kg bw. Similar constituted groups of 5 mice/sex received the vehicle (0.5 % carboxymethyl cellulose) or the positive control (30 mg/kg bw cyclophosphamide).
Animals of the test group were sacrificed by cervical dislocation at 24, 48 and 72 hours after treatment and bone marrow smears were prepared. Positive control animals were sacrificed 24 hours after treatment, the vehicle control group was sacrificed 48 hours after dosing.

3. Slide preparation:
Immediately after sacrifice, the right femoral bone was dissected, the proximal end of the femur was cut and marrow cells were flushed out with foetal calf serum. After whirl-mixing, the bone marrow suspensions were centrifuged and smears were prepared. The specimens were fixed in methanol and stained with May-Grünwald/Giemsa

4. Slide evaluation:
Slides were randomly coded and examined by microscopical analysis. About 2000 polychromatic erythrocytes (PCE) per animal were scored for the presence of micronuclei. The percentage of PCE in 200 erythrocytes was calculated. In addition, the number of micronucleated normochromatic erythrocytes (NCE) was determined during the counting of 2000 PCE.

5. Statistics:
The number of micronucleated PCE in the test group were compared to the number found in the vehicle group. Statistical analysis was performed using the one-way analysis of variance performed on the values transformed to normal scores according to Blom's method[18].

6. Acceptance criteria:
Acceptance criteria were not specified in the study report.

7. Evaluation criteria:
Evaluation criteria were not specified in the study report.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS
Analytical determinations were not performed in the study, as not compulsory by the test guideline.

B. PRELIMINARY TOXICITY STUDY
Clinical signs of systemic toxicity have not been described in the study report. Bone marrow toxicity, evident as a shifted ratio of polychromatic erythrocytes has not been observed. The percentage of PCE was between 34 and 43 % for both, test and control group. Based on the results of the preliminary toxicity study, 5000 mg/kg bw was selected as dose level for the main micronucleus test.

C. MAIN MICRONUCLEUS TEST
Systemic toxicity:
Mortality:
No mortality reported.

Clinical signs of toxicity:

[18] Blom (1958): Statistical Estimates and Transformed Beta Variables, New York: John Wiley and Sons, Inc

Clinical signs of toxicity were not described in the study report.

Evaluation of bone marrow slides:
There was no difference in the ratio of polychromatic to normochromatic erythrocytes when compared to vehicle controls for any sampling time point, demonstrating that there was no bone marrow toxicity evident. Values were in the range of 37.7 – 42.2 % and fell into the normal range of the laboratory (data not shown).

There was no statistically significant increase in the incidence of micronucleated PCE in test item-treated animals at any sampling time point when compared to vehicle control. The frequency of micronuclei in the solvent and positive control groups were in accordance with the laboratory's historical control data, confirming the sensitivity of the test and demonstrating the capability of the test animals to respond to mutagenic substances.

Table 5.4.2-14: Mutagenicity test: Micronucleus test with Glyphosate ([redacted] 1991b), summary of genotoxicity data

Treatment	Dose (mg/kg bw)	Sampling time	Males		Females	
			mPCE ± SD /2000 PCE	mean% PCE ± SD /200 erythrocytes	mPCE ± SD /2000 PCE	mean% PCE ± SD /200 erythrocytes
CMC	0.5 %	48 h	3.00 ± 1.41	36.00 ± 5.15#	2.40 ± 0.55	39.80 ± 1.30#
Test item	5000	24 h	3.40 ± 1.14	35.60 ± 2.97	3.00 ± 1.41	40.00 ± 3.32#
	5000	48 h	2.20 ± 0.84	37.00 ± 2.24	3.40 ± 1.67	40.40 ± 1.95
	5000	72 h	1.80 ± 1.30	37.40 ± 3.44	1.60 ± 1.14	37.80 ± 3.63
CPA	30	24 h	48.60 ± 4.98	41.20 ± 3.03	47.80 ± 9.96	43.00 ± 5.70

Mean values and standard deviations (SD) were calculated based on the raw data given in the study report.
mPCE: number of micronuclei observed in 2000 PCE
%PCE: PCE in percent of total erythrocytes
Calculations were performed with raw data values of poor legibility.
CMC: carboxymethyl cellulose, solvent control; CPA: cyclophosphamide, positive control

III. CONCLUSION

Based on the experimental findings glyphosate technical is negative for cytogenetic effects in bone marrow in mice *in vivo*.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Negative for clastogenic/aneugenic effects in the bone marrow of male and female mice i*n vivo*.

The study was performed under GLP conditions and in accordance with OECD guideline 474 (1983). When compared to the currently valid OECD guideline 474 (2016), a number of deviations became evident. The number of polychromatic erythrocytes investigated was 2000/animal, corresponding to the number required by the previous guideline (1997). In addition, the percentage of polychromatic erythrocytes among total erythrocytes was determined for 200 erythrocytes instead of 500 erythrocytes. The deviations were considered to be of minor degree and to not compromise the validity of the study. Bone marrow toxicity, indicated by a reduced polychromatic to normochromatic erythrocyte ratio or

signs of systemic toxicity, was not evident, but the test was performed at dose levels exceeding the limit concentrations specified in the current guideline. The study is therefore considered valid and acceptable.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.4.1/013
Report author	[redacted]
Report year	1989
Report title	Mutagenicity study of glyphosate in NMRI mice using the micronucleus test
Report No	Not reported
Document No	Not reported
Guidelines followed in study	No guideline followed. The study was conducted according to the main criteria of OECD 474 (1983).
GLP	No, not conducted under GLP/ Officially recognised testing facilities. When the study was conducted, GLP was not compulsory.
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	Glyphosate active ingredient (batch and purity not reported) was tested for induction of micronuclei in the bone marrow of NMRI mice. Based on the results of a preliminary toxicity study (data not shown), three groups of 7 mice/sex were treated with 2000 mg/kg bw glyphosate. The test substance was dissolved in water plus Tween 80 and administered as a single oral dose by gavage. In addition, groups of 5 mice/sex were included as control animals and received either the solvent (water plus Tween 80) by oral gavage or the positive control cyclophosphamide (100 mg/kg bw) by intraperitoneal injection. Glyphosate-treated animals were sacrificed 24, 48 and 72 hours after treatment and bone marrow slides were prepared. Sampling of the solvent control group was performed 48 hours after dosing and sampling of the positive control group was conducted 24 hours after treatment. Smears were prepared from the femoral bone marrow of each animal. About 1000 polychromatic erythrocytes (PCE) per animal were scored for the presence of micronuclei. In addition, the amount of normochromatic erythrocytes (NCE) among 1000 PCE was determined.
Short description of results:	Mortality and clinical signs of toxicity were not reported. However, from the result tables it became obvious that 1/7 glyphosate-treated males of the 48-hour sampling group died during the course of the study. In addition, no results were scored for 1/7 glyphosate-treated males of the 72 hour sampling group. It was not specified why no scoring was performed for the animal, therefore it cannot be excluded that mortality has occurred. Without a dose-response relationship it can be assumed that if mortality occurred, it was not related to treatment.

	There was no significant increase in the number of NCE among 1000 PCE when compared to solvent controls for any sampling time point, indicating that no bone marrow toxicity was evident. In addition, there was no statistically significant increase in the frequency of micronucleated PCE (mPCE) or NCE at any sampling time point. A marked increase in the incidence of mPCE was observed for the positive control. Based on the experimental findings and under the conditions of the test, glyphosate active ingredient did not induce micronuclei in the bone marrow of male and female NMRI mice *in vivo*.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	The study was considered not acceptable, due to a large number of guideline deviations and reporting deficiencies. The test material purity and batch no. were not indicated. According to the current guideline OECD 474 (2016), at least 4000 polychromatic erythrocytes per animal should be evaluated for the presence of micronuclei. In the present study only 1000 polychromatic erythrocytes were evaluated. The guideline further recommends to include a negative/ vehicle control for every sampling time point or at least at the first sampling time point. In the present study the sampling of the solvent control animals was performed 48 hours after treatment, which represents the second sampling time point. Bone marrow exposure, indicated by a reduced polychromatic to normochromatic erythrocyte ratio, was not observed and systemic toxicity was not reported. In addition, mortality was not reported, however, from the result tables it became obvious that 1/7 glyphosate-treated males of the 48-hour sampling group died during the course of the study. In addition, no results were scored for 1/7 glyphosate-treated males of the 72 hour sampling group. It was not specified why no scoring was performed for the animal, therefore it cannot be excluded that mortality has occurred. In addition, evaluation criteria were not specified in the study report and no data on proficiency and/or historical control data were provided. The deviations were considered to compromise the validity of the study.
Reasons why the study report is not available for submission	Not applicable
Category study in AIR5 dossier (L-docs)	Category 3b

Micronucleus tests in the bone marrow of rats *in vivo*:

1. Information on the study

Data point	CA 5.4.2/014
Report author	[redacted]
Report year	2009
Report title	Micronucleus test of Glyphosate TC in Bone Marrow Cells of the CD Rat by oral administration
Report No	23917
Document No	Not reported
Guidelines followed in study	OECD 474 (1997); Commission Directive 2000/32/EC B.12 (2000), USA EPA, JMAFF

Deviations from current test guideline OECD 474 (2016)	According to the current guideline OECD 474 (2016), at least 4000 polychromatic erythrocytes per animal should be evaluated for the presence of micronuclei. In the present study 2000 erythrocytes were evaluated (requirement of previous OECD guideline (1997)). Bone marrow exposure, indicated by a reduced polychromatic to normochromatic erythrocyte ratio, was not confirmed and there was no systemic toxicity observed, but dose levels included limit concentrations specified in the current guideline. In addition, historical control data were provided for solvent controls, but not for positive controls.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid[#]
Category study in AIR 5 dossier (L docs)	Category 2a

#: Following the feedback from the Assessment Group Glyphosate in March 2020, the Notifier is aware of concerns regarding work conducted at this specific contract laboratory ([redacted]). For the sake of completeness, this study is included in the dossier submission since it was part of the data package of previous EU evaluation processes.

2. Full summary

Glyphosate technical (batch: 20080801, purity: 98.8 %) was tested for genotoxicity in CD rats using the micronucleus test. Based on the results of a preliminary toxicity study in which no signs of toxicity nor mortality were observed up to a dose level of 2000 mg/kg bw, 2000 mg/kg bw were selected as top dose for the main micronucleus study. Groups of 5 rats per sex were administered a single oral dose of 500, 1000 and 2000 mg glyphosate technical/kg bw dissolved in 0.8 % aqueous hydroxypropylmethyl cellulose at a constant dosage volume of 20 mL/kg bw. Vehicle control animals treated with 0.8 % aqueous hydroxypropylmethyl cellulose in an identical manner and positive control group animals receiving an intraperitoneal injection of 27 mg/kg bw cyclophosphamide were included in the experiment. All animals were observed for clinical signs of toxicity. Bone marrow sampling was performed 24 hours after treatment for all dose groups including the vehicle and positive controls and additionally after 48 hours for the control and high dose group. 2000 polychromatic erythrocytes (PCE) per animal were scored for the incidence of micronucleated PCE. In addition, the ratio of PCE to normochromatic erythrocytes (NCE) was determined for each animal by counting a total of 1000 erythrocytes.

Oral administration of glyphosate technical up to 2000 mg/kg bw did not induce any signs of systemic toxicity in the animals.

There was no difference in the ratio of PCE to NCE observed in test item treated rats when compared to the vehicle controls, indicating that no relevant cytotoxicity in bone marrow occurred. No increase in micronucleated PCE was observed in the treated groups when compared to the corresponding vehicle control group for any dose level and sampling time points. The positive reference item group exhibited a statistically significant increase in the number of micronucleated PCE, demonstrating the functionality of the test system.

Based on the results of the test and under the experimental conditions chosen, there is no evidence for the test item to induce clastogenicity/aneugenicity in rats *in vivo*.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material: Glyphosate Technical

Identification:	37/064/08
Description:	White solid
Lot/Batch #:	20080801
Purity:	98.8 % (w/w)
Stability of test compound:	The stability of the test item at storage conditions (at room temperature, in the dark) was guaranteed until the expiry date 01 Aug 2010. The stability of the test item in vehicle was not specified.
Solvent (vehicle) used:	0.8 % aqueous hydroxypropylmethyl cellulose

2. Control materials

Solvent (vehicle) control:	0.8 % aqueous hydroxypropylmethyl cellulose, 20 mL/kg bw administered by oral gavage
Positive control:	Cyclophosphamide, 27 mg/kg bw in 0.9 % NaCl, 20 mL/kg bw administered by intraperitoneal injection

3. Test animals:

Species:	Rat
Strain:	CD
Sex:	Males and females
Source:	[redacted]
Age at dosing:	32-33 days (males) and 33 - 34 days (females)
Weight at dosing:	106-132 g (males) and 88 – 111 g (females)
Acclimation period:	At least 5 days
Diet/Food:	Commercial ssniff® R/M-H V1534 diet (ssniff Spezialdiäten GmbH, Soest, Germany), *ad libitum*, feeding was discontinued approx. 16 hours before administration
Water:	Tap water, *ad libitum*
Housing:	In groups of 2 - 3/sex in Makrolon type III plus cages with granulated textured wood bedding

4. Environmental conditions:

Temperature:	22 ± 3 °C
Humidity:	55 ± 15 %
Photoperiod:	12-hour light and dark cycle

5. Test concentrations and treatment groups:

a) Preliminary toxicity study

Dose levels:	500, 1000 and 2000 mg/kg bw
Dose volume:	20 mL/kg bw
Number of animals:	1/sex
Route of administration:	Oral gavage

b) Main micronucleus test

Dose levels:	500, 1000 and 2000 mg/kg bw
Dose volume:	20 mL/kg bw
Number of animals:	500 and 1000 mg/kg bw: 5/sex 2000 mg/kg bw: 10/sex
Route of administration:	Oral gavage

B: STUDY DESIGN AND METHODS

1. Dates of experimental work: 04 Feb – 06 Mar 2009
Finalisation date: 18 May 2009

2. Animal assignment and treatment:

Preliminary toxicity study:
A preliminary study on acute oral toxicity was performed in male and female rats. One animal per sex received a single dose of 500, 1000 or 2000 mg/kg bw by oral gavage at a constant dosage volume of 20 mL/kg bw. The animals were observed for clinical signs of toxicity and mortality for a period of 3 days.

Main micronucleus test:
Based on the results of the preliminary toxicity study, the dose levels for the main micronucleus test were chosen. The top dose was defined as the dose producing signs of toxicity such that higher dose levels based on the same dosing regimen would be expected to produce lethality or as the dose which produces indications of bone marrow toxicity.

Groups of five rats each received a single oral dose of 500, 1000 and 2000 mg/kg bw glyphosate technical, which was administered at a constant dosage volume of 20 mL/kg bw. Similar constituted groups of control animals received the vehicle (0.8 % aqueous hydroxypropylmethyl cellulose) by oral gavage or the positive control (27 mg/kg bw in 0.9 % NaCl) by intraperitoneal injection. All animals were observed for clinical signs of toxicity before sacrifice.

Bone marrow sampling was performed 24 hours after treatment for all dose groups including the vehicle and positive control and additionally after 48 hours for the control and high dose group only.

3. Slide preparation:

After sacrifice, the femurs of the rats were excised and the bone marrow was flushed out with calf serum. The cells were centrifuged, re-suspended in a drop of calf serum and smears of 30 – 60 mm length were prepared. The smears were air-dried, fixed in methanol and stained with Mayers Haemaleum. Afterwards, the slides were rinsed with cold tap water and further stained with 0.5 % (w/v) ethanolic eosin. The slides were again left to air-dry, cleared in xylene and mounted.

4. Slide evaluation:

The slides were coded and randomized before microscopical analysis. 2000 polychromatic erythrocytes (PCE) per animal were scored for the incidence of micronuclei. In addition, the ratio of PCE to normochromatic erythrocytes (NCE) was determined for each animal by counting a total of 1000 erythrocytes.

5. Statistics:

The frequency of micronuclei and the ratio of PCE/NCE was analysed by comparing glyphosate treated samples to those of the positive and the solvent control. Statistical assessment was carried out using the chi-square test corrected for continuity. For each group, interindividual variation in the numbers of micronucleated PCE was estimated by means of a heterogeneity chi-square test. The numbers of micronucleated PCE in each treated group (males and females, separately and combined) were then compared with the numbers in the vehicle control groups by using a 2 x 2 contingency table to determine chi-square. Probability values of $p \leq 0.05$ were accepted as significant.

6. Acceptance criteria:

The micronucleus test was considered valid when the following criteria were met:

- The heterogeneity chi-square test provided evidence of acceptable variability between animals within a group.
- The incidence of micronucleated PCE in the vehicle control groups fell within or close to the historical vehicle control range of the laboratory.
- At least 7 animals (males and females together) out of each group at each kill were available for analysis.
- The positive reference chemical (CPA) induced clear and statistically significant increases in the frequencies of micronucleated PCE.

7. Evaluation criteria:

The test substance was considered as clearly positive if the following criteria were met:

- A statistically significant increase in the frequency of micronucleated PCE occurred for at least one dose at one sampling time.
- The frequency of micronucleated PCE at such a point exceeded the laboratory's historical control range.
- Corroborating evidence was obtained, for example, increased but statistically insignificant frequencies or micronucleated PCE at other doses or kill times, or dose response profiles.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Analytical determinations were not performed in this study, as not compulsory by the test guideline.

B. PRELIMINARY TOXICITY STUDY

No signs of systemic toxicity or mortality were observed up to the limit dose of 2000 mg/kg bw. Based on the results of the preliminary toxicity study, the dose levels for the main study were selected to be 500, 1000 and 2000 mg/kg bw.

C. MAIN MICRONUCLEUS TEST

Systemic toxicity:

Mortality:

No mortality occurred.

Clinical signs of toxicity:

There were no clinical signs of toxicity observed after administration of glyphosate technical up to the highest reasonable dose level of 2000 mg/kg bw until 48 hours after dosing.

Evaluation of bone marrow slides:

No difference in the ratio of PCE/NCE compared to the vehicle controls was observed in male and female rats treated with glyphosate technical, indicating that no relevant cytotoxicity in bone marrow occurred.

No test item-related increase in micronucleated PCE was observed in any treated group as compared to the corresponding vehicle control group at any of the two sampling time points.
The positive reference item group exhibited a statistically significant increase in the number of micronucleated PCE, demonstrating validity and sensitivity of the test system.

Table 5.4.2-15: Micronucleus test of Glyphosate TC in Bone Marrow Cells of the CD Rat by oral administration ([redacted] 2009), summary of genotoxicity data

Treatment	Dose (mg/kg bw)	Sampling time	Males		Females	
			mPCE ± SD /1000 PCE	PCE/NCE[$]	mPCE ± SD /1000 PCE	PCE/NCE[$]
HPMC	20 mL/kg	24 h	0.8 ± 0.6	0.65 ± 0.10	0.9 ± 0.2	0.65 ± 0.05
	20 mL/kg	48 h	1.0 ± 0.9	0.79 ± 0.20	1.1 ± 0.7	0.84 ± 0.11
HCD[#] mean			1.97	0.87	1.86	0.76
range			0.4 - 5.7	0.26 - 2.94	0.4 - 4.7	0.32 - 1.47
Test item	500	24 h	0.5 ± 0.6	0.65 ± 0.07	0.6 ± 0.7	0.65 ± 0.14
	1000	24 h	0.4 ± 0.2	0.77 ± 0.21	0.8 ± 0.4	0.56 ± 0.08
	2000	24 h	0.6 ± 0.4	0.72 ± 0.18	0.4 ± 0.4	0.59 ± 0.10
	2000	48 h	0.8 ± 0.4	0.98 ± 0.14	0.4 ± 0.4	0.74 ± 0.13
CPA	27	24 h	15.1 ± 5.2*	0.51 ± 0.05	12.0 ± 2.4*	0.55 ± 0.16

mPCE: micronucleated polychromatic erythrocytes, NCE: normochromatic erythrocytes

HPMC: 0.8 % hydroxypropylmethylcellulose, vehicle control

CPA: cyclophosphamide, positive control

[#] HCD Historical control data from the laboratory, average of group means from the most recent background data in 2009, data from 24, 48 and 72 h samplings combined

[$] per 1000 counted cells

* Statistical significance at $p \leq 0.05$ (chi square test)

III. CONCLUSION

Under the conditions of the test and based on the experimental results of the present study, glyphosate technical tested up to the limit dose of 2000 mg/kg bw did not show mutagenic properties in the rat bone marrow micronucleus test. Therefore, the test item was considered to be negative for clastogenicity/aneugenicity in rats.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Negative for clastogenic/aneugenic effects in the bone marrow of CD rats *in vivo*.

The study was performed in compliance with GLP and in accordance with OECD guideline 474 (1997). There were only minor deviations when compared to the current OECD guideline 474 (2016). The number of polychromatic erythrocytes investigated per animal was 2000 (instead of 4000), which was the number recommended by the previous OECD guideline (1997). Bone marrow exposure, indicated by a reduced ratio of polychromatic to normochromatic erythrocytes, was not shown and no systemic toxicity was observed. However, test item doses included the limit dose of 2000 mg/kg bw. Therefore,

the study was considered valid and acceptable.

Assessment and conclusion by RMS:

Chromosomal aberration tests in the bone marrow of mice *in vivo*:

1. Information on the study

Data point	CA 5.4.2/015
Report author	[redacted]
Report year	1994
Report title	Genetic toxicology: *In vivo* mammalian bone marrow cytogenetic test – Chromosomal analysis
Report No	890-MUT-CH.AB
Document No	Not reported
Guidelines followed in study	OECD 475 (1984)
Deviations from current test guideline OECD 475 (2016)	Only 50 metaphase cells/ mouse were investigated for chromosomal aberrations, whereas the currently valid OECD guideline 475 (2016) suggests a total of 200 metaphase cells to be analysed for each animal. The mitotic index was determined for 100 cells instead of 1000 cells. Analysis of metaphase cells was only performed for control group animals and for animals of the high dose group, were toxicity was observed. There was no scoring for chromosome aberrations for lower dose levels where no toxicity occurred. The animals were treated twice, but the use of the second dose was not justified. The cell cycle arrest time was insufficient and the sampling time after the second dose was later (24 hours plus additional 1.5 hours cell cycle arrest) than specified in the current guideline (24 hours after 2nd dose including cell cycle arrest). Acceptance and evaluation criteria were not specified in the study report. Historical control data were not provided.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Glyphosate (batch: 046, purity: 96.8 %) was investigated for its potential to induce chromosomal aberrations in bone marrow in male and female Swiss albino mice. The test item was dissolved in refined groundnut oil and administered by oral gavage. Based on the results of a preliminary toxicity study, in which no signs of systemic toxicity were noted at 5000 mg/kg bw/day, the same dose level was also chosen for the *in vivo* bone marrow chromosomal aberration study. Groups of 5 mice/sex received 50, 500 or 5000 mg/kg bw/day for two consecutive days at a constant dosage volume of 10 mL/kg bw. Similar constituted groups of mice received the solvent or the positive control (50 mg/kg bw/day cyclophosphamide).

The animals were observed for clinical signs of toxicity and mortality twice a day and individual body weights were recorded on study Days 1, 2 and at sacrifice.

24 hours after the second treatment, the animals received an intraperitoneal injection of 0.4 % colchicine. 90 minutes following the injection of colchicine, the animals were sacrificed and bone marrow slides were prepared.
A total of 50 metaphases per mouse (250 per sex per condition) were scored for the presence of chromosomal aberrations and the number of dividing cells (mitotic index) was determined for 1000 cells per animal.

Upon treatment with glyphosate, 2/5 males of the high dose group were dull and had soft stool, which was considered to be incidental. In addition, there was a statistically significant reduction of body weight in females of the high dose group (26.8 ± 0.98 g instead of 32.2 ± 2.13 g in control animals). In animals of both sex, there was a statistically significant reduction in the mitotic index at 5000 mg/kg bw/day, indicating that bone marrow toxicity was evident.

There was no statistically significant and biologically relevant increase in the number of aberrant metaphases or in the incidence of individual chromosomal aberrations observed at the highest dose level of 5000 mg/kg bw/day when compared to solvent control animals. A statistically significant increase in the incidence of gaps in females was observed at 5000 mg/kg bw/day, however, the observations were considered to be without toxicological relevance. As the frequency of aberrant metaphase cells upon treatment with glyphosate was not increased at the highest dose level tested, the slides of the low and the mid dose groups were not investigated.

The frequency of aberrant metaphase cells in solvent control animals was as expected and the number of aberrant metaphases and incidences of aberration types was significantly high in cyclophosphamide treated animals. In line with significant toxicity in the bone marrow of cyclophosphamide treated animals, the results confirm the sensitivity of the animals to respond to chromosomal damaging agents.

Under the conditions of the test and based on the experimental findings, glyphosate did not increase the frequency of chromosomal aberrant metaphases in the bone marrow of male and female mice and was therefore considered negative for cytogenicity *in vivo*.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material: Glyphosate (N-(Phosphonomethyl) glycine)

Identification:	Not specified
Description:	Odorless white crystals
Lot/Batch #:	046
Purity:	96.8 %
Stability of test compound:	The stability of the test item at storage conditions (at ambient temperature) was guaranteed until the expiry date July 1994. The stability of the test item in solvent was not specified. Dosing formulations were prepared fresh prior to treatment. Homogeneity of the test compound suspension was maintained by constant stirring/mixing in mortar during treatment.
Solvent (vehicle) used:	Refined groundnut (peanut) oil

2. Control materials

Solvent (vehicle) control:	Refined groundnut (peanut) oil
Positive control:	Cyclophosphamide (Endoxan-ASTA), 50 mg/kg bw/day

3. Test animals:

Species:	Mouse
Strain:	Swiss albino
Sex:	Male and female
Source:	[redacted]
Age at dosing:	8 - 12 weeks
Weight at dosing:	32 – 38 g (males) and 28 – 32 g (females)
Acclimation period:	At least one week
Fasting period prior administration:	Gold Mohur pelleted mice feed (Lipton India Ltd., Bangalore, India), *ad libitum*
Diet/Food:	Protected, deep borewell water, passed through activated charcoal filter and exposed to UV rays in Aquaguard on-line water filter-cum-purifier, *ad libitum*
Water:	In groups of 5/sex in sterilised standard polypropylene cages measuring 290 x 220 x 140 mm with paddy husk bedding
Housing:	Mouse

4. Environmental conditions:

Temperature:	22 ± 3 °C
Humidity:	40 - 70 %
Photoperiod:	12-hour light and dark cycle

5. Test concentrations and treatment groups:

a) Preliminary toxicity study

Dose levels:	2000 and 3000 mg/kg bw/day
Concentrations:	Not specified
Dose volume:	Not specified
Number of animals:	2/sex/group
Route of administration:	Oral gavage

b) Main cytogenicity test

Dose levels:	50, 500 and 5000 mg/kg bw/day
Concentrations:	5.1, 51.5 and 334.0 mg/mL
Dose volume:	10 mL/kg bw (low and mid dose), 15 mL/kg bw (high dose)
Number of animals:	5/sex/group
Route of administration:	Oral gavage

B: STUDY DESIGN AND METHODS

1. Dates of experimental work: 11 Jan – 09 Feb 1993
Finalisation date: 22 Jan 1994

2. Animal assignment and treatment:

Preliminary toxicity study:
In a range-finding study two male and two female mice were administered dose levels of 2000 and 3000 mg/kg bw/day for two consecutive days to determine the maximum tolerated dose (MTD). Based on the results of the preliminary toxicity study, dose levels for the bone marrow chromosomal aberration study were chosen.

Main micronucleus test:
Groups of five mice per sex were administered for two consecutive days at doses of 50, 500 and 5000 mg/kg bw/day. The test item was dissolved in refined groundnut (peanut) oil and administered via oral gavage at a constant dosage volume of 10 mL/kg bw. Similar constituted groups of 5 mice/sex were treated with the solvent or positive control (50 mg/kg bw/day cyclophosphamide).
The animals were observed for clinical signs of toxicity and mortality twice a day and individual body weights were recorded on study Days 1, 2 and at sacrifice.

24 hours after the second treatment, the animals received an intraperitoneal injection of 0.4 % colchicine at a dosage volume of 10 mL/kg bw. 90 minutes later, the animals were sacrificed by cervical dislocation and bone marrow slides were prepared.

3. Slide preparation:
After sacrifice, the femur bones from both sides were removed, dissected and bone marrow cells were flushed out with 0.56 % potassium chloride solution. The cell suspensions underwent hypotonic treatment at 37 °C for 15 minutes, followed by centrifugation and drop-wise re-suspension in freshly prepared methanol and glacial acetic acid (3:1) fixative. The cells were fixed two times for 10 minutes, followed by 1 hour fixation in the refrigerator. The cell suspension was dropped onto a clean chilled slide and flame dried. Staining was performed with 2 % Giemsa for 10 minutes, afterwards the slides were rinsed with distilled water, blow dried, immersed in xylene and coverslip mounted with DPX.

4. Metaphase analysis:
Slides were coded and 50 metaphase cells per animal (250 per condition) were scored for chromosomal aberrations. Number and frequency of metaphase cells were recorded and evaluated for aberrations such chromatid or chromosome gaps, breaks, acentric fragments, ring chromosomes, multiple chromatid breaks, pulverization, polyploidy and exchange figures.

In addition, toxicity was evaluated based on the mitotic index, representing the frequency of metaphase cells in 100 cells per slide.

5. Statistics:
Intra-group body weight changes during the treatment period were compared by paired t-test. Chromosomal aberrations in treated and positive control animals versus solvent control animals were statistically analysed by Z-test.

6. Acceptance criteria:
Acceptance criteria were not specified in the study report.

7. Evaluation criteria:
Evaluation criteria were not specified in the study report.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS
Analytical determinations were not performed in the study, as not compulsory by the test guideline.

B. PRELIMINARY TOXICITY STUDY

There were no clinical signs of toxicity and no changes in body weight observed. The maximum tolerated dose (MTD) was determined to be > 3000 mg/kg bw/day. Based on the results of the dose-range finding study, the dose levels for the bone marrow cytogenicity study were selected.

C. BONE MARROW CHROMOSOMAL ABERRATION TEST

Systemic toxicity:

Mortality:

No mortality occurred.

Clinical signs of toxicity:

There were no treatment-related signs of systemic toxicity at 50 and 500 mg/kg bw/day. In the high dose group, 2/5 males were dull and had soft stool. The findings were considered toxicologically not relevant.

Body weight:

At sacrifice, there was a statistically significant reduction in body weight development of females of the high dose group. The observation was attributed to treatment and considered toxicologically relevant.

Table 5.4.2-16: Genetic toxicology: *In vivo* mammalian bone marrow cytogenetic test – Chromosomal analysis ([redacted] 1994), body weight changes

Group	Dose [mg/kg bw/day]	Males			Females		
		Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Solvent		35.2 ± 2.04	35.6 ± 1.49	36.0 ± 1.79	28.8 ± 0.98	29.2 ± 0.98	26.8 ± 1.60
Test item	50	36.0 ± 1.26	35.6 ± 0.80	35.2 ± 1.60	28.4 ± 1.60	34.0 ± 1.79	32.3 ± 2.13
	500	34.8 ± 1.60	34.0 ± 1.79	32.3 ± 2.13	29.6 ± 0.80	28.8 ± 0.98	29.2 ± 0.98
	5000	34.8 ± 1.60	36.0 ± 1.26	35.2 ± 1.60	30.8 ± 0.98	28.8 ± 1.60	26.8 ± 0.98*
CPA	50	36.8 ± 1.60	36.8 ± 1.60	35.4 ± 2.30	29.6 ± 1.49	29.6 ± 1.49	29.2 ± 1.60

CPA: Cyclophosphamide, positive control

* Statistically significantly decreased compared to control by paired t-test.

Evaluation of bone marrow slides:

There was no statistically significant and biologically relevant increase in the number of aberrant metaphases or in the incidence of individual chromosomal aberrations observed at the highest dose level of 5000 mg/kg bw/day when compared to solvent control animals.

A statistically significant increase in the incidence of gaps in females was observed at 5000 mg/kg bw/day; in both, males and females of the 5000 mg/kg bw/day group there was once incidence of each chromosome exchange figure (statistically not significant). The observations were both considered to be without toxicological relevance.

In addition, there was a statistically significant reduction in the mitotic index at 5000 mg/kg bw/day, indicating that bone marrow toxicity was evident.

As the frequency of aberrant metaphase cells upon treatment with glyphosate was not increased at the highest dose level tested, the slides of the low and the mid dose groups were not investigated.

The frequency of aberrant metaphase cells in solvent control animals was as expected and the number of aberrant metaphases and incidences of aberration types was significantly high in cyclophosphamide

treated animals. In line with significant toxicity in the bone marrow of cyclophosphamide treated animals, the results confirm the sensitivity of the animals to respond to chromosomal damaging agents.

Table 5.4.2-17: Genetic toxicology: *In vivo* mammalian bone marrow cytogenetic test – Chromosomal analysis ([redacted] 1994), summary of genotoxicity data in rats

Compound	Dose [mg/kg bw/day]	Sampling time point	Number of animals (♂ + ♀)	No. of metaphases scored	Genotoxicity					Judge	Mitotic index [%]
					No. structural aberrant cells		% structural aberrant cells		PI		
					incl. gaps	**excl. Gaps**	incl. gaps	**excl. gaps**	No. polyploid cells		
Males											
Solvent (HBSS)	/	24 h	5	250	18	**12**	7.20	**4.80**	0	negative	13.34
Test item	5000	24 h	5	250	15	**10**	6.00	**4.00**	1	negative	8.87*
Positive control (CPA)	50	24 h	5	250	164*	**139***	65.5*	**55.6***	2	positive	14.68
Females											
Solvent (HBSS)	/	24 h	5	250	15	**10**	6.00	**4.00**	0	negative	17.42
Test item	5000	24 h	5	250	27	**11**	10.80	**4.40**	1	negative	9.54*
Positive control (CPA)	50	24 h	5	250	171*	**155***	68.4*	**62***	0	positive	5.53*
Both sexes combined											
Solvent (HBSS)	/	24 h	10	500	33	**22**	13.20	**8.80**	0	negative	15.30
Test item	5000	24 h	10	500	42	**21**	16.80	**8.40**	2	negative	9.20*
Positive control (CPA)	50	24 h	10	501	335*	**294***	134*	**117.6***	2	positive	10.10*

CPA: Cyclophosphamide

*: Statistically significantly when compared to control by "Z" test

PI: polyploid cells

III. CONCLUSION:

Based on the results of the present study, glyphosate did not increase the frequency of chromosomal aberrant metaphases in the bone marrow of male and female mice. Thus, the test item was considered negative for cytogenicity *in vivo* under the conditions of the test.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Negative for chromosome aberrations in the bone marrow of male and female Swiss albino mice *in vivo*.

The study was performed in compliance with GLP and according to OECD guideline 475 (1984). When compared to the current OECD guideline 475 (2016), a number of deviations became evident. The number of metaphase cells scored for chromosome aberrations was 50, whereas 200 metaphases should be evaluated according to the current guideline. Likewise, the mitotic index was determined for 100 cells instead of 1000 cells. The animals were treated twice, but the use of the second dose was not justified.

Bone marrow exposure was indicated by a reduction in the mitotic index and clinical signs of toxicity and impaired body weight analysis were observed in animals of the high dose group. Analysis of metaphase cells was only performed for control group animals and for animals of the high dose group, were toxicity was observed. There was no scoring for chromosome aberrations for lower dose levels where no toxicity occurred. However, as no induction of chromosomal aberrations was observed at this concentration, it can be assumed that the number of chromosome aberrations was comparatively low at lower test item doses. Further deviations to the current guideline were considered to be of minor degree. Due to the large number of deviations and because only a very low number of metaphase cells was investigated per animal, the study was considered to provide supporting information.

Evidence of bone marrow exposure was used to read across to micronucleus assays in mice of the same strain reported by the same study authors.

Assessment and conclusion by RMS:

Chromosomal aberration tests in the bone marrow of rats *in vivo*:

1. Information on the study

Data point	CA 5.4.2/016
Report author	[redacted]
Report year	1983
Report title	*In Vivo* Bone Marrow Cytogenetics Study of Glyphosate in Sprague-Dawley Rats
Report No	830083
Document No	M-645019-01-1
Guidelines followed in study	No guideline followed, study was conducted similar to OECD 475 (2016)
Deviations from current test guideline OECD 475 (2016)	The test item was administered by intraperitoneal injection, which does not represent an intended route for human exposure. In addition, the test was conducted as limit test with a dose level of 1000 mg/kg bw, whereas the current OECD TG 475 suggests a dose level of 2000 mg/kg bw. There was no evidence that the test item was administered at a dose level which induces toxicity, as no clinical signs of systemic toxicity were observed and no bone marrow toxicity became evident. Furthermore, no body weight data or individual toxicity data were reported. In addition, the mitotic index as an indicator for cytotoxicity was not calculated and the number of polyploid

	cells was not determined. A total of 50 metaphase cells/rat were investigated for chromosomal aberrations, whereas the currently valid OECD guideline 475 (2016) suggests that at least 200 metaphases should be analysed for each animal. In addition, sampling time points for bone marrow cells was 6, 12 and 24 hours, but a latter sampling time point (24 hours after the first sampling time point) was not included. The test was conducted with 3 rats per sex (6 rats per dose level), whereas the current OECD TG 475 recommends to conduct the test in 5 rats/sex or in 5 rats of one sex. The weight of the animals was not reported. In addition, acceptance and evaluation criteria were not specified in the study report. Historical control data were not provided.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, not conducted under GLP /Officially recognised testing facilities. When the study was performed, GLP was not compulsory.
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

A mammalian bone marrow chromosomal aberration test was performed in male and female Sprague-Dawley rats in order to investigate clastogenic effects of glyphosate (batch: T830044, purity: 98.7 %) *in vivo*.

The test substance was dissolved in Hank's Buffered Salt Solution (HBSS) and administered to 3 groups of fasted animals via intraperitoneal injection. All three groups of 3 rats per sex received a test item dose of 1000 mg/kg bw. Similarly constituted groups of rats received the vehicle (HBSS) or the positive control cyclophosphamide and served as controls. About 4, 10 and 22 hours following treatment, each one group of test item and solvent control animals was injected with 2 mg/kg bw colchicine. Two hours after colchicine administration (6, 12 and 24 hours after treatment), the animals were sacrificed and bone marrow slides were prepared for each animal. Bone marrow samples for the positive control group animals were prepared from the 24 hour sampling time only.

A total of 50 metaphases per animal (300 per sex per condition) were scored for the presence of chromosomal aberrations.

Clinical signs of systemic toxicity were not reported for either control or glyphosate treated animals and no signs of bone marrow toxicity became evident.

A significant increase in the number of aberrant metaphases was not observed for any of the three sampling time points upon treatment with glyphosate. The type and frequency of any chromosomal aberrations in glyphosate-treated rats was comparable to those of solvent control animals.

In contrast, rats treated with the positive control cyclophosphamide showed a strong increase in the frequency of structural chromosome aberrations about 24 hours after treatment. In addition, a reduction in metaphase cells was observed for these animals, indicating that bone marrow toxicity was evident. As solvent and positive control animals showed the expected results, the sensitivity of the test system and the capability of the animals to respond to clastogenic substances was demonstrated.

Based on the results of the experiment and under the tested conditions, glyphosate was considered negative for clastogenicity in male and female rats *in vivo*.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material:

Identification:	Glyphosate
Description:	White powder
Lot/Batch #:	T830044
Purity:	98.7 %
Stability of test compound:	The stability of the test item was not reported. Storage at room temperature was recommended. The stability of the test item in solvent was not specified.
Solvent (vehicle) used:	Hank's Buffered Salt Solution (HBSS)

2. Control materials

Solvent (vehicle) control:	Hank's Buffered Salt Solution (HBSS)
Positive control:	Cyclophosphamide, 25 mg/kg bw in HBSS

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley (Crl: CD®(SD)BR)
Sex:	Male and female
Source:	[redacted]
Age at dosing:	Approx. 9 weeks
Weight at dosing:	Not specified
Acclimation period:	Approx. 7 days
Fasting period prior administration:	14 – 24 hours
Diet/Food:	Purina Laboratory Rodent Chow® No. 5002 (Ralston-Purina Company, St. Louis, Missouri, USA), *ad libitum*
Water:	Tap water supplied by the public water system, *ad libitum*
Housing:	In stainless steel mesh cages suspended over absorbent paper bedding

4. Environmental conditions:

Temperature:	21 - 23°C
Humidity:	35 -60 %
Photoperiod:	12-hour light and dark cycle

5. Test concentrations and treatment groups:

Dose levels:	1000 mg/kg bw
Concentrations:	100 mg/mL
Dose volume:	10 mL/kg bw
Number of animals:	6 males and 6 females
Route of administration:	Intraperitoneal injection

B: STUDY DESIGN AND METHODS

1. Dates of experimental work: 08 – 12 Aug 1983
Finalisation date: 20 Oct 1983

2. Animal assignment and treatment:

Three groups of 3 rats per sex (6 rats/ group in total) received an intraperitoneal injection of 1000 mg/kg bw glyphosate, which was administered at a constant dosage volume of 10 mL/kg bw. Prior to treatment, the animals were fasted for 14-24 hours. Similar constituted groups of control animals received the vehicle (HBSS) or the positive control (25 mg/kg bw cyclophosphamide in HBSS).

About 4, 10 and 22 hours following treatment, each one group of test item and solvent control animals was injected with 2 mg/kg bw colchicine. Two hours after colchicine administration (6, 12 and 24 hours after treatment), the animals of the test item and the solvent control group were sacrificed by CO_2 asphyxiation and severance of the spinal cord. Bone marrow slides from the positive control group were sampled 24 hours after treatment only.

3. Slide preparation:

Immediately after sacrifice, both femurs of the animals were dissected and marrow was aspirated from each femur and mixed with HBSS. The cells were pelleted by centrifugation and re-suspended in 0.075 M potassium chloride. After removal of gross debris, the cells were incubated in a water bath at 37 °C for 30 minutes, and subsequently fixed with Cornoy's fixative (methanol/glacial acetic acid 3:1 v/v). The cells were centrifuged again, re-suspended in fixative, dropped onto clean wet slides and flamed to facilitate spreading of chromosomes. The slides were air-dried and stained with 2 % Giemsa solution for 15-20 minutes, rinsed with water, air-dried again and covered with cover slips.

4. Metaphase analysis:

Slides were randomly coded and approximately 50 mitotic cells per animal (300 per condition) were scored for chromosomal aberrations. Number and frequency of metaphase cells were recorded and evaluated for structural chromosomal aberrations such as dicentric chromosomes, ring chromosomes, chromosome deletions, gaps. In addition, chromatid aberrations and the number of aneuploidy cells were recorded.

5. Statistics:

The student's t-test was used for data analysis.

6. Acceptance criteria:

Acceptance criteria were not specified in the study report.

7. Evaluation criteria:

Evaluation criteria were not specified in the study report.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Analytical determinations were not performed.

B. BONE MARROW CHROMOSOMAL ABERRATION TEST

Systemic toxicity:

Mortality:

No mortality occurred.

Clinical signs of toxicity:

No clinical signs of systemic toxicity were reported.

Evaluation of bone marrow slides:

A significant increase in the number of aberrant metaphases was not observed for any of the three sampling time points upon treatment with glyphosate. Although a small number of chromatid aberrations was noted 6, 12 and 24 hours after treatment, the frequency was comparable for glyphosate-treated and solvent control animals. In addition, there was no evidence for bone marrow toxicity in any of the test item treated groups.

A huge increase in the frequency of chromosomal aberrant cells, accompanied with strong evidence for bone marrow toxicity was observed for the positive control cyclophosphamide 24 hours after treatment, demonstrating the animal's capability to respond to clastogenic substances and confirming the sensitivity of the test.

Table 5.4.2-18: *In Vivo* Bone Marrow Cytogenetics Study of Glyphosate in Sprague-Dawley Rats ([redacted], 1983), summary of genotoxicity data

Compound	Dose [mg/kg bw]	Sampling time point	Number of animals (♂ + ♀)	No. of metaphases scored	Genotoxicity							
					No. structural aberrant cells	% structural aberrant cells	No. of aberrations		Rel. No. of structural aberrations §		Numerical aberrations	Judge
					incl. gaps#	incl. gaps	incl. gaps	excl. gaps	incl. gaps	excl. Gaps	No. aneugenic cells$	
Solvent (HBSS)	/	6 h	6	600	12	2.00	13	8	2.17	1.33	35	negative
		12 h	6	575	7	1.22	5	2	0.87	0.35	48	negative
		24 h	6	565	10	1.77	12	4	2.12	0.71	46	negative
Test item	1000	6 h	6	600	16	2.67	18	6	3.00	1.00	43	negative
		12 h	6	577	13	2.25	14	5	2.43	0.87	42	negative
		24 h	6	492	13	2.64	13	7	2.64	1.42	35	negative
Positive control (CP)	25	24 h	6	277	113	40.79	351	314	126.71	113.36	23	positive

CP: Cyclophosphamide

#: Calculated based on raw data from study report as follows: no of cells scored - no of normal cells (incl. aneuploid cells)

$: Almost all aneuploid cells were minus one chromosome, and since slides had been flamed, these cells were considered to be

Table 5.4.2-18: *In Vivo* Bone Marrow Cytogenetics Study of Glyphosate in Sprague-Dawley Rats (■, 1983), summary of genotoxicity data

Compound	Dose [mg/kg bw]	Sampling time point	Number of animals (♂ + ♀)	No. of metaphases scored	Genotoxicity							Judge
					No. structural aberrant cells	% structural aberrant cells	No. of aberrations		Rel. No. of structural aberrations §		Numerical aberrations	
					incl. gaps#	incl. gaps	incl. gaps	excl. gaps	incl. gaps	excl. Gaps	No. aneugenic cells$	

technical artefacts

§: in 100 metaphases, calculated as follows: = (100/number of metaphases scored) x number of aberrations

III. CONCLUSION

Based on the results of the present study, glyphosate did not increase the frequency of chromosomal aberrant metaphases in the bone marrow of male and female rats. Thus, the test item was considered negative for clastogenicity in vivo under the conditions of the test.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Negative for chromosome aberrations in the bone marrow of male and female Sprague-Dawley rats *in vivo*.

The study was conducted not compliant with GLP but similar to OECD guideline 475 (2016). However, a number of deviations became evident. The test item was administered by intraperitoneal injection, which does not represent an intended route for human exposure. In addition, the test was conducted as limit test with a dose level of 1000 mg/kg bw, whereas the current OECD TG 475 suggests a higher dose level of 2000 mg/kg bw. There was no evidence that the test item was administered at a dose level which induced toxicity, as no clinical signs of systemic toxicity were observed and no bone marrow toxicity became evident. However, due to the route of exposure selected (intraperitoneal injection) it can be assumed that the bone marrow was exposed. In addition, the mitotic index as an indicator for cytotoxicity was not calculated and the number of polyploid cells was not determined. A total of 50 metaphase cells/rat were investigated for chromosomal aberrations, whereas the currently valid OECD guideline 475 (2016) suggests that at least 200 metaphases should be analysed for each animal.

Further deviations were considered to be of minor degree. Due to the large number of guideline deviations, the study was considered to provide supporting information.

Assessment and conclusion by RMS:

CA 5.4.3 *In vivo* studies in germ cells

In the previous 2001 EU glyphosate evaluation genotoxic effects on germ cells were examined in dominant

lethal assays in rats and mice. In both species no genotoxic effect of glyphosate on germinal tissues was found. No new studies were performed since the last review.

Table 5.4.3-1: Summary of *in vivo* genotoxicity testing with glyphosate acid in germ cells

Annex Point	Study	Study type Test system, Dosing and Mating	Substance Dose levels Purity	Reference list-related category§	Result
CA 5.4.3/001 CA 5.4.3/002 CA 5.4.3/003	[redacted] 1992	Dominant lethal Wistar rats (30 ♂/group, 30 ♀/group and mating interval) Oral gavage (single treatment) 10 successive one-week mating periods (1:1 sex ratio)	Glyphosate 200, 1000 and 5000 mg/kg bw Purity: 96.8 %	Supportive, Category 2a	negative
CA 5.4.3/004	[redacted], 1982	Dominant lethal CFY rats (20 ♂/group, 40 ♀/group and mating interval) Oral feed for 8 weeks via the diet 4 successive one-week mating periods (1:2 sex ratio)	Glyphosate active principle 10, 30 and 100 mg/kg bw/day (nominal dose) Purity: not reported	Supportive, Category 3a	negative
CA 5.4.3/005	[redacted] 1980	Dominant lethal CD-1 mice (10 ♂/group/20 ♀/group and mating interval) Oral gavage (single treatment) 8 successive one-week mating periods (1:2 sex ratio)	Technical Glyphosate 200, 800 and 2000 mg/kg bw Purity: 98.7 %	Supportive, Category 2a	negative

§: The category describes the acceptability/reliability of the study within the AIR 5 submission. This category does not describe hazard classification. (for details please refer to the Doc ID: 110054-B-GRG_Jun_2020)

1. Information on the study

Data point	CA 5.4.3/001, CA 5.4.3/002 and CA 5.4.3/003
Report author	[redacted]
Report year	1992
Report title	Dominant lethal test in Wistar rats
Report No	TOXI: 888-DLT
Document No	Not reported
Guidelines followed in study	OECD 478 (1984)
Deviations from current test guideline OECD 478 (2016)	Dose levels were spaced with a factor of 5, which is above the factors (2 – 4) specified in the current OECD guideline 478 (2016). Necropsy was performed on Day 16 after pairing rather than on gestation Day 16. The number of implantations per group for each mating period was below the recommended number of 400 implants per group as required according to OECD 478 (2016). Fetal body weights were not recorded. No information on historical control data was provided. There was no raw data on individual animals treated with the positive control provided. The mean pre- and post-implantation losses per dam were not calculated, but percentages of pre-implantation losses, as well as percentages of post-implantation losses (corresponding to the dominant lethal factor) were reported. Acceptance and evaluation criteria were not specified in the study report. Statistical assessment of under- and overdispersion was not performed.
Previous evaluation	Yes, accepted in RAR (2015)

GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Glyphosate (batch: 1071-83-6, purity: 96.8 %) was investigated in a dominant lethal study in Wistar rats. The test item was dissolved in refined groundnut oil and administered by oral gavage to groups of 30 males at dose levels of 200, 1000 and 5000 mg/kg bw (single dose) and a constant dosage volume of 10 mL/kg bw. A similar constituted group of males received the solvent control. Two positive control groups of 5 males received ethylmethanesulphonate either administered at a treatment regime of 100 mg/kg bw/day for 5 consecutive days or as single dose of 500 mg/kg bw.

Immediately after dosing, the males were paired with untreated virgin females (mating ratio 1:1) for 6 days. On the 8th day after treatment, the males were again paired with a new batch of virgin females. The procedure was repeated for 10 consecutive weeks. All animals were observed twice daily for clinical signs. Body weights were recorded for males prior to treatment, on Days 1, 2, 4 and 6 and weekly thereafter. Females were weighed prior to mating and at terminal sacrifice. The females from each mating interval were sacrificed on gestation Day 16. The uterus and ovaries were dissected and investigated for the number of *corpora lutea*, implantations, early resorptions, fetal and embryonic resorptions and the number of live implants. All males were subjected to gross necropsy following the last pairing.

Oral treatment with glyphosate did not induce mortality in any animal. Clinical signs of toxicity were observed predominantly for animals of the high dose group throughout the whole study period and comprised nasal discharge, rough hair coat, snuffling, soft stool/diarrhea and urine incontinence. In addition, the body weight of sires was decreased in the 1000 and 5000 mg/kg bw dose group during the first four days of treatment. Both observations were attributed to treatment and considered toxicologically relevant.

At terminal necropsy, unilateral testicular atrophy was observed in 3/30 males of the high dose group. Male fertility was not impaired upon treatment with glyphosate.

Investigation of the female uteri contents revealed an acute toxic effect of glyphosate at a dose level of 5000 mg/kg bw after the first mating. The number and percentage of pregnant females and the number of implantations per dam were significantly lower than for control animals. In line with these findings, there was an increased incidence of early resorptions and of pre- and post-implantation losses in the animals of this group. In the first mating group, there were no adverse effects on the number of *corpora lutea*, the number of fetal and embryonic resorptions and the number of live implants per dam.

Fertility indices during the remaining 9 study weeks were not considered to be affected by treatment. Although changes of statistical significance were observed for the number of implantations, the number of early, fetal and embryonic resorptions, the number of live implants and the number of pre- and post-implantation losses, these fluctuations and highly variable values for dominant lethality were observed among all dose groups and without any relation to dose or duration of treatment.

Treatment with the positive control ethylmethanesulphonate had statistically significant dominant lethal effects for both treatment regimes. A significant decrease in the incidence of pregnancy to 0 % was observed in the third mating interval and a gradual recovery back to normal by 9 to 10 weeks. In addition, there was an increased incidence of small moles /fetal resorptions during the first week, which

gradually recovered to normal by week 9.

Based on the experimental findings and under the conditions of the test, treatment of males with glyphosate was considered to induce acute toxic effects at 5000 mg/kg bw in male and female animals during the first mating period. Under the conditions of the test, glyphosate did not induce dominant lethal effects in Wistar rats and was therefore considered non-genotoxic to germ cells *in vivo*.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material:

Identification:	Glyphosate
Description:	Odourless, white crystals
Lot/Batch #:	1071-83-6
Purity:	96.8 %
Stability of test compound:	Under storage conditions (at ambient temperature) stability was guaranteed for more than two years. Dosing formulations were prepared fresh prior to treatment.
Solvent (vehicle) used:	Refined groundnut (peanut) oil

2. Control materials

Solvent (vehicle) control:	Refined groundnut (peanut) oil
Positive control:	Ethylmethanesulphonate, 100 mg/kg bw/day for 5 days in distilled water or 500 mg/kg bw as single dose in distilled water

3. Test animals:

Species:	Rat
Strain:	Wistar
Sex:	Males and females
Source:	Bred at [redacted]
Age at study initiation:	20 – 24 weeks
Weight at study initiation:	> 250 g (males) and > 150 g (females)
Acclimation period:	At least 10 days
Diet/Food:	Pelleted rat feed (M/S Lipton India Ltd., Bangalore, India), *ad libitum*
Water:	Deep borewell water, passed through activated charcoal filter and exposed to UV rays in Aquaguard on-line water filter-cum-purifier, *ad libitum*

Housing:	Before treatment: In groups of 15-20/sex in suspended stainless steel wire mesh cages with clean paddy husk bedding. During mating: On a 1:1 basis (male:female) in a standard polypropylene cages measuring 290 x 220 x 140 mm) with sterilized paddy husk bedding. Post mating: Females were housed in groups of 5 in serial order from the same group in standard polypropylene cages measuring 430 x 270 x 150 mm) with sterilized paddy husk bedding.

4. Environmental conditions:

Temperature:	22 ± 3 °C
Humidity:	52 - 70 %
Air changes:	Approximately 10 - 15/hour
Photoperiod:	12-hour light and dark cycle

5. Test concentrations and treatment groups:

Dose levels:	200, 1000 and 5000 mg/kg bw
Dose volume:	10 mL/kg bw
Number of animals:	30 males and 300 females per group (30 females per mating interval)
Route of administration:	Oral gavage

B: STUDY DESIGN AND METHODS

1. Dates of experimental work: 03 Sep 1991 – 31 Jan 1992
Finalisation date: 04 Nov 1992

2. Animal assignment and treatment:

Groups of 30 males per dose received a single administration of glyphosate by oral gavage at dose levels of 200, 1000 and 5000 mg/kg bw at a constant dosage volume of 10 mL/kg bw. Similar constituted groups received the solvent (refined groundnut oil) or the positive control ethylmethane sulfonate. The positive control substance was dissolved in distilled water and administered to two groups of 5 males at a treatment regimen of 100 mg/kg bw/day for 5 consecutive days and as single dose of 500 mg/kg bw.

Immediately after dosing, the males were paired with untreated virgin females (mating ratio 1:1) for 6 days. On Day 8 after treatment, the males were again paired with a new batch of virgin females. The procedure was repeated for 10 consecutive weeks. The females from each mating interval were sacrificed on gestation Day 16.

3. Observations

All animals were observed twice daily during the first week and once daily during the remaining period for signs of toxicity and mortality. The time of onset, degree and duration of clinical signs was recorded.

4. Body weight

The body weight of male animals was recorded prior to treatment, on Days 1, 2, 4 and 6 and weekly thereafter. For females, the initial body weight and the body weight at terminal sacrifice were noted.

5. Sacrifice

All animals were sacrificed under ether anaesthesia.
Males were sacrificed after 10 mating intervals and investigated for gross necropsy changes in the visceral organs. From all sires the following organs were collected and preserved in 10 % neutral buffered formalin: testes, epididymides, seminal vesicles and prostate.

Females from each mating interval were sacrificed on gestation Day 16. The uterus along with the ovaries was dissected and the following parameters were recorded prior to preservation in 10 % neutral buffered formalin:

- Number of *corpora lutea* in each ovary
- Number of implantations
- Number of early resorptions
- Number and percentage of small moles/embryonic resorptions
- Number and percentage of large moles/fetal resorptions
- Number and percentage of live implants

Furthermore, the percentage of pre- and post-implantation losses were determined according to the following formulas:

$$\text{Pre} - \text{implantation loss [\%]} = \frac{\text{Total number of corpora lutea} - \text{Number of implantations}}{\text{Total number of corpora lutea}} \times 100$$

$$\text{Post} - \text{implantation loss [\%]} = \frac{\text{Number of early} + \text{late embryonic deaths}}{\text{Total number of implantations}} \times 100$$

The percentage of post-implantation losses corresponds to the dominant lethal factor.

6. Statistics:

The weekly male body weight, the number of *corpora lutea*, the number of implantations, the number of early resorptions, the number and percentage of small and large moles, the number and percentage of live implants and the percentage of pre- and post-implantation losses were statistically analysed using the Bartlett's test for homogeneity, analysis of variance and Dunnett's multiple pairwise comparison. The number of early resorptions were compared by Mann Whitney test; pregnancy indices were analysed by "Z"-test. Results of the statistical analysis were designated in comparison to the solvent control group.

7. Acceptance criteria:

Acceptance criteria were not specified in the study report.

8. Evaluation criteria:

Evaluation criteria were not specified in the study report.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Analytical determinations were not performed in this study, as not required by the test guideline.

B. OBSERVATIONS

Systemic toxicity:

Mortality:

One sire of the positive control group in the second dose regime (single treatment with 500 mg/kg bw)

died during study week 4. There were no deaths in the dose groups treated with glyphosate.

Clinical signs of toxicity:
Clinical signs of toxicity were observed among all treatment and control groups. Commonly and frequently observed symptoms included nasal discharge, rough hair coat, snuffling, soft stool/diarrhea and urine incontinence. The incidence was increased at 5000 mg/kg bw, which was attributed to treatment and considered toxicologically relevant. In animals of the high dose group, the clinical signs were observed throughout the entire study period.

Table 5.4.3-2: Dominant lethal test in Wistar rats ([redacted] 1992), clinical signs of toxicity observed in sires

Clinical sign of toxicity	Dose group [mg/kg bw]			
	0	200	1000	5000
Nasal discharge	1/30	0/30	2/30	9/30
Rough hair coat	0/30	0/30	0/30	4/30
Snuffling	3/30	0/30	0/30	10/30
Soft stool / diarrhea	1/30	0/30	1/30	5/30
Urine incontinence	0/30	0/30	5/30	1/30

C. BODY WEIGHT AND BODY WEIGHT DEVELOPMENT OF SIRES

Starting body weights of all glyphosate treated groups were statistically significantly higher than those of the solvent control group. The difference continued during the entire study period until final sacrifice. Upon treatment with the test item, body weight of sires was decreased in the 1000 and 5000 mg/kg bw group during the first four days of treatment, but was comparable to those of solvent control treated animals thereafter. The observation was considered treatment-related.

Table 5.4.3-3: Dominant lethal test in Wistar rats ([redacted] 1992), body weights in sires

		Dose group [mg/kg bw]			
		0	200	1000	5000
Days in study week 1	**0**	270 ± 26	303 ± 11*	329 ± 34*	314 ± 32*
	1	285 ± 26	305 ± 13*	317 ± 34*	303 ± 32*
	2	292 ± 25	310 ± 14*	323 ± 34*	297 ± 30*
	4	297 ± 26	313 ± 14*	325 ± 32*	308 ± 32*
	6	304 ± 26	321 ± 15*	332 ± 32*	316 ± 31*
Study week no.	**2**	305 ± 25	320 ± 15*	332 ± 32*	317 ± 32*
	3	322 ± 27	330 ± 18*	350 ± 30*	333 ± 34*
	4	331 ± 32	341 ± 20	350 ± 33	334 ± 33
	5	331 ± 32	344 ± 29*	359 ± 32*	352 ± 35*
	6	342 ± 34	348 ± 25	363 ± 33	352 ± 39
	7	350 ± 34	358 ± 29	360 ± 32	358 ± 33
	8	346 ± 34	362 ± 28*	374 ± 31*	364 ± 35*
	9	351 ± 35	359 ± 30*	376 ± 31*	369 ± 39*
	10	339 ± 43	357 ± 34*	376 ± 34*	371 ± 39*
Sacrifice		347 ± 38	359 ± 33*	388 ± 34*	371 ± 40*

Table 5.4.3-3: Dominant lethal test in Wistar rats ([redacted] 1992), body weights in sires

	Dose group [mg/kg bw]			
	0	200	1000	5000

* Statistically significant difference from control group by Dunnett's test

D. NECROPSY OF SIRES

At terminal sacrifice, unilateral testicular atrophy was noted in 3/30 sires of the high dose group. The effect was considered treatment-related. There were no further gross lesions in visceral organs and the reproductive system observed.

E. FERTILITY INDICES

In the first mating interval (study week 1), the number and percentage of pregnant females was reduced in the high dose group (statistically not significant, refer to 5.4.3-4). The effect was explained by acute toxicity of the test item and in line with increased incidences of early resorptions (refer to Table 5.4.3-7), pre- and post-implantation losses (refer to Table 5.4.3-11 and Table 5.4.3-12). Pregnancy status at 200 and 1000 mg/kg bw was not affected by treatment. Within the remaining 9 mating intervals, there were no changes in the number and percentage of pregnant females.

Table 5.4.3-4: Dominant lethal test in Wistar rats ales ([redacted] 1992), number and percentage of pregnant females

	Group				
	1	2	3	4	PC
	Dose [mg/kg bw]				
	0	200	1000	5000	100/500[x]
No. of pregnant females (%)					
Mating study week					
1	29 (97)	28 (93)	29 (97)	23* (77) ↓	10 (100)
2	25 (83)	29 (97)	23 (77)	26 (87)	8 (80)
3	27 (90)	26 (87)	26 (87)	25 (83)	0 (0)
4	28 (93)	29 (97)	26 (87)	26 (87)	2 (22)
5	27 (90)	29 (97)	28 (93)	27 (90)	6 (67)
6	27 (90)	27 (90)	26 (87)	29 (97)	7 (78)
7	28 (93)	23 (77)	29 (97)	25 (83)	7 (78)
8	28 (93)	26 (87)	27 (90)	29 (97)	8 (89)
9	24 (80)	26 (87)	28 (93)	30 (100)	8 (89)
10	25 (83)	59 (97)	28 (93)	29 (97)	9 (100)

PC: positive control EMS: Ethylmethanesulphonate, [x]combined data for both treatment regimens (100 mg/kg bw/day for 5 days in distilled water or 500 mg/kg bw as single dose in distilled water)

* $p < 0.05$ statistical significance when compared to controls, ↓ decrease, ↑ increase

The number of *corpora lutea* per dam was not affected by treatment (refer to Table 5.4.3-5). A decreased number of *corpora lutea* at 1000 mg/kg bw among the females of the first mating interval and a significant

increase in the number of *corpora lutea* at 5000 mg/kg bw in the females of the third mating interval were considered incidental.

Table 5.4.3-5: Dominant lethal test in Wistar rats ([redacted] 1992), number of *corpora lutea*

	Group				
	1	2	3	4	PC
	Dose [mg/kg bw]				
	0	200	1000	5000	100/500^x
No. of *corpora lutea*: Mean per dam ± SD					
Mating study week					
1	12.4 ± 1.66	12.1 ± 1.27	11.1 ± 2.03* ↓	12.4 ± 3.58	11.0 ± 2.14*
2	11.6 ± 1.98	11.5 ± 1.96	10.4 ± 1.41	12.3 ± 2.26	12.0 ± 6.65
3	10.9 ± 1.99	11.2 ± 2.54	9.88 ± 1.58	12.4 ± 1.44* ↑	0.0 ± 0.0
4	10.3 ± 1.70	11.9 ± 2.01	12.2 ± 2.51	11.7 ± 2.61	12.0 ± 5.08*
5	11.7 ±1.54	10.6 ± 1.68	12.4 ± 2.13	12.1 ± 1.40	12.3 ± 6.74
6	11.4 ± 2.04	11.2 ± 2.27	11.4 ± 1.72	11.7 ± 2.44	10.7 ± 5.32
7	14.6 ± 1.95	12.4 ± 1.78	12.9 ± 2.43	11.3 ± 1.67	12.7 ± 6.23*
8	13.2 ± 2.11	13.8 ± 2.26	11.0 ± 1.34	10.6 ± 2.01	12.3 ± 5.29
9	14.2 ± 2.53	12.0 ± 2.16	10.6 ± 1.67	12.1 ± 1.53	11.6 ± 5.17*
10	12.1 ± 2.15	12.1 ± 1.98	12.8 ± 2.38	13.0 ± 1.77	12.6 ± 4.45

PC: positive control EMS: Ethylmethanesulphonate, positive control, ^{x}combined data for both treatment regimens (100 mg/kg bw/day for 5 days in distilled water or 500 mg/kg bw as single dose in distilled water)

*: p < 0.05 and ** p < 0.01 statistical significance when compared to controls, ↓ decrease, ↑ increase

Upon treatment with glyphosate, the number of implantations per dam showed fluctuations among all dose groups over the entire study period (refer to Table 5.4.3-6). The statistically significant decrease at 5000 mg/kg bw in females of the first mating interval was attributed to the acute toxic effects caused by the test item and of toxicological relevance. Further, there was a significant increase in the number of implantations per dam during study week 4 at 200 mg/kg bw, a significant decrease in all test item treated groups in study weeks 7 and 8 and in the mid dose group (1000 mg/kg bw) during study week 9. The statistically significant observations in test item-treated groups during mating intervals 4, 7, 8 and 9 were attributed to the high variability of implantation sites among solvent control animals rather than to treatment with glyphosate and therefore considered to be incidental.

Table 5.4.3-6: Dominant lethal test in Wistar rats ([redacted] 1992), number of implantation sites per dam

	Group				
	1	2	3	4	PC
	Dose [mg/kg bw]				
	0	200	1000	5000	100/500^x
No. of implantation sites: Mean per dam ± SD					
Mating study week					
1	11.7 ± 2.39	11.4 ± 1.83	10.8 ± 2.02	8.91 ± 3.60* ↓	9.10 ± 3.17*
2	10.3 ± 2.94	11.2 ± 2.54	9.70 ± 2.10	11.9 ± 2.41	2.63 ± 2.42*
3	10.1 ± 2.16	10.5 ± 2.94	8.92 ± 2.37	11.0 ± 2.21	0.0 ± 0.0
4	9.39 ± 2.10	10.6 ± 2.40* ↑	9.73 ± 3.74	10.2 ± 3.10	2.00 ± 0.97*

Table 5.4.3-6: Dominant lethal test in Wistar rats ([redacted] 1992), number of implantation sites per dam

	Group				
	1	2	3	4	PC
	Dose [mg/kg bw]				
	0	200	1000	5000	100/500[x]
5	10.8 ± 1.69	10.5 ± 1.81	11.1 ± 3.11	10.7 ± .034	4.83 ± 3.90*
6	10.4 ± 2.21	10.4 ± 2.62	10.7 ± 2.79	10.1 ± 2.64	7.57 ± 4.62*
7	13.5 ± 2.36	10.1 ± 3.50*↓	11.0 ± 4.06* ↓	9.88 ± 2.65* ↓	11.4 ± 6.22*
8	11.7 ± 3.40	12.0 ± 3.19	10.4 ± 1.42* ↓	9.41 ± 2.29* ↓	10.0 ± 5.12*
9	12.0 ± 3.75	11.0 ± 2.51	9.57 ± 2.10* ↓	11.1 ± 2.83	11.1 ± 5.13
10	10.8 ± 2.66	11.3 ± 2.90	10.9 ± 3.38	12.3 ± 2.75	11.9 ± 4.19

PC: positive control EMS: Ethylmethanesulphonate, positive control, [x]combined data for both treatment regimens (100 mg/kg bw/day for 5 days in distilled water or 500 mg/kg bw as single dose in distilled water)
*: $p < 0.05$ and ** $p < 0.01$ statistical significance when compared to controls, ↓ decrease, ↑ increase

A statistically significant increase in the number of early resorptions was noted during study week 1 (first mating) at 5000 mg/kg bw, during study week 3 (third mating) at ≥ 1000 mg/kg bw and during study week 4 (fourth mating) at 1000 mg/kg bw. The effect was not reproducible in the next mating interval. Due to a lack of a dose-dependency, the observation was not attributed to treatment.

Table 5.4.3-7: Dominant lethal test in Wistar rats ([redacted] 1992), number of total early resorptions per group

	Group				
	1	2	3	4	PC
	Dose [mg/kg bw]				
	0	200	1000	5000	100/500[x]
No. of early resorptions: Number of total early resorptions per group[#]					
Mating study week					
1	0.0	0.0	0.0	25.0* ↑	0.0
2	0.0	0.0	0.0	0.0	0.0
3	0.0	0.0	10.0	8.0	0.0
4	0.0	0.0	24.0* ↑	0.0	0.0
5	0.0	0.0	0.0	1.0	0.0
6	0.0	0.0	0.0	0.0	0.0
7	0.0	1.0	0.0	0.0	0.0
8	0.0	0.0	0.0	0.0	0.0
9	0.0	0.0	0.0	0.0	0.0
10	0.0	0.0	0.0	0.0	0.0

PC: positive control EMS: Ethylmethanesulphonate, positive control, [x]combined data for both treatment regimens (100 mg/kg bw/day for 5 days in distilled water or 500 mg/kg bw as single dose in distilled water)
*: $p < 0.05$ and ** $p < 0.01$ statistical significance when compared to controls, ↓ decrease, ↑ increase
[#]: reported as mean number of early resorptions per dam in study report

There was a statistically significant increase in the incidence of small moles / fetal resorptions at ≥ 1000

mg/kg bw during weeks 1 and 7, at 1000 mg/kg bw during week 5, at 5000 mg/kg bw during weeks 6 and 8 and in all glyphosate treated groups during week 9 (refer to Table 5.4.3-8). In addition, strong fluctuations in the percentages of fetal resorptions were noted over the entire study period. The observations were not dose-related and inconsistent throughout the study period and therefore not attributed to treatment.

Table 5.4.3-8: Dominant lethal test in Wistar rats ([redacted] 1992), mean number of fetal resorptions per dam and percentage of fetal resorptions per group (small moles)

	Group				
	1	2	3	4	PC
	Dose [mg/kg bw]				
	0	200	1000	5000	100/500[x]
Small moles / fetal resorptions: Mean per dam ± SD					
Mating study week					
1	0.38 ± 0.68	0.29 ± 0.53	0.62 ± 0.86* ↑	0.78 ± 1.17* ↑	2.80 ± 2.71*
2	0.92 ± 2.71	0.34 ± 0.67* ↓	0.35 ± 0.57* ↓	0.88 ± 1.63	1.80 ± 1.72
3	0.96 ± 2.59	0.58 ± 1.17* ↓	0.85 ± 1.32	0.72 ± 0.89	0.0 ± 0.0
4	0.71 ± 0.98	0.55 ± 0.91	0.62 ± 0.90	0.81 ± 1.27	2.00 ± 0.97*
5	0.59 ± 0.97	0.69 ± 0.81	0.86 ± 1.48* ↑	0.59 ± 1.05	0.83 ± 1.58
6	0.37 ± 0.56	0.44 ± 0.80	0.46 ± 0.58	1.10 ± 1.47* ↑	1.00 ± 1.25
7	0.43 ± 0.84	0.48 ± 0.49	0.62 ± 0.98* ↑	1.01 ± 1.14* ↑	0.71 ± 1.08
8	0.57 ± 1.26	0.12 ± 0.33* ↓	0.67 ± 0.88	1.00 ± 1.25* ↑	0.50 ± 0.70
9	0.13 ± 0.34	0.81 ± 1.39* ↑	0.32 ± 0.67* ↑	0.87 ± 0.97* ↑	0.25 ± 0.42
10	0.52 ± 0.77	0.21 ± 0.41* ↓	0.07 ± 0.26* ↓	0.45 ± 0.57	0.56 ± 0.85
Percentage of small moles / fetal resorptions					
Mating study week					
1	3.04 ± 5.33	2.48 ± 5.04	5.46 ± 7.54* ↑	10.9 ± 18.2* ↑	33.5 ± 34.7*
2	11.7 ± 29.5	3.05 ± 5.79* ↓	3.22 ± 5.30* ↓	7.87 ± 16.1* ↓	82.3 ± 42.1*
3	10.6 ± 27.3	6.22 ± 12.8* ↓	10.5 ± 14.4	8.32 ± 14.4* ↓	0.0 ± 0.0
4	7.85 ± 11.3	5.14 ± 8.59* ↓	10.1 ± 21.0* ↑	6.55 ± 8.97* ↓	100 ± 42.2*
5	5.21 ± 8.52	6.34 ± 7.25* ↑	9.11 ± 18.4* ↑	7.44 ± 16.5* ↑	16.7 ± 31.6*
6	3.17 ± 4.79	3.88 ± 6.76	5.45 ± 10.3* ↑	11.0 ± 16.7* ↑	12.2 ± 13.5*
7	3.15 ± 6.18	10.4 ± 22.6* ↑	8.30 ± 19.8* ↑	11.1 ± 13.6* ↑	10.0 ± 16.4*
8	6.05 ± 14.0	0.87 ± 2.45* ↓	6.15 ± 7.97	13.0 ± 21.0* ↑	4.3 ± 5.98*
9	0.99 ± 2.67	8.87 ± 18.2*	3.58 ± 8.03*	7.72 ± 8.74*	2.34 ± 4.02*
10	4.41 ± 6.15	2.39 ± 5.52*	0.60 ± 2.21*	5.11 ± 9.88*	4.26 ± 6.77

PC: positive control EMS: Ethylmethanesulphonate, positive control, [x]combined data for both treatment regimens (100 mg/kg bw/day for 5 days in distilled water or 500 mg/kg bw as single dose in distilled water)

*: $p < 0.05$ and ** $p < 0.01$ statistical significance when compared to controls, ↓ decrease, ↑ increase

A statistically significant increase in the mean number of embryonic resorptions per dam was observed in the low and mid dose group only for mating intervals 3, 4 and 10 (refer to Table 5.4.3-9 Table below). In addition, the percentage of embryonic resorptions was statistically significantly increased at 5000 mg/kg bw after the first mating, at 200 mg/kg bw after the 2nd, 3rd and 8[th] mating and at 200 and 1000 mg/kg bw during weeks 4 and 10. Without any dose-response relationship, the observations were considered to be incidental and not related to treatment.

Table 5.4.3-9: Dominant lethal test in Wistar rats ([redacted] 1992) mean number of embryonic resorptions per dam and percentage of embryonic resorptions per group (large moles)

	Group				
	1	2	3	4	PC
	Dose [mg/kg bw]				
	0	200	1000	5000	100/500[x]
Large moles / embryonic resorptions: Mean per dam ± SD					
Mating study week					
1	0.31 ± 0.60	0.36 ± 0.56	0.0 ± 0.0	0.17 ± 0.49	0.60 ± 1.50
2	0.52 ± 0.82	0.52 ± 0.74	0.09 ± 0.42* ↓	0.12 ± 0.33* ↓	0.50 ± 0.84
3	0.37 ± 0.63	0.62 ± 0.98	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
4	0.11 ± 0.31	0.38 ± 0.68* ↑	0.38 ± 0.98* ↑	0.15 ± 0.54	0.0 ± 0.0
5	0.44 ± 0.97	0.28 ± 0.59* ↓	0.25 ± 0.52* ↓	0.30 ± 1.35	1.17 ± 1.25
6	0.44 ± 0.97	0.19 ± 0.40	0.35 ± 0.80	0.0 ± 0.0	0.43 ± 0.67
7	0.75 ± 0.75	0.74 ± 1.18	0.52 ± 0.78	0.0 ± 0.0	1.14 ± 1.23
8	0.79 ± 0.88	0.62 ± 0.80* ↓	0.26 ± 0.53* ↓	0.03 ± 0.19* ↓	1.00 ± 1.03
9	1.46 ± 1.56	0.69 ± 0.97	0.29 ± 0.53* ↓	0.0 ± 0.0* ↓	0.50 ± 0.70
10	0.16 ± 0.47	0.38 ± 0.73* ↑	0.36 ± 0.62* ↑	0.17 ± 0.38	0.44 ± 0.70
Percentage of large moles / embryonic resorptions					
Mating study week					
1	2.69 ± 5.64	3.31 ± 5.36	0.0 ± 0.0* ↓	3.35 ± 10.9* ↑	10.9 ± 29.8*
2	5.50 ± 9.47	7.09 ± 18.8* ↑	0.65 ± 3.13* ↓	1.08 ± 3.25* ↓	14.6 ± 25.0
3	3.91 ± 6.51	5.54 ± 9.03* ↑	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
4	0.91 ± 2.70	3.63 ± 6.33* ↑	3.98 ± 11.1* ↑	1.38 ± 5.02	0.0 ± 0.0*
5	3.84 ± 8.04	2.58 ± 5.43* ↓	2.10 ± 4.46* ↓	2.52 ± 11.3* ↓	32.0 ± 33.3*
6	4.25 ± 9.45	2.13 ± 5.02* ↓	4.84 ± 15.1	0.0 ± 0.0* ↓	3.86 ± 5.93
7	5.90 ± 6.22	6.33 ± 9.44	4.61 ± 6.75* ↓	0.0 ± 0.0	11.6 ± 11.7*
8	7.18 ± 9.69	9.48 ± 20.7* ↑	2.57 ± 5.40* ↓	0.45 ± 2.41* ↓	11.4 ± 13.4*
9	12.1 ± 12.5	7.08 ± 9.45* ↓	3.01 ± 5.53* ↓	0.0 ± 0.0* ↓	3.73 ± 5.23*
10	1.36 ± 3.88	3.78 ± 7.58* ↑	3.01 ± 5.17* ↑	1.25 ± 2.80	3.93 ± 6.64*

PC: positive control EMS: Ethylmethanesulphonate, positive control, [x]combined data for both treatment regimens (100 mg/kg bw/day for 5 days in distilled water or 500 mg/kg bw as single dose in distilled water)

*: $p < 0.05$ and **: $p < 0.01$ statistical significance when compared to controls, ↓ decrease, ↑ increase

Increases and decreases in the incidence and percentage of live implants were observed in test item-treated and solvent control-treated animals and occurred without any dose-relation and without consistency over the 10 mating intervals. Thus, the changes were as well considered unrelated to treatment (refer to Table 5.4.3-10).

Table 5.4.3-10: Dominant lethal test in Wistar rats ([redacted] 1992), mean number of live implants per dam and per centage of live implants per group

	Group				
	1	2	3	4	PC
	Dose [mg/kg bw]				
	0	200	1000	5000	100/500[x]
Live implants: Mean per dam ± SD					
Mating study week					
1	11.0 ± 2.38	10.8 ± 1.97	10.2 ± 1.98	6.87 ± 5.08* ↓	6.50 ± 4.79*
2	8.88 ± 3.78	10.3 ± 2.67* ↑	9.26 ± 1.89	10.9 ± 3.01* ↑	0.25 ± 0.63*
3	8.81 ± 3.48	9.35 ± 3.11	7.69 ± 2.96	9.92 ± 3.23	0.0 ± 0.0
4	8.57 ± 2.15	9.69 ± 2.52* ↑	7.81 ± 5.01	9.23 ± 2.42	0.0 ± 0.0*
5	9.74 ± 1.83	9.52 ± 1.60	9.96 ± 3.58	9.78 ± 3.61	2.83 ± 3.20*
6	9.63 ± 2.17	9.78 ± 2.47	9.92 ± 2.99	9.03 ± 2.86	6.14 ± 3.68*
7	12.3 ± 2.77	8.83 ± 3.52	9.86 ± 4.02	8.84 ± 2.72	9.57 ± 5.72*
8	10.4 ± 3.87	11.3 ± 3.53	9.48 ± 1.63	8.38 ± 2.78* ↓	8.50 ± 4.54*
9	10.4 ± 3.59	9.46 ± 3.42	8.96 ± 2.24	10.2 ± 2.85	10.4 ± 4.72
10	10.1 ± 2.49	10.7 ± 2.93	10.5 ± 3.32	11.7 ± 2.77	10.9 ± 3.91
Percentage of live implants					
Mating study week					
1	94.3 ± 7.27	94.3 ± 6.69	94.6 ± 7.53	64.9 ± 43.3* ↓	55.6 ± 39.0*
2	82.8 ± 28.7	89.8 ± 18.8* ↑	96.1 ± 6.66* ↑	91.2 ± 16.0* ↑	3.13 ± 7.91*
3	85.4 ± 26.8	88.2 ± 15.1	85.7 ± 22.6	87.7 ± 23.2	0.0 ± 0.0
4	91.4 ± 10.8	91.2 ± 9.58	71.0 ± 38.0* ↓	92.1 ± 9.32	0.0 ± 0.0*
5	91.0 ± 12.4	91.1 ± 8.32	88.8 ± 18.2	89.4 ± 19.0	51.3 ± 41.9*
6	92.7 ± 10.7	94.1 ± 7.47	89.7 ± 16.9* ↓	89.0 ± 16.7* ↓	84.0 ± 42.2*
7	90.9 ± 10.2	82.90 ± 22.0* ↓	87.1 ± 19.1* ↓	89.0 ± 13.5	78.3 ± 43.7*
8	87.7 ± 16.4	89.7 ± 20.4	91.3 ± 10.3* ↑	86.6 ± 20.8	84.4 ± 37.6*
9	86.9 ± 12.6	84.1 ± 23.4* ↓	93.5 ± 8.92*	92.3 ± 8.74* ↑	94.0 ± 40.1*
10	94.2 ± 6.86	93.9 ± 8.73	96.4 ± 6.64	93.7 ± 9.98	91.9 ± 30.7*

PC: positive control EMS: Ethylmethanesulphonate, positive control, [x]combined data for both treatment regimens (100 mg/kg bw/day for 5 days in distilled water or 500 mg/kg bw as single dose in distilled water)

*: $p < 0.05$ and ** $p < 0.01$ statistical significance when compared to controls, ↓ decrease, ↑ increase

Statistically significant increases in the percentages of pre-implantation losses were noted at 5000 mg/kg bw for the first mating interval, for all or most treatment groups during weeks 3, 4, 5 and 7 and statistically significantly decreased in all or in the majority of glyphosate treated groups during weeks 2, 8, 9 and 10. There was no dose-response relationship evident at any mating interval. Thus, the effects on the percentage of pre-implantation losses were not considered to be related to treatment with glyphosate.

Table 5.4.3-11: Dominant lethal test in Wistar rats ([redacted] 1992), percentage of pre-implantation losses

	Group				
	1	2	3	4	PC
	Dose [mg/kg bw]				
	0	200	1000	5000	100/500[x]
Percentage of pre-implantation losses (%): Mean per dam ± SD					
Mating study week					
1	6.21 ± 13.5	5.54 ± 11.7	2.37 ± 5.34* ↓	24.2 ± 30.2* ↑	12.3 ± 19.5*
2	10.6 ± 20.1	3.69 ± 14.1* ↓	7.75 ± 12.0* ↓	3.98 ± 7.31* ↓	73.0 ± 38.8*
3	6.60 ± 9.65	6.81 ± 13.0	10.1 ± 17.8* ↑	10.9 ± 16.5* ↑	0.0 ± 0.0
4	8.36 ± 14.6	9.97 ± 14.6* ↑	21.3 ± 24.7* ↑	12.8 ± 19.9* ↑	82.5 ± 35.1*
5	7.57 ± 10.7	1.49 ± 3.82*↓	11.6 ± 18.0* ↑	12.1 ± 21.9* ↑	60.8 ± 40.3*
6	8.56 ± 10.5	6.59 ± 14.6* ↓	7.56 ± 17.8	12.5 ± 18.5* ↑	30.0 ± 29.5*
7	7.79 ± 11.1	18.2 ± 26.4* ↑	15.0 ± 25.1* ↑	13.1 ± 19.1* ↑	10.7 ± 21.1*
8	12.1 ± 18.9	13.8 ± 19.8* ↑	5.62 ± 6.59* ↓	9.72 ± 18.9* ↓	19.1 ± 22.4*
9	16.8 ± 21.4	8.12 ± 13.9* ↓	9.47 ± 14.6* ↓	9.20 ± 18.7* ↓	4.63 ± 8.77*
10	11.3 ± 14.3	7.20 ± 16.4* ↓	14.8 ± 24.5* ↑	6.17 ± 17.5* ↓	4.92 ± 6.92*

PC: positive control EMS: Ethylmethanesulphonate, positive control, [x]combined data for both treatment regimens (100 mg/kg bw/day for 5 days in distilled water or 500 mg/kg bw as single dose in distilled water)

*: $p < 0.05$ and ** $p < 0.01$ statistical significance when compared to controls, ↓ decrease, ↑ increase

The percentage of post-implantation loss, corresponding to the dominant lethal factor, was statistically significantly increased in the high dose group of the first mating interval, which was attributed to acute toxicity in the animals. The dominant lethal factor was further statistically significantly increased in the mid and high dose group after the 6th mating and for all glyphosate treated groups after the 7th mating. For the females of the remaining mating intervals, the dominant lethal factor was either not affected or lower than in solvent control animals. None of the observations was dose-related or consistent over the ten mating periods.

Table 5.4.3-12: Dominant lethal test in Wistar rats ([redacted] 1992), percentage of post-implantation losses, corresponding to dominant lethal factors

	Group				
	1	2	3	4	PC
	Dose [mg/kg bw]				
	0	200	1000	5000	100/500[x]
Percentage of post-implantation losses[#] (%)					
Mating study week					
1	5.71 ± 7.28	5.76 ± 6.74	5.46 ± 7.54	35.1 ± 43.3* ↑	44.4 ± 39.0*
2	17.2 ± 28.8	10.2 ± 18.8*	3.89 ± 6.67* ↓	8.93 ± 16.0* ↓	96.9 ± 41.6*
3	14.5 ± 26.8	11.8 ± 15.1* ↓	14.3 ± 22.6	12.3 ± 23.2	0.0 ± 0.0
4	8.76 ± 10.9	8.79 ± 9.58	29.0 ± 38.0* ↑	7.93 ± 9.32	100 ± 42.2*

Table 5.4.3-12: Dominant lethal test in Wistar rats ([redacted] 1992), percentage of post-implantation losses, corresponding to dominant lethal factors

	Group				
	1	2	3	4	PC
	Dose [mg/kg bw]				
	0	200	1000	5000	100/500[x]
5	9.07 ± 12.4	8.88 ± 8.34	11.2 ± 18.1* ↑	10.6 ± 19.0	48.7 ± 41.0*
6	7.43 ± 10.8	6.03 ± 7.55* ↓	10.3 ± 16.9* ↑	11.0 ± 16.7* ↑	16.0 ± 14.0*
7	9.10 ± 10.2	17.2 ± 22.0* ↑	12.9 ± 19.1* ↑	11.1 ± 13.6* ↑	21.7 ± 24.2*
8	13.2 ± 15.9	10.3 ± 20.5* ↓	8.71 ± 10.3* ↓	13.4 ± 20.8	15.7 ± 13.8*
9	13.1 ± 12.6	16.0 ± 23.4* ↑	6.59 ± 8.95* ↓	7.72 ± 8.74* ↓	6.01 ± 6.58*
10	5.77 ± 6.86	6.17 ± 8.74	3.59 ± 6.62	6.34 ± 9.98	8.22 ± 10.3*

PC: positive control EMS: Ethylmethanesulphonate, positive control, [x]combined data for both treatment regimens (100 mg/kg bw/day for 5 days in distilled water or 500 mg/kg bw as single dose in distilled water)

[#]: Corresponds to the dominant lethal factor

*: $p < 0.05$ and ** $p < 0.01$ statistical significance when compared to controls, ↓ decrease, ↑ increase

Treatment with the positive control ethylmethane sulphonate induced statistically significant dominant lethal effects and demonstrated the validity of the test. There was a statistically significant decrease in the incidence of pregnancy to 0 % observed in the third mating interval and a gradual recovery back to normal by 9 to 10 weeks (refer to Table 5.4.3-4). In addition, there was an increased incidence of small moles / fetal resorptions during the first week (mating period 1), which gradually recovered to normal by week 9 (mating period 9, refer to Table 5.4.3-8).

III. CONCLUSION

Under the conditions of the test, glyphosate did not induce dominant lethal effects in Wistar rats up to a dose level of 5000 mg/kg bw. Treatment of males with 5000 mg/kg bw was associated with clinical signs of toxicity and reduced body weight in males, as well as with acute toxicity after the first mating in females. Toxicity in female rats became evident as a reduction in the incidence of pregnancy and an increase in the incidences of early resorption and pre- and post-implantation losses. Based on the experimental findings, glyphosate was considered non-genotoxic to germ cells *in vivo*.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Negative for genotoxicity to germ cells *in vivo*. The test item did not induce any dominant lethal effects.

At 5000 mg/kg bw, treatment with glyphosate caused acute toxic effects in females of the first mating group, which affected the fertility indices pregnancy, early resorption, pre- and post-implantation losses.

The study was performed under GLP and in accordance with OECD guideline 478 (1984). When compared to the current OECD guideline (2016), a number of deviations became evident. The spacing factor between dose levels was too high (5 instead of 2 – 4) and the number of implantations per group for each mating period was below the recommended number of 400 implants per group as required

according to OECD 478 (2016). Fetal body weights were not recorded and no information on historical control data was provided. Further, there was no raw data on individual animals treated with the positive control provided. The mean pre- and post-implantation losses per dam were not calculated, but percentages of pre-implantation losses, as well as percentages of post-implantation losses (corresponding to the dominant lethal factor) were reported. Further deviations were considered to be of minor degree. Due to the large number of deviations to the current OECD guideline, the study was considered to provide supporting information.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.4.3/004
Report author	[redacted]
Report year	1982
Report title	Mutagenic testing of glyphosate in rat by dominant lethal test
Report No	Not reported
Document No	Not reported
Guidelines followed in study	No guideline followed, study was conducted similarly to OECD 478 (2016)
Deviations from current test guideline OECD 478 (2016)	When compared to OECD 478 (2016), clinical signs of toxicity and mortality were not reported and no body weights were recorded. The selected dose levels were comparably low, but a scientific justification for the selection of dose levels was not given. For some mating intervals, the number of implantations per group for each mating period was below the recommended number of 400 implants per group. Therefore, the number of animals mated was presumably not sufficient. Fetal body weights were not recorded and no historical control data were provided. Necropsy of sires was not performed and resorptions, termed as "dead implants" in study report were not distinguished as early or late resorptions. In addition, pregnant females were sacrificed on gestation Day 18, whereas OECD 478 (2016) recommends to sacrifice the females between gestation Days 14 – 15 for rats. Raw data on individual animals were not provided, therefore standard deviations were not calculated. In addition, analytical determinations on the test items stability, homogeneity or concentration in the diet were not performed. Acceptance and evaluation criteria were not specified in the study report.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, not conducted under GLP /Officially recognised testing facilities. When the study was performed, GLP was not compulsory.
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (I docs)	Category 3a

2. Full summary

The mutagenic potential of glyphosate active principle (batch: 00260481, purity: not reported) was investigated in a dominant lethal study in CFY rats.

The test item was incorporated into the ground diet and administered via the feed to male animals for a period of 8 weeks. Dose levels of 10, 30 and 100 mg glyphosate/kg food were applied, corresponding to a mean actual achieved test substance intake of 6.8, 20.5 and 70.4 mg/kg bw/day. A group of control males received the normal diet without test item and a group of positive control animals was administered 5 mg cyclophosphamide/kg food, corresponding to a mean actual achieved intake of 3.7 mg/kg bw/day.

After dosing, each male was paired with untreated females (mating ratio 1 : 2) for one week. After separation, the males were paired with two new females for a second week. Females were continually replaced after one week until 4 mating intervals were completed.

Information on clinical signs of toxicity, mortality or individual and group mean body weights were not included in the study. Food consumption and mean achieved compound intake was recorded on a weekly base. Analytical determinations on stability, homogeneity and concentration of the test item in the diet were not conducted.

Females were sacrificed on Day 18 of gestation. The uterus and ovaries were dissected and investigated for the number of *corpora lutea*, the number of implantations, the number of dead implants (resorptions) and the number of viable fetuses.

Investigations of the female uterine contents revealed no treatment-related findings. The number of pregnant females, the number of *corpora lutea* and implantation sites and the number of resorptions were not affected for any mating interval upon treatment with glyphosate. The number of viable fetuses was comparable for females mated with glyphosate-treated and solvent control-treated males. In addition, the number of females having one or more dead fetus did not increase. The percentage of pre-implantation loss was considered not to be affected by treatment. Fluctuations were noted in females of all dose groups, but most groups showed a decrease in pre-implantation losses. However, the findings were not consistent during the 4-week mating period and a dose-response relationship was not observed. The percentage of post-implantation loss, corresponding to the dominant lethal factor, was comparable for all glyphosate and control groups.

Treatment with cyclophosphamide resulted in a strong increase in the number of dead implants / resorptions in the females of all mating intervals and a reduction in the number of viable fetuses, especially in the females of the first mating interval. Cyclophosphamide proved to be highly mutagenic and induced clear dominant lethal effects, demonstrating the sensitivity and validity of the test.

Based on the experimental findings, glyphosate active principle has no mutagenic potential in germ cells *in vivo*.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: N-phosphonomethyl-glycine

Identification: Glyphosate active principle

Description: Not specified

Lot/Batch #:	00260481
Purity:	Not specified
Stability of test compound:	The stability of the test item at storage conditions or in vehicle (feed) was not specified.
Solvent (vehicle) used:	The test item was incorporated into the diet (LATI laboratory rodent food)

2. Control materials

Solvent (vehicle) control:	LATI laboratory rodent food
Positive control:	Cyclophosphamide, 5 mg/kg food (nominal concentration), corresponding to 3.7 mg/kg bw/day (actual dose received)

3. Test animals:

Species:	Rat
Strain:	CFY
Sex:	Males and females
Source:	[redacted]
Age at study initiation:	12 months
Weight at study initiation:	Not specified
Acclimation period:	Not specified
Diet/Food:	Standard LATI rat and mouse food (Lati, Gödöllö, Hungary)
Water:	Not specified
Housing:	In groups of 5/sex in shoebox type plastic cages with wood shaving bedding. During mating: Each male was cohabitated with 2 females.

4. Environmental conditions:

Environmental conditions were not specified.

5. Test concentrations and treatment groups:

Dose levels:	10, 30 and 100 mg/kg food (nominal dose), corresponding to 6.8, 20.5 and 70.4 mg/kg bw/day (actual dose, calculated based on average food intake)
Treatment duration:	8 weeks
Number of animals:	The total number of animals used was 100 males and 900 females. The number of animals used per mating interval was not explicitly specified in the study report. As the animals were divided into 5 groups, it can be assumed that 20 males per group were used. There is a discrepancy in the mentioned number of 900 females used and the information that each male was mated during the 4 week mating period with 2 females per week, leading to a total number of 800 females used in the study.
Route of administration:	Oral, feed

B: STUDY DESIGN AND METHODS

1. Finalisation date: 1982

2. Animal assignment and treatment:

The test item was incorporated into the ground diet and administered via the feed to male animals for a period of 8 weeks. Dose levels of 10, 30 and 100 mg/kg food were applied, corresponding to an actual mean daily intake of 6.8, 20.5 and 70.4 mg/kg bw/day, as calculated based on the average weekly food intake per group. A group of control males received the normal diet without test item and a group of positive control animals was administered 5 mg/kg food cyclophosphamide, corresponding to 3.7 mg/kg bw/day (actual dose received).
After dosing, each male was paired during 4 weeks with two females per week (mating ratio 1 : 2).

3. Observations

Information on observations regarding mortality or clinical signs of toxicity were not included in the study report.

4. Body weight

Information on individual or group mean body weights of animals in the respective groups were not included in the study report.

5. Food consumption and compound intake

Food consumption and test substance intake were recorded for each dose group in mg/kg bw/week.

6. Sacrifice

Female rats were sacrificed on the 18th day of gestation. The uteri and ovaries of the animals were dissected and investigated for the following parameters:

- Number of *corpora lutea*
- Number of implantations
- Number of resorptions
- Number of viable and nonviable fetuses

Furthermore, the percentage of pre- and post-implantation loss was determined as follows:

$$\text{Pre} - \text{implantation loss [\%]} = \frac{\text{Total number of corpora lutea} - \text{Number of implantations}}{\text{Total number of corpora lutea}} \times 100$$

$$\text{Post} - \text{implantation loss [\%]} = \frac{\text{Number of implantations} - \text{Number of live fetuses} *}{\text{Total number of implantations}} \times 100$$

* fetuses (gestation day 18) were termed embryos in study report

The percentage of post-implantation losses corresponds to the dominant lethal factor.

Macroscopical examination of male rats was not included in the study report.

7. Statistics:

Statistical analysis was not described in the study report.

8. Acceptance criteria:

Acceptance criteria were not specified in the study report.

9. Evaluation criteria:

Evaluation criteria were not specified in the study report.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Analytical determinations on the stability, concentration and homogeneity of the test substance in the diet were not performed in the study.

B. OBSERVATIONS

Systemic toxicity:

Mortality:

Information on mortality was not provided in the study report.

Clinical signs of toxicity:

Clinical signs of toxicity were not reported.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

There were no treatment-related effects on food consumption of males during the 8-week treatment period. The average intake of test substance per group was 47.3 ± 2.9, 143.3 ± 11.9 and 492.9 ± 35.4 mg/kg bw/week, calculated based on the average food intake (g/kg bw/week) over all treatment weeks. Based on this, the corresponding mean daily doses of 6.8, 20.5 and 70.4 mg/kg bw/day were calculated.

D. BODY WEIGHT AND BODY WEIGHT DEVELOPMENT OF SIRES

Body weights were not recorded during the course of the study.

E. NECROPSY OF SIRES

Information on macroscopical or microscopical analysis of sacrificed males was not included.

F. FERTILITY INDICES

Treatment with glyphosate did not affect the number of mated males and females (refer to Table 5.4.3-13 and Table 5.4.3-14). The number of pregnant females was comparable for all groups.

Table 5.4.3-13: Mutagenic testing of glyphosate in rat by dominant lethal test ([redacted], 1982), number of males mated

	Group				
	1	2	3	4	PC
	Dose [mg/kg bw/day]				
	0	6.8	20.5	70.4	3.7
No. of males mated					
Mating study week					
1	15	20	19	18	20
2	18	19	19	19	20
3	18	17	19	19	20
4	17	17	18	19	18

PC: positive control Cyclophosphamide

Table 5.4.3-14: Mutagenic testing of glyphosate in rat by dominant lethal test ([redacted], 1982), number of pregnant females

	Group				
	1	**2**	**3**	**4**	**PC**
	Dose [mg/kg bw/day]				
	0	**6.8**	**20.5**	**70.4**	**3.7**
No. of pregnant females					
Mating study week					
1	23	33	29	29	32
2	30	31	30	36	34
3	29	26	28	29	32
4	28	25	32	33	31

PC: positive control Cyclophosphamide

In addition, the number of *corpora lutea* and the number of implantations (refer to Table 5.4.3-15 and Table 5.4.3-16) were not affected by treatment.

Table 5.4.3-15: Mutagenic testing of glyphosate in rat by dominant lethal test ([redacted], 1982), number of *corpora lutea*

	Group				
	1	**2**	**3**	**4**	**PC**
	Dose [mg/kg bw/day]				
	0	**6.8**	**20.5**	**70.4**	**3.7**
No. of corpora lutea: Mean per dam					
Mating study week					
1	16.7	16.2	17.0	17.2	16.6
2	16.6	15.3	16.5	16.0	17.1
3	16.2	16.3	16.2	17.2	17.5
4	15.9	16.5	16.8	15.8	16.1

PC: positive control Cyclophosphamide

Table 5.4.3-16: Mutagenic testing of glyphosate in rat by dominant lethal test ([redacted], 1982), number of implantation sites

	Group				
	1	**2**	**3**	**4**	**PC**
	Dose [mg/kg bw/day]				
	0	**6.8**	**20.5**	**70.4**	**3.7**
No. of implantation sites: Mean per dam					
Mating study week					
1	13.6	14.8	15.0	13.7	13.6
2	13.5	14.2	14.3	13.6	14.4

Table 5.4.3-16: Mutagenic testing of glyphosate in rat by dominant lethal test ([redacted], 1982), number of implantation sites

	Group				
	1	2	3	4	PC
	Dose [mg/kg bw/day]				
	0	6.8	20.5	70.4	3.7
3	13.5	13.7	14.5	13.5	14.1
4	13.6	15.0	14.7	13.9	14.7

PC: positive control Cyclophosphamide

The amount of resorptions (termed "dead implants" in study report) was comparable to those of control animals for all mating intervals (refer to Table 5.4.3-17). The number of females having one or more dead fetus of early death did not increase either.

Table 5.4.3-17: Mutagenic testing of glyphosate in rat by dominant lethal test ([redacted], 1982), number of dead implants / resorptions

	Group				
	1	2	3	4	PC
	Dose [mg/kg bw/day]				
	0	6.8	20.5	70.4	3.7
Number of dead implants / resorptions: Mean per dam					
Mating study week					
1	1.7	1.1	1.3	1.3	7.7
2	1.3	1.2	1.2	1.4	6.2
3	1.7	1.5	1.1	1.5	4.2
4	1.7	2.2	1.1	1.5	3.5

PC: positive control Cyclophosphamide

The number of viable fetuses (termed "live implants" or "live embryos" in study report) in glyphosate-treated animals was comparable to those of control animals.

Table 5.4.3-18: Mutagenic testing of glyphosate in rat by dominant lethal test ([redacted], 1982), number of live implants / viable foetuses

	Group				
	1	2	3	4	PC
	Dose [mg/kg bw/day]				
	0	6.8	20.5	70.4	3.7
Number of live implants / viable fetuses: Mean per dam					
Mating study week					
1	11.8	13.7	13.7	12.4	5.8

Table 5.4.3-18: Mutagenic testing of glyphosate in rat by dominant lethal test ([redacted], 1982), number of live implants / viable foetuses

	Group				
	1	2	3	4	PC
	Dose [mg/kg bw/day]				
	0	6.8	20.5	70.4	3.7
2	12.2	13.1	13.1	12.3	8.2
3	11.9	12.2	13.4	12.0	9.9
4	11.9	12.8	13.6	12.5	11.2

PC: positive control Cyclophosphamide

Further, the percentage of pre -implantation losses (refer to Table 5.4.3-19) was not considered to be affected upon treatment with glyphosate. Fluctuations were noted in females of all dose groups, but most groups showed a decrease in pre-implantation losses. However, the findings were not consistent during the 4-weeks mating period and a dose-response relationship was not observed.

Table 5.4.3-19: Mutagenic testing of glyphosate in rat by dominant lethal test ([redacted], 1982), percentage of pre-implantation losses

	Group				
	1	2	3	4	PC
	Dose [mg/kg bw/day]				
	0	6.8	20.5	70.4	3.7
Percentage of pre-implantation losses (%)					
Mating study week					
1	19.0	9.0 ↓	11.6 ↓	20.2	18.1
2	18.5	7.6 ↓	13.3 ↓	14.9 ↓	16.0
3	16.8	16.3	10.6 ↓	21.8 ↑	19.5
4	14.4	9.2 ↓	12.5 ↓	11.7 ↓	8.4

PC: positive control Cyclophosphamide

The percentage of post-implantation losses, corresponding to the dominant lethal factor, was not affected by treatment (refer to Table 5.4.3-20).

Treatment with the positive control cyclophosphamide markedly increased the number of dead implants / resorptions in the females of all mating intervals (refer to Table 5.4.3-17) and markedly reduced the number of viable fetuses, especially in the females of the first mating interval (refer to Table 5.4.3-18). Treatment with cyclophosphamide induced clear dominant lethal effects, demonstrating the sensitivity and validity of the test.

Table 5.4.3-20: Mutagenic testing of glyphosate in rat by dominant lethal test ([redacted], 1982), percentage of post-implantation losses, corresponding to dominant lethal factors

	Group				
	1	2	3	4	PC
	Dose [mg/kg bw/day]				
	0	6.8	20.5	70.4	3.7
Percentage of post-implantation losses[#] (%):					
Mating study week					
1	12.8	7.2	8.7	9.8	56.9
2	9.6	7.7	8.4	10.0	42.9
3	12.0	10.4	7.2	10.7	29.9
4	12.6	14.4	7.6	10.0	23.9

PC: positive control Cyclophosphamide

[#]: Corresponds to the dominant lethal factor

III. CONCLUSION

Based on the experimental findings and under the conditions of the test, glyphosate active principle did not cause dominant lethal effects in CFY rats after 8-week dietary administration. Therefore, glyphosate active principle is considered non-genotoxic to germ cells *in vivo*.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Negative for mutagenic effects in the dominant lethal assay in rats.

The study is not compliant with GLP but was performed according to the main criteria of OECD guideline 478 (2016). The study is considered supplementary due to serious reporting deficiencies, e.g. the purity of the test compound was not given and it is not unequivocally clear how many males and females were allocated to the individual test groups. Further methodology deficiencies are the number of implants (< 400/group) indicating the number of animals mated was not sufficient. In addition, the dose levels appear to be too low for definitive assessment. It is known from other studies that much higher doses can be applied. However, it is the only dominant lethal test with repeated dietary administration, and, therefore, the study may provide some additional information.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.4.3/005
Report author	[redacted]
Report year	1980
Report title	Dominant lethal mutagenicity assay with technical Glyphosate in mice
Report No	401-064

Document No	M-643921-01-1
Guidelines followed in study	No guideline followed, study was conducted similarly to OECD 478 (2016)
Deviations from current test guideline OECD 478 (2016)	The number of implantations per group for each mating period was below the recommended number of 400 implants per group. It can be assumed that the number of males and females used for mating was too low. Fetal body weights were not recorded. No historical control data were provided. No dominant lethal frequency was calculated in the study report. Acceptance and evaluation criteria were not specified. Statistical analysis did not include the assessment of under- and overdispersion.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, not conducted under GLP /Officially recognised testing facilities. When the study was performed, GLP was not compulsory.
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The mutagenic potential of glyphosate (batch: XHJ-64, purity: 98.7 %) was investigated in a dominant lethal study in CD-1 mice. The test item was dissolved in aqueous 0.5 % Methocel® and administered by oral gavage to groups of 10 males at dose levels of 200, 800 and 2000 mg/kg bw (single dose) at a constant dosage volume of 10 mL/kg bw. A similar constituted group of males received the solvent control (vehicle) or the positive control Cytoxan®. The positive control substance was administered via intraperitoneal injection as single dose of 240 mg/kg bw at a constant dosage volume of 12 mL/kg bw.

Immediately after dosing, each male was paired with untreated virgin females (mating ratio 1:2) for 7 days. After separation, each male was then paired with two new females for a second week. Females continued to be replaced in this manner for eight weeks so that each male was mated with a total of 16 females. All animals were observed twice daily for mortality and overt changes in appearance and behaviour. Body weights were recorded for males prior to treatment and weekly thereafter. Females were sacrificed 13 days after mid-week of their caging and presumptive mating. The uterus and ovaries were dissected and investigated for the number of *corpora lutea*, the number of implantations, the number of early and late resorptions and the number and localisation of viable and non-viable fetuses.

Four unscheduled deaths were observed during the course of the study. One female mated with a male of the 200 mg/kg bw group died in study week 3, one male of the 800 mg/kg bw group died in study week 5, one male of the 2000 mg/kg bw group died in week 6 and one female mated with a male of the 2000 mg/kg bw dose group died in study week 2. Necropsy of the decedent animals revealed no treatment-related findings, therefore the observations were not attributed to treatment but considered to be incidental. There were no treatment-related clinical signs of toxicity and no differences in body weight development in males over the entire study period.

Investigations of the female uterine contents revealed no treatment-related findings. The number of pregnant females, the number of *corpora lutea* and implantation sites and the number of early and late resorptions were not affected for any mating interval upon treatment with glyphosate. There was a slight but statistically significant decrease in the number of viable fetuses in females of the 800 mg/kg bw group mated during study week 1 and in females of the 2000 mg/kg bw group mated during study week 3. As no increase in early fetal deaths were observed in these groups, the findings were not attributed to glyphosate treatment and considered to be incidental. Pre- and post-implantation losses and calculated dominant lethal factors were comparable for treated and control groups.

Treatment with the positive control Cytoxan® induced a statistically significant decrease in the number of viable fetuses and an increase in the proportion of early fetal deaths, indicated by a statistically significant increase in the mean number of early resorptions and post-implantation loss observed during the first 3 weeks of mating. These results indicated a dominant lethal effect and an effect at the postmeiotic stage of spermatogenesis, thereby demonstrating the validity of the test system.

Under the conditions of the test, there was no mutagenic potential identified for glyphosate in the dominant lethal assay and is therefore considered non-genotoxic to germ cells *in vivo*.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material:

Identification:	Technical Glyphosate
Description:	White powder
Lot/Batch #:	XHJ-64
Purity:	98.7 %
Stability of test compound:	The stability of the test item at storage conditions or in vehicle was not specified.
Solvent (vehicle) used:	Aqueous 0.5 % Methocel®

2. Control materials

Solvent (vehicle) control:	Aqueous 0.5 % Methocel®
Positive control:	Cytoxan®, 240 mg/kg bw dissolved in sterile water

3. Test animals:

Species:	Mouse
Strain:	CD-1
Sex:	Males and females
Source:	[redacted]
Age at study initiation:	70 - 110 days
Weight at study initiation:	36 – 45 g (males) (weight of females not specified)
Acclimation period:	At least 10 days
Diet/Food:	Purina® Certified Rodent Chow® 5002, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually (except during mating) in suspended wire mesh cages. During mating: Each male was cohabitated with 2 females.

4. Environmental conditions:

The animals were maintained in a temperature-, humidity- and light controlled room

5. Test concentrations and treatment groups:

Dose levels:	200, 800 and 2000 mg/kg bw
Dose volume:	10 mL/kg bw
Number of animals:	10 males and 160 females per group (2 females per mating interval)
Route of administration:	Oral gavage

B: STUDY DESIGN AND METHODS

1. Dates of experimental work: 04 Jun – 09 Aug 1979
Finalisation date: 16 Apr 1980

2. Animal assignment and treatment:

Groups of 10 males per dose received a single administration of glyphosate by oral gavage at dose levels of 200, 800 and 2000 mg/kg bw at a constant dosage volume of 10 mL/kg bw. Similar constituted groups received the solvent (0.5 % aqueous Methocel®). The positive control Cytoxan® was administered via intraperitoneal injection as single dose of 240 mg/kg bw at a constant dosage volume of 12 mL/kg bw.

Immediately after dosing, each male was paired with untreated virgin females (mating ratio 1: 2) for 7 days. After separation, each male was then paired with two new females for a second week. Females continued to be replaced in this manner for eight weeks so that each male was mated with a total of 16 females.

3. Observations

All animals were observed twice daily for mortality and overt changes in appearance and behaviour. All males were given a detailed observation to determine if any clinical signs of toxicity were present.

4. Body weight

The body weight of male animals was recorded prior to treatment and weekly thereafter for 9 weeks.

5. Sacrifice

All mice not surviving to the scheduled sacrifice were necropsied. Males were sacrificed on the last day of mating.

Females were sacrificed by carbon dioxide inhalation thirteen days after mid-week of their caging and presumptive mating. The uterus and ovaries were removed by an abdominal incision and investigated for the following parameters:

- Number of *corpora lutea* per dam
- Number of implantations
- Number of early and late resorptions
- Number and location of viable and nonviable fetuses

The thoracic and abdominal cavities and organs of the dams were examined for grossly evident morphological changes.

Furthermore, the number of post-implantation losses was determined. The number of pre-implantation losses was determined based on the raw data provided in the study report.

Mutagenic effects in the dominant lethal assay were assessed by comparing the number of early fetal

deaths in the treated groups versus the control groups. For all groups, the dominant lethal factor was calculated as follows:

$$\text{Dominant lethal factor [\%]} = \frac{\text{Number of post} - \text{implantation losses}}{\text{Total number of implantations}} \times 100$$

6. Statistics:

All statistical analysis compared the treatment groups (test article and positive control) with the vehicle control group.

Fetal deaths per dam and post-implantation losses were compared by the Mann-Whitney U-test. The number of dams with fetal deaths was compared using the chi-square test with Yates' correction for 2 x 2 contingency tables and/or Fisher's exact probability test. The mean number of live fetuses and corpora lutea were compared by analysis of variance (one-way classification) Bartlett's test for homogeneity of variances and the appropriate t-test (for equal or unequal variances) using Dunnett's multiple comparison tables to judge significance of differences.

7. Acceptance criteria:

Acceptance criteria were not specified in the study report.

8. Evaluation criteria:

Evaluation criteria were not specified in the study report.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Analytical determinations were not performed in the study, as not required by the test guideline.

B. OBSERVATIONS

Systemic toxicity:

Mortality:

Four unscheduled deaths occurred during the course of the study. One female mated with a male of the 200 mg/kg bw group died in study week 3, one male of the 800 mg/kg bw group died in study week 5, one male of the 2000 mg/kg bw group died in week 6 and one female mated with a male of the 2000 mg/kg bw dose group died in study week 2. The cause of death could not be determined at necropsy. The observations on mortality were not attributed to treatment and considered to be incidental.

Clinical signs of toxicity:

There were no test item-related signs of systemic toxicity observed. Incidental findings included alopecia, matting of the hair coat and enlargement of the genital area. These findings were observed randomly throughout various weeks of the study among all treatment groups. Due to the random occurrence and small number of total animals affected, the findings were not related to treatment.

C. BODY WEIGHT AND BODY WEIGHT DEVELOPMENT OF SIRES

There were no biologically meaningful differences in body weight and body weight gain in any of the treatment groups when compared to control animals over the entire study period.

D. NECROPSY OF SIRES

Necropsy of unscheduled sires revealed no abnormal findings which were related to treatment.

E. FERTILITY INDICES

Treatment with glyphosate did not affect the number and percentage of pregnant females throughout the course of the study (refer to Table 5.4.3-21). In addition, the number of *corpora lutea* (refer to Table

5.4.3-22) and the number of implantations (refer to) were not affected by treatment.

Table 5.4.3-21: Summary of results obtained in the dominant lethal assay, number and percentage of pregnant females ([redacted], 1980)

	Group				
	1	2	3	4	PC
	Dose [mg/kg bw]				
	0	200	800	2000	240
No. of pregnant females (%)					
Mating study week					
1	17 (85)	15 (75)	16 (80)	17 (85)	15 (75)
2	17 (85)	16 (80)	16 (80)	18 (90)	18 (90)
3	14 (70)	18 (90)	18 (90)	17 (85)	16 (80)
4	19 (95)	13 (65)	17 (85)	17 (85)	14 (70)
5	20 (100)	12 (60)	17 (85)	18 (90)	18 (90)
6	18 (90)	14 (70)	19 (95)	17 (85)	15 (75)
7	19 (95)	19 (95)	18 (90)	15 (75)	12 (60)
8	18 (90)	14 (70)	18 (90)	20 (100)	16 (80)

PC: positive control Cytoxan

Table 5.4.3-22: Summary of results obtained in the dominant lethal assay, number of *corpora lutea* [redacted], 1980)

	Group				
	1	2	3	4	PC
	Dose [mg/kg bw]				
	0	200	800	2000	240
No. of corpora lutea: Mean per dam ± SD					
Mating study week					
1	12.9 ± 1.68	11.1 ± 2.27	11.8 ± 1.68	11.2 ± 2.17	11.5 ± 2.80
2	11.2 ± 2.70	11.4 ± 1.55	11.1 ± 2.49	11.4 ± 1.42	9.1 ± 3.44**
3	13.3 ± 1.38	14.4 ± 2.67	13.1 ± 1.51	12.3 ± 1.16	10.9 ± 2.25**
4	12.5 ± 2.41	11.9 ± 2.95	13.2 ± 2.35	12.0 ± 4.28	13.5 ± 1.05
5	13.3 ± 2.00	12.3 ± 1.57	12.2 ± 2.13	12.6 ± 1.54	13.1 ± 1.44
6	13.8 ± 1.77	13.9 ± 1.20	12.9 ± 1.83	14.1 ± 2.29	11.4 ± 1.50
7	13.5 ± 1.84	13.3 ± 3.18	13.2 ± 2.87	13.6 ± 1.80	11.8 ± 2.44
8	12.7 ± 1.45	14.1 ± 1.88	12.4 ± 3.32	13.9 ± 2.43	13.5 ± 1.87

PC: positive control Cytoxan

** $p < 0.01$ statistical significance when compared to controls

Table 5.4.3-23: Summary of results obtained in the dominant lethal assay, number of implantation sites ([redacted], 1980)

	Group				
	1	2	3	4	PC
	Dose [mg/kg bw]				
	0	200	800	2000	240
No. of implantation sites: Mean per dam ± SD					
Mating study week					
1	12.5 ± 1.28	11.3 ± 2.74	11.6 ± 1.78	12.2 ± 2.43	9.1 ± 2.95
2	12.2 ± 2.30	12.5 ± 1.90	11.6 ± 1.89	13.1 ± 1.25	7.4 ± 3.18
3	12.6 ± 1.22	13.4 ± 1.46	12.2 ± 1.83	11.6 ± 1.17	9.8 ± 2.17
4	12.1 ± 2.31	11.6 ± 2.90	12.5 ± 2.48	10.8 ± 3.81	12.7 ± 1.44
5	12.5 ± 2.12	11.6 ± 2.27	12.0 ± 2.26	11.8 ± 1.90	12.3 ± 2.09
6	12.8 ± 2.36	13.4 ± 1.39	12.2 ± 2.16	12.3 ± 2.55	13.1 ± 1.44
7	13.2 ± 1.51	12.1 ± 2.55	11.8 ± 3.28	13.0 ± 1.96	11.4 ± 2.91
8	12.0 ± 2.06	12.9 ± 2.91	11.2 ± 2.98	11.9 ± 2.41	12.6 ± 1.91

PC: positive control Cytoxan

The amount of early and late resorptions was comparable to those of control animals for all mating intervals (refer to Tables below). There were no non-viable fetuses in any dose group of any mating interval. However, there was a slight but statistically significant decrease in the number of viable fetuses in females of the 800 mg/kg bw group mated during study week 1 and in females of the 2000 mg/kg bw group mated during study week 3 (refer to Table 5.4.3-26). As no increase in early fetal deaths were observed in these groups, the findings were not attributed to glyphosate treatment and considered to be incidental.

Table 5.4.3-24: Summary of results obtained in the dominant lethal assay, number of early resorptions ([redacted], 1980)

	Group				
	1	2	3	4	PC
	Dose [mg/kg bw]				
	0	200	800	2000	240
Early resorptions: Mean per dam					
Mating study week					
1	0.6 ± 0.93	0.7 ± 1.54	0.8 ± 1.11	0.4 ± 0.62	4.9 ± 2.12**
2	0.9 ± 2.03	1.0 ± 1.41	0.7 ± 1.46	0.8 ± 0.90	4.1 ± 1.89**
3	0.5 ± 0.76	1.3 ± 1.53	0.8 ± 1.00	0.6 ± 0.80	4.4 ± 3.18**
4	0.8 ± 1.27	0.6 ± 1.00	1.2 ± 1.67	0.9 ± 0.86	1.5 ± 2.07
5	0.4 ± 0.83	0.5 ± 0.72	1.3 ± 3.39	0.9 ± 1.28	0.5 ± 0.52
6	0.8 ± 1.48	0.6 ± 0.77	0.6 ± 0.79	0.9 ± 1.22	1.2 ± 2.01
7	1.3 ± 1.41	0.6 ± 0.90	0.4 ± 0.61	0.5 ± 0.64	1.0 ± 1.21
8	0.5 ± 0.99	0.9 ± 1.13	0.6 ± 0.76	1.1 ± 1.53	0.7 ± 1.59

PC: positive control Cytoxan

Table 5.4.3-24: Summary of results obtained in the dominant lethal assay, number of early resorptions ([redacted], 1980)

	Group				
	1	2	3	4	PC
	Dose [mg/kg bw]				
	0	200	800	2000	240

** $p < 0.01$ statistical significance when compared to controls

Table 5.4.3-25: Summary of results obtained in the dominant lethal assay, number of late resorptions ([redacted], 1980)

	Group				
	1	2	3	4	PC
	Dose [mg/kg bw]				
	0	200	800	2000	240
Late resorptions: Mean per dam ± SD					
Mating study week					
1	0.0 ± 0.00	0.1 ± 0.35	0.3 ± 0.70	0.1 ± 0.33	0.1 ± 0.35
2	0.2 ± 0.33	0.1 ± 0.25	0.8 ± 2.81	0.2 ± 0.44	0.0 ± 0.00
3	0.1 ± 0.27	0.1 ± 0.24	0.1 ± 0.24	0.8 ± 3.15	0.6 ± 2.00
4	0.9 ± 3.21	0.2 ± 0.39	0.0 ± 0.00	0.0 ± 0.00	0.2 ± 0.60
5	0.8 ± 3.21	0.3 ± 0.59	0.1 ± 0.33	0.4 ± 0.78	0.1 ± 0.29
6	0.2 ± 0.55	0.2 ± 0.37	0.1 ± 0.33	0.1 ± 0.26	0.3 ± 0.61
7	0.2 ± 0.50	0.1 ± 0.23	0.3 ± 0.75	0.3 ± 0.59	0.2 ± 0.39
8	0.1 ± 0.32	1.3 ± 3.56	0.3 ± 0.44	0.6 ± 1.26	0.5 ± 1.87

PC: positive control Cytoxan

Table 5.4.3-26: Summary of results obtained in the dominant lethal assay, number of viable fetuses ([redacted], 1980)

	Group				
	1	2	3	4	PC
	Dose [mg/kg bw]				
	0	200	800	2000	240
Viable fetuses: Mean per dam ± SD					
Mating study week					
1	11.8 ± 1.38	10.5 ± 3.58	10.5 ± 1.83*	11.6 ± 2.32	4.1 ± 3.77**
2	11.1 ± 3.35	11.4 ± 2.31	10.1 ± 3.26	12.1 ± 1.89	3.3 ± 3.34**
3	12.0 ± 1.30	12.0 ± 2.42	11.4 ± 2.15	10.3 ± 2.95*	4.8 ± 3.17**
4	10.4 ± 3.76	10.8 ± 3.24	11.3 ± 3.10	9.9 ± 4.46	10.9 ± 2.87
5	11.3 ± 3.27	10.8 ± 1.95	10.4 ± 4.09	10.4 ± 2.94	11.7 ± 2.06
6	11.8 ± 2.43	12.6 ± 1.42	11.4 ± 2.09	11.3 ± 3.04	11.6 ± 1.87

Table 5.4.3-26: Summary of results obtained in the dominant lethal assay, number of viable fetuses ([redacted], 1980)

	Group				
	1	2	3	4	PC
	Dose [mg/kg bw]				
	0	200	800	2000	240
7	11.8 ± 1.84	11.4 ± 2.76	11.1 ± 3.05	12.3 ± 1.98	10.3 ± 3.08
8	11.4 ± 2.20	10.7 ± 4.25	10.4 ± 2.94	10.3 ± 2.91	11.4 ± 3.88

PC: positive control Cytoxan

*: $p < 0.05$ and ** $p < 0.01$ statistical significance when compared to controls

Further, the incidence of pre- and post-implantation losses (refer to Tables below) was not considered to be affected upon treatment with glyphosate and the retrospectively calculated dominant lethal factors (refer to Table 5.4.3-29) were comparable for glyphosate treated and control animals.

Table 5.4.3-27: Summary of results obtained in the dominant lethal assay, number of pre-implantation losses ([redacted], 1980)

	Group				
	1	2	3	4	PC
	Dose [mg/kg bw]				
	0	200	800	2000	240
Pre-implantation loss$: Mean per dam ± SD					
Mating study week					
1	0.47 ± 0.80	0.93 ± 2.15	0.19 ± 0.40	1.06 ± 1.30	2.33 ± 3.33
2	1.00 ± 1.12	0.94 ± 1.29	1.56 ± 1.85	0.35 ± 0.61	1.69 ± 2.39
3	0.71 ± 0.73	1.00 ± 2.15	0.83 ± 0.92	0.65 ± 0.79	1.13 ± 1.15
4	0.37 ± 0.68	0.35 ± 0.61	0.71 ± 0.77	1.12 ± 2.12	0.77 ± 0.73
5	0.84 ± 0.96	0.71 ± 1.49	0.18 ± 0.64	0.78 ± 1.11	0.83 ± 1.40
6	0.94 ± 1.55	0.53 ± 1.02	0.71 ± 0.92	1.80 ± 2.08	0.29 ± 0.61
7	0.32 ± 0.75	1.26 ± 2.31	1.39 ± 2.06	0.60 ± 0.74	0.42 ± 0.67
8	0.72 ± 1.67	1.22 ± 1.77	1.20 ± 1.11	2.00 ± 2.58	0.86 ± 1.70

PC: positive control Cytoxan

$: Calculated based on raw data provided in study report as follows: (number of corpora lutea - number of implants)/number of pregnant females

*: $p < 0.05$ and ** $p < 0.01$ statistical significance when compared to controls

Table 5.4.3-28: Summary of results obtained in the dominant lethal assay, number of post-implantation losses ([redacted], 1980)

	Group				
	1	2	3	4	PC
	Dose [mg/kg bw]				
	0	200	800	2000	240
Post-implantation loss: Mean per dam ± SD					
Mating study week					
1	0.6 ± 0.93	0.8 ± 1.52	1.1 ± 1.20	0.5 ± 0.72	5.1 ± 2.12**
2	1.1 ± 2.05	1.1 ± 1.39	1.5 ± 3.22	1.0 ± 1.06	4.1 ± 1.89**
3	0.6 ± 0.76	1.4 ± 1.50	0.8 ± 0.99	1.4 ± 3.10	4.9 ± 3.36**
4	1.7 ± 3.35	0.8 ± 1.03	1.2 ± 1.67	0.9 ± 0.86	1.6 ± 2.42
5	1.2 ± 3.25	0.8 ± 0.88	1.6 ± 3.47	1.3 ± 1.88	0.6 ± 0.51
6	1.0 ± 1.36	0.7 ± 0.99	0.8 ± 0.75	1.0 ± 1.20	1.5 ± 1.91
7	1.4 ± 1.54	0.6 ± 0.90	0.7 ± 0.97	0.7 ± 0.70	1.2 ± 1.19
8	0.6 ± 0.98	2.2 ± 3.47	0.8 ± 1.01	1.6 ± 2.19	1.2 ± 2.52

PC: positive control Cytoxan

** $p < 0.01$ statistical significance when compared to controls

Treatment with the positive control Cytoxan® induced statistically significant dominant lethal effects and effect at the postmeiotic stage of spermatogenesis and demonstrated the validity of the test. When compared to the vehicle control group, there was a statistically significant decrease in the number of viable fetuses and an increase in the proportion of early fetal deaths, indicated by a statistically significant increase in the mean number of early resorptions and post-implantation loss observed during the first 3 weeks of mating.

Table 5.4.3-29: Summary of results obtained in the dominant lethal assay, dominant lethal factors ([redacted], 1980)

	Group				
	1	2	3	4	PC
	Dose [mg/kg bw]				
	0	200	800	2000	240
Dominant lethal factor					
Mating study week					
1	5.19	7.10	9.68	4.35	55.47
2	8.70	8.50	12.98	7.66	55.46
3	4.55	10.13	6.82	11.62	50.64
4	14.35	6.60	9.43	7.95	13.94
5	9.28	7.11	13.24	11.32	4.76
6	8.23	5.51	6.28	8.11	11.48
7	10.76	5.24	5.66	5.64	10.22
8	5.09	16.81	7.17	13.61	9.60

PC: positive control Cytoxan

[#]: Calculated as follows: (total post implantation loss per female / total implantations per female) x 100

In addition, there was a lower number of implantations observed during the first three weeks of mating after treatment with Cytoxan® (refer to
), with a statistically significant decrease at mating weeks 2 and 3 when compared to control animals (refer to Table 5.4.3-21). The observations were attributed to a decreased ovulation rate, as seen by a lower number of corpora lutea during these weeks (refer to Table 5.4.3-22).

III. CONCLUSION

Under the conditions of the test, glyphosate had no mutagenic potential in CD-1 mice in the dominant lethal assay up to a dose level of 2000 mg/kg bw. Based on the experimental findings, glyphosate was considered non-genotoxic to germ cells *in vivo*.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Negative for mutagenic effects in the dominant lethal assay in mice.

The experimental performance of the study was not conducted under GLP criteria, but matched the descriptions of OECD guideline 478 (2016). However, there was a large number of deviations when compared to the current guideline. The number of implantations per group for each mating period was below the recommended number of 400 implants per group. It can be assumed that the number of males and females used for mating was too low. Fetal body weights were not recorded and no historical control data were provided. No dominant lethal frequency was calculated in the study report, but as the raw data were provided in the study report, dominant lethal factors could be calculated retrospectively for this evaluation. Further deviations were of minor degree and considered to not compromise the outcome of the study. Due to the large number of deviations, the study was considered to provide supporting information.

Assessment and conclusion by RMS:

Literature evaluation

A literature search for the active substance glyphosate was performed in accordance to the provisions of the EFSA Guidance "Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) 1107/2009" and updated Appendix to this Guidance document. The following publications were found relevant and reliable for this section and the summaries are thus presented below and are part of the general discussion at the beginning of the section.

Table 5.4-1: Overview on literature found relevant for evaluation of glyphosate in section 5.4

Annex Point	Study	Study type Parameters investigated Test system	Substance Dose levels Exposure	Reliability & restriction comments	Result
CA 5.4/001	Adler-Flindt *et al*., 2019	Cytotoxicity assay Balb/3T3 mouse fibroblast cells and in human foreskin	Glyphosate isopropylamine salt Purity: not reported	Reliable with restriction Glyphosate salt tested not sufficiently	3T3 cells: IC_{50} = 954.8 ± 117.1 µg/mL hFF cells: IC_{50} = 1211 ± 885.7 µg/mL

Table 5.4-1: Overview on literature found relevant for evaluation of glyphosate in section 5.4

Annex Point	Study	Study type Parameters investigated Test system	Substance Dose levels Exposure	Reliability & restriction comments	Result
		fibroblasts (hFF)	Up to 1000 µg/mL (information on ± S9 mix not reported) 72 h exposure	characterized IC 50 standard deviations in human fibroblasts for glyphosate and GBH are excessively large	
CA 5.4/002	Ilyushina *et al*., 2018	Micronucleus test *in vivo* CD1 mice, 5/sex/group Sampling: 22 h after 2nd dose	Glyphosate (4 batches tested) Purities: 95.7, 98.3, 95.1 and 95.8 % 2000 mg/kg bw/day or MTD Oral gavage on 2 consecutive days	Reliable with restriction Sources of glyphosate not reported	Micronucleus test: negative (all 4 batches)
CA 5.4/003	Nagy *et al*., 2019	Comet assay *in vitro* human mononuclear white blood (HMWB) cells	Glyphosate Purity: not reported 1 – 1000 µM ± S9 mix 4 h exposure	Reliable with restriction Glyphosate tested not characterized No positive control utilized	Comet assay: negative Cytotoxicity: no cytotoxicity observed
CA 5.4/004	De Almeida *et al*., 2018	Comet assay *in vitro* breast cancer cells (MCF7 and MDA-MB-231) and endometrial cancer cells (HEC1A)	Glyphosate Purity: 99.5 % 500 – 1000 µg/mL (information on S9 mix not reported) 4 h exposure	Reliable with restriction Study conducted at physiologically unrealistic concentrations, > 1mM	Comet assay: positive in HEC1A and MDA-MB-231 cells (500 and 1000 µg/mL), negative in MCF7 cells Cytotoxicity: at ≥ 75 µg/mL in HEC1A cells, no cytotoxicity in MCF7 and MDA-MB-231 cells
CA 5.4/005	Ilyushina *et al*., 2018	Micronucleus test *in vivo* CD1 mice, 5/sex/group Sampling: 22 h after 2nd dose	Glyphosate acid (3 batches from 3 sources tested) Purities: 96.6, 95.8 and 95.7 % 500, 1000 and 2000 mg/kg bw/day Oral gavage on 2 consecutive days	Reliable with restriction Source of three glyphosate batches not revealed Insufficient detail on methodology	Micronucleus test: positive (technical batch I; likely due to 0.13 % formaldehyde as impurity) negative (technical batches II and III)
CA 5.4/006	Santovito *et al*., 2018	CBMN test Chromosome aberration test (CA) *in vitro* human peripheral lymphocytes	Glyphosate Purity: not reported 0.0125 – 0.5 µg/mL (information on S9 mix not reported)	Reliable with restriction Glyphosate tested not sufficiently characterized Deficiencies relative to OECD test guideline	CBMN: positive CA: positive

Table 5.4-1: Overview on literature found relevant for evaluation of glyphosate in section 5.4

Annex Point	Study	Study type Parameters investigated Test system	Substance Dose levels Exposure	Reliability & restriction comments	Result
			CBMN: 72 h exposure CA: 52 h exposure		
CA 5.4/007	Kasuba *et al*., 2017	Comet assay CBMN cytome assay Oxidative stress[#] *in vitro* In HepG2 cells	Glyphosate Purity: not reported 0.5 – 3.5 µg/mL (information on S9 mix not reported) Comet assay: 4 and 24 h exposure MN: 4 and 24 h exposure	Reliable with restriction Findings at low *in vitro* concentrations not concordant with high dose *in vivo* studies	Comet assay: negative CBMN: equivocal Proliferation: slight increase after 4 h treatment Oxidative stress: no oxidative DNA damage
CA 5.4/008	Kwiatkowska *et al*., 2017	Comet assay / DNA repair Methylation of p16 and p53 promotor regions Global DNA methylation *in vitro* human peripheral blood mononuclear cells	Glyphosate Purity: 95 % 0.25 – 10 mM (information on S9 mix not reported) 24 h exposure	Reliable with restriction Lowest dose *in vitro* with positive finding (0.5 mM) is higher than *in vivo* plasma concentration with negative result (0.3 mM) dosed at 2000 mg/kg limit	Comet assay: -DNA damage: positive at ≥ 0.5 mM -DNA repair: increase after 120 min recovery Methylation of p16 and p53 promotor regions: increased methylation of p53 promotor at ≥ 0.25 mM Global DNA methylation: decreased at 0.25 mM
CA 5.4/009	Suárez-Larios *et al.*, 2017	Induction of DNA double strand breaks (immunofluorescence of γ-phosphorylated H2AX foci) Induction of proteins involved in DNA recombination (Westernblot analysis) *in vitro* human peripheral blood lymphocytes	Glyphosate Purity: not reported 0.4 – 50 µM (information on S9 mix not reported) 1.5 h exposure	Reliable with restriction Glyphosate tested not sufficiently characterized Results not concordant with high dose *in vivo* studies	Induction of DNA double strand breaks: positive at ≥ 0.33 µM Induction of proteins involved in DNA recombination: positive Cytotoxicity: 100 – 70 % viability at 1.25 – 5 µM
CA 5.4/010	Townsend *et al*., 2017	Comet assay *in vitro* Burkitt's Lymphoma (Raji) cells	Glyphosate Purity: 95 % 0.1 µM – 15 mM (information on S9 mix not reported) 10 – 120 min exposure	Reliable with restriction Positive findings only at doses not physiologically feasible *in vivo* No positive control data	Comet assay: positive at ≥ 1 mM Cytotoxicity: at ≥ 10 mM

Table 5.4-1: Overview on literature found relevant for evaluation of glyphosate in section 5.4

Annex Point	Study	Study type Parameters investigated Test system	Substance Dose levels Exposure	Reliability & restriction comments	Result
CA 5.4/011	Roustan *et al*., 2014	Micronucleus assay Photoactivation Intracellular ROS *in vitro* CHO-K1 cells	Glyphosate Purity: not reported 5 – 100 µg/mL ± S9 mix 3 h exposure	Reliable with restriction Test substances not sufficiently characterized No historical control data	Micronucleus assay: negative (-S9) positive (+S9; > 10 µg/mL) Photoactivation: positive Oxidative stress: negative
CA 5.4/012	Mañas *et al*., 2013	Comet assay (blood and liver) Oxidative stress[##] (liver, kidney, lung and heart) *in vivo* Balb C mice	Glyphosate Purity: 96 %, 40 or 400 mg/kg bw/day 14 day administration in drinking water	Reliable with restriction Only two doses No dose-response Doses much lower than other *in vivo* studies with negative results	Comet assay: positive (40 and 400 mg/kg bw/day) Oxidative stress: Negative, except decrease in SOD activity in heart, increase in CAT activity in liver
CA 5.4/013	Koller *et al*., 2012	Comet assay CBMN cytome assay *in vitro* buccal epithelial cells (TR146)	Glyphosate Purity: 95 % 0 – 2000 mg/L 20 min exposure	Reliable with restriction No reported slide coding for blinded scoring Positive finding concentration (20-2000 mg/L), yet noted significant effects on necrosis and apoptosis markers were observed at 20 mg/L in parallel experiments	Comet assay: positive (≥ 20 mg/L) CBMN: positive (≥ 10 mg/L) Cytotoxicity: at ≥ 80 mg/L

[#]: Oxidative stress parameters investigated: lipid peroxidation (TBARs), total antioxidant capacity, ROS, GSH, GSH peroxidase activity
[##]: Oxidative stress parameters investigated: TBARs, SOD and CAT activity
CA: Chromosome aberration; CAT: Catalase; CBMN: Cytokinesis block micronucleus assay; GSH: Glutathion; MTD: Maximum tolerated dose; ROS: Reactive oxygen species; SCGE: single cell gel electrophoresis; SOD: Superoxid dismutase; TBARs: Thiobarbituric active reactive substances

1. Information on the study

Data point:	CA 5.4/001
Report author	Adler-Flindt, S. *et al.*
Report year	2019
Report title	Comparative cytotoxicity of plant protection products and their active ingredients
Document No	doi.org/10.1016/j.tiv.2018.10.020 ISSN: 0887-2333

Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Yes/Reliable with restrictions

4. Full summary of the study according to OECD format

In this study PPPs were analyzed for the correlation of GHS classifications resulting mainly from in vivo LD50-values with classifications obtained from calculated LD50-values using the CLP calculation method (CM). Accordingly, the CM predicted 80 % of the PPPs correctly. However 31 % of classified products were not identified revealing a considerable inaccuracy of this method. Based on these results ten PPPs and corresponding ASs were further tested in a cytotoxicity assay employing 3T3 and hFF cells (one PPP and corresponding AS were tested in HepaRG cells).

Materials and methods

Chemicals - Glyphosate isopropylamine salt (MON 0138) and RoundUP LB Plus (360 g/L A.I., MON 52276) was purchased from Monsanto Agrar Deutschland GmbH, Düsseldorf, Germany. Glyphosate was received dissolved in water at a concentration of 620 g/L.

Culture of Balb/3T3 cells, hFF cells and HepaRG cells - Mouse fibroblast cells (Balb/3T3) and human foreskin fibroblast cells (hFF) were cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10 % (v/v) foetal bovine serum (FBS) and 50 U/mL penicillin/streptomycin, and passaged every 3 to 4 days at a split-ratio of approximately 1:20 for 3T3 cells and 1:8 for hFF cells after enzymatic dissociation with trypsin-EDTA. Differentiated HepaRG cells were received in 96-well plates and were grown for 2 weeks in Williams's medium containing 10 % foetal calf serum (FCS), 100 U/mL penicillin/streptomycin, 0.05 % human insulin and 50 µM hydrocortisone hemisuccinate. For differentiation, cells were incubated for a another 2 weeks in differentiation medium containing in addition to the above components 1.7 % DMSO.

Proliferation test - To determine the optimal seeding density for the 48-hour toxicity assay, proliferation tests were performed in 2 independent runs. 3T3 and hFF cells were seeded in test medium (DMEM) supplemented with 10 % (v/v) Panexin NTA serum substitute and 50 U/mL penicillin/streptomycin, into 96-well plates, in a 2-fold dilution series, with the highest cell density being 1.6×10^4 cells/well. After 48 hours the viability of the cells was assessed by measuring the reduction of resazurin to the fluorescent resorufin. The fluorescence signal was measured at 530 nm (excitation) and at 590 nm (emission) using a multimode plate reader.

Cytotoxicity test - 3T3 and hFF cells were dissociated into single cells and seeded into 96-well plates in 100 µL per well of DMEM supplemented with 10 % (v/v) Panexin NTA serum substitute and 50 U/mL penicillin/streptomycin, at a density of 2,000 cells per well for 3T3 cells and 4,000 cells per well for hFF cells. Differentiated HepaRG cells were received in 96-well plates already seeded in a density of 9,000 cells per well. The test medium for HepaRG cells was based on phenol-red-free Williams's medium containing 2 % foetal calf serum (FCS), 100 U/mL penicillin/streptomycin, 0.05 % human insulin and 50 µM hydrocortisone hemisuccinate. After 24 hours, 100 µL of test medium (containing the double required final concentration of the test substance) were added to each well (day 0). After 48 hours cell viability was assessed by measuring the reduction of resazurin to the fluorescent resorufin. The fluorescence signal was measured at 530 nm (excitation) and at 590 nm (emission) using a multimode plate reader.

Testing of glyphosate and MON 52276 - At least two independent runs of each experiment were performed.

The highest tested concentration for glyphosate isopropylamine salt was 1000 µg/mL.

Results
Proliferation assay - Proliferation tests were performed to determine the optimal seeding density for the 48-hour toxicity assay. The cells were seeded in a 2-fold dilution series into 96-well plates, and reduction of resazurin into the fluorescent resorufin was measured after 48 hours. The proliferation assay revealed an optimal seeding concentration for the cytotoxicity test of 2,000 cells per well for 3T3 cells and 4,000 cells per well for hFF cells.

Testing of glyphosate and MON 52276 - Two controls, saccharin as the negative control and 5-FU as the positive control, were tested for 48 hours on 3T3 and hFF cells. The treatment of both cell types with saccharin did not significantly reduce cell viability up to a concentration of 1,000 µg/mL. In contrast, the treatment with 5-FU resulted in a noticeable reduction of viability, with different IC_{50} values for the two cell types, i.e. 0.06 ± 0.01 µg/mL for 3T3 cells and 0.14 ± 0.05 µg/mL for hFF cells. The IC_{50} for glyphosate isopropylamine salt was 954.8 ± 117.1 µg/mL for 3T3 cells and 1211 ± 885.7 µg/mL for hFF cells. The IC_{50} for MON 52276 was 313.2 ± 29.3 µg/mL for 3T3 cells and 361.6 ± 612 µg/mL for hFF cells. The ratio of the AUC under the % viability vs concentration curve of glyphosate isopropylamine salt over MON 52276 is 1.7 for 3T3 cells and 1.3 for hFF cells. This indicates that the treatment of hFF cells with glyphosate and its formulation Roundup did not result in significant differences between cytotoxicity curves. The ratio of the AUCs of glyphosate over MON 52276 for both cell types was below a factor 2 and could thus be regarded as minor.

Conclusion
In this study, glyphosate isopropylamine salt, amongst other pesticides, and its corresponding formulation MON 52276 were tested for cytotoxicity in 3T3 cells and hFF cells. The IC_{50} for glyphosate isopropylamine salt was 954.8 ± 117.1 µg/mL for 3T3 cells and 1211 ± 885.7 µg/mL for hFF cells and the IC_{50} for MON 52276 was 313.2 ± 29.3 µg/mL for 3T3 cells and 361.6 ± 612 µg/mL for hFF cells. The ratio of the AUCs of glyphosate over MON 52276 for both cell types was below a factor 2 and could thus be regarded as minor.

4. Assessment and conclusion

Assessment and conclusion by applicant:
It was the intention of this study to evaluate the GHS classification of pesticide formulations for acute toxicity based on calculated LD_{50} values using the CLP calculation method (CM). Because of the considerable inaccuracy of this method the *in vitro* cytotoxicity of 10 pesticide formulations was compared against that of the active ingredient using mouse (3T3) and human (hFF) fibroblasts. In this exercise the IC_{50} for glyphosate isopropylamine salt was found to be 954.8 ± 117.1 µg/mL for 3T3 cells and 1211 ± 885.7 µg/mL for hFF cells and the IC_{50} for MON 52276 was 313.2 ± 29.3 µg/mL for 3T3 cells and 361.6 ± 612 µg/mL for hFF cells. The difference in cytotoxicity (expressed as the AUC of the % viability vs concentration curve) between glyphosate and MON 52276 could be regarded as minor.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate isopropylamine salt used was not sufficiently characterized and the standard deviation of the IC_{50} of glyphosate (1211 ± 885.7 µg/mL) and MON 52276 (361.6 ± 612 µg/mL) for human fibroblasts is too large.

Assessment and conclusion by RMS:

Reliability criteria for *in vitro* toxicology studies

Publication: Adler-Flindt *et al.*, 2019	Criteria	Comments

	met? Y/N/?	
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	N	Glyphosate, isopropylamine salt, purity not reported. Source: Monsanto Agrar Deutschland GmbH, Düsseldorf, Germany.
Only glyphosate acid or one of its salts is the tested substance	N	MON 52276 (RoundUP LB Plus, 360 g/L A.I.). Source : Monsanto Agrar Deutschland GmbH, Düsseldorf, Germany. Other pesticides and their formulations were tested as well.
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	Y	Mouse (3T3) and human (hFF) fibroblasts.
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	NA	
Test concentrations in physiologically acceptable range (< 1 mM)	Y (for local contact)	Test concentrations up to 1000 µg/mL which is beyond the systemic physiological range but not when applied dermally.
Cytotoxicity tests reported	Y	
Positive and negative controls	N	Saccharin was used as the negative control and 5-FU as the positive control.
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	Y	IC_{50} were calculated.
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Reliability not assignable		
Not reliable		

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate isopropylamine salt used was not sufficiently characterized and the standard deviation of the IC_{50} of glyphosate (1211 ± 885.7 µg/mL) and MON 52276 (361.6 ± 612 µg/mL) for human fibroblasts is too large.

1. Information on the study

Data point:	CA 5.4/002
Report author	Ilyushina, N. *et al.*
Report year	2018
Report title	Maximum tolerated doses and erythropoiesis effects in the mouse bone marrow by 79 pesticides' technical materials assessed with the micronucleus assay
Document No	doi.org/10.1016/j.toxrep.2018.12.006 ISSN: 2214-7500
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary of the study according to OECD format

Effects of technical materials of pesticide active ingredients, belonging to various chemical classes, on erythropoiesis in mouse bone marrow were studied as part of the research on the pesticide mutagenic activity in micronucleus test. The purpose of the present study was to estimate the toxic action of the test substances on the target organ and the validity of the results of the micronucleus assay under conditions of erythropoiesis suppression.

Materials and methods

Chemicals - Four glyphosate batches were tested with a purity of respectively 95.7, 98.3, 95.1, and 95.8 %.

Animals – CD-1 mice were purchased from "Andreevka" Branch of the Federal Government Budgetary Establishment of Science "Scientific Center of Biomedical Technologies" of the Federal Bio-Medical Agency of the Russian Federation. The acclimation period was 7 days. Mice had access to drinking water and feed ad libitum, and were maintained under a 12:12-hour light/dark photoperiod at 22 – 22.5 °C and 36 - 40 % humidity.

Mammalian Erythrocyte Micronucleus Test (OECD TG 474) - At least 5 groups of minimum 5 mice per sex were used. Each assay included a positive control group (40 mg/kg bw cyclophosphamide), a negative control group (1 % potato starch in water), and 3 treatment groups. Glyphosate was administered orally by gavage once a day for 2 subsequent days (24 hours apart) at a volume of 10 mL/kg bw. The maximum dose in the main experiment was either 2,000 mg/kg bw or the MTD as determined in a preliminary dose-finding experiment. Mice were sacrificed 22 hours after the second administration by cervical dislocation, then femurs were removed and bone marrow was harvested. To assess the effect of glyphosate on erythropoiesis, the ratio of polychromatic erythrocytes (PCEs) to the sum of PCEs and normochromatic erythrocytes (NCEs) was determined by counting at least 500 cells (PCEs + NCEs) per animal (at least 250 cells for each slide) under a Nikon Eclipse Ci-L microscope. At least 4,000 PCEs were counted per animal by two different researchers.

Statistical analysis - Statistical analysis was performed using SPSS Statistics v. 22.0 software. The statistical significance of the difference in the proportion of PCEs/(PCEs + NCEs) between the highest dose group and the concurrent negative control group was evaluated using the independent samples t-test for each study.

Results
It should be noted that the negative control values slightly varied from experiment to experiment, and that the historical negative controls of the laboratory were 0,50 ± 0,06 and 0,52 ± 0,06 PCEs/(PCEs+NCEs) females and males, respectively. Cyclophosphamide did not cause a significant decrease in the proportion PCEs/(PCEs+NCEs) in comparison with the negative control. The 4 glyphosate batches tested at the limit dose of 2,000 mg/kg bw in CD-1 mice according to the protocol of the *in vivo* micronucleus assay compliant with OECD test guideline 474 did not reveal any effect on erythropoiesis in the bone marrow.

Conclusion
Glyphosate tested at the limit dose of 2,000 mg/kg bw in mice in the *in vivo* micronucleus assay did not show any effect on erythropoiesis in the bone marrow.

3. Assessment and conclusion

Assessment and conclusion by applicant:
Along with 51 other pesticides 4 batches of glyphosate with purities ranging from 95.1 to 98.3 % were investigated for their effect on erythropoiesis in mice. To assess the toxicity of glyphosate on the bone marrow the *in vivo* micronucleus test in the mouse according to OECD test guideline 474 was conducted at the limit dose of 2,000 mg/kg bw. No effect of glyphosate on erythropoiesis was found.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the source of the glyphosate batches used was not reported and no suitable positive control was used in the micronucleus test. The test conducted was in compliance with OECD test guideline 474.

Assessment and conclusion by RMS:

Reliability criteria for *in vivo* toxicology studies

Publication: Ilyushina *et al*., 2018	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	Y	OECD TG 474, in vivo MN assay only used with the purpose to assess toxicity to erythropoiesis in the bone marrow.
Study performed according to GLP	N	Not stated.
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and	Y?	4 batches were tested

reported (i.e. purity, source, content, storage conditions)		with purity of 95.7, 98.3, 95.1, and 95.8 %. Source was not mentioned.
Only glyphosate acid or one of its salts is the tested substance	N	51 other pesticides were tested as well.
AMPA is the tested substance	N	
Study		
Test species clearly and completely described	Y	
Test conditions clearly and completely described	Y	
Route and mode of administration described	Y	
Dose levels reported	Y	Only limit dose of 2,000 mg/kg bw was considered.
Number of animals used per dose level reported	Y	At least 5 groups of minimum 5 mice per sex.
Method of analysis described for analysis test media	N	
Validation of the analytical method	N	
Analytical verifications of test media	N	
Complete reporting of effects observed	Y?	Only effect on erythropoiesis was reported.
Statistical methods described	Y	
Historical control data of the laboratory reported	Y	
Dose-effect relationship reported	N	Not possible since only one dose was used.
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Reliability not assignable		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the source of the glyphosate batches used was not reported. The test conducted was in compliance with OECD test guideline 474.		

1. Information on the study

Data point:	CA 5.4/003
Report author	Nagy, K. *et al.*
Report year	2019
Report title	Comparative cyto- and genotoxicity assessment of glyphosate and glyphosate-based herbicides in human peripheral white blood cells
Document No	doi.org/10.1016/j.envres.2019.108851 E-ISSN: 1096-0953
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	None
GLP/Officially recognised testing	No, not conducted under GLP/Officially recognised testing

facilities	facilities.
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary of the study according to OECD format

This study investigates the cyto- and genotoxic potential of the active ingredient glyphosate and GBHs in human mononuclear white blood (HMWB) cells. HMWB cells were treated for 4 h at 37 °C with increasing concentrations (1–1000 µM) of glyphosate alone and in three GBHs (Roundup Mega, Fozat 480 and Glyfos) to test cytotoxic effect with fluorescent colabelling and genotoxic effect with comet assay. In addition, each concentration was tested with and without metabolic activation using human liver S9 fraction.

Materials and methods
Chemicals; Analytical-grade glyphosate (N-(phosphonomethyl) glycine, CAS No: 1071-83-6) was purchased from VWR International Kft (Debrecen, Hungary) and samples of three GBHs, i.e.
• Roundup Mega containing 551 g/L or 42 % (w/w) potassium salt of glyphosate (CAS No: 70901-12-1; equivalent to 450 g/L glyphosate) and 7 % (w/w) ethoxylated etheralkylamine (CAS No: 68478-96-6);
• Fozat 480 containing 480 g/L or 41 % (w/w) isopropylammonium salt of glyphosate (CAS No: 38641-94-0; equivalent to 360 g/L glyphosate) and < 5 % (w/w) hygroscopic substances;

• Glyfos containing 480 g/L or 42 % (w/w) isopropylammonium salt of glyphosate (equivalent to 360 g/L glyphosate) and 9 % (w/w) polyethoxylated tallow amine (CAS No: 61791-26-2);

were kindly provided by pesticide applicators. Composition data for each formulation were retrieved from the material safety data sheets (MSDS). Chemicals used for the assays and human liver-derived metabolic activation system (S9 fraction) were obtained from Sigma-Aldrich Chemie GmbH (Heidelberg, Germany). Cell culture medium and its supplements were obtained from Biowest (Nuaillè, France). The acetomethoxy derivative of calcein (Calcein AM) and propodium iodide (PI) fluorescent dyes were purchased from Biotium (Hayward, CA, USA). Heparin-containing vacutainers were purchased from BD Vacutainer Systems (Plymouth, UK).

Cell cultures; Human peripheral whole blood samples were obtained by venipuncture and collected into heparin-containing vacutainer tubes from four non-smoking, healthy volunteers (three males and one female, aged 20–40 years) without known previous contact with pesticides, mutagens or carcinogens. Cultures were prepared within 1 h of phlebotomy. Human mononuclear white blood (HMWB) cells were prepared from erythrocytes by density-gradient centrifugation over Histopaque-1077 gradient. The buffy coat was aspirated and re-suspended in RPMI 1640 medium containing 10 % foetal calf serum (FCS).

Cell treatment; HMWB cells were treated in the cell-culture medium with increasing concentrations (1 µM, 10 µM, 100 µM, 250 µM, 500 µM, 750 µM and 1 mM) of glyphosate alone and in three GBHs in a way that the concentrations of glyphosate in GBHs were equivalent. Concentrations were chosen based on the results from previous in vitro studies performed on human lymphocytes. The stock solutions and the dilution series were made in phosphate-buffered saline (PBS) and adjusted with 1 M NaOH to pH 7.2. Aliquots of different concentrations of glyphosate and GBH solutions, as well as PBS as negative control, were added to the cell cultures and incubated for 4 h at 37 °C. The PBS content was always < 10 % (v/v) in the cell culture medium. The experiments were conducted in the presence and absence of S9 fraction. 100 µL of the working S9 mix containing 10 % (v/v) of S9 fraction was composed of 8 mM MgCl2, 33 mM KCl, 100 mM sodium phosphate buffer pH 7.4, 5 mM glucose-6-phosphate, and 4 mM NADP was added to the S9+ samples. 100 µM hydrogen peroxide was used as a positive control.

Cytotoxicity assay; After treatment, aliquots of samples were immediately subjected to cytotoxicity test. Calcein AM and propidium iodide (PI) fluorescent dyes were used to colabel the cells. Calcein AM is a non-polar compound that passively crosses the plasma membrane of living cells, where it is cleaved by

intracellular esterases to reveal a very polar derivative of fluorescein (calcein) that remains trapped in the cytoplasm. PI is a DNA intercalating dye, which is able to permeate membranes of dead and dying cells but cannot penetrate plasma membranes of live healthy cells. Both fluorescent dyes were dissolved in PBS (pH 7.2) to a final concentration of 2 µM each. 200 µL of this working solution added to the cell pellets (1 × 105 cells), and incubated for 30 min at 4 °C, protected from light. The labelled cells were washed and re-suspended in ice-cold PBS buffer. 40 µL of the cell suspension was put on a microscope slide for immediate microscopic examination at 100x magnification using a Zeiss Axioplan epifluorescent microscope. FITC filter for Calcein AM and TRITC filter for PI was applied to excite the colabelled cells. Survival rate was determined by visual examination of 10 randomly selected non-overlapping fields per slide. Each field contained 10 to 30 images. Cell viability was expressed as the mean of the percentages of living cells from repeated experiments. The proportions of living cells observed in technical replicates were subjected to statistical analysis.

Genotoxicity assay; The alkaline version of the comet assay was performed according to the methodology of Collins (2004). Following treatment, samples were centrifuged and HMWB cells were resuspended in serum-free medium at a cell density of 2000 cells/µL. Degreased frosted slides were coated with two layers: 1 % normal melting point agarose (NMA) covered with 0.75 %, low melting point agarose (LMA) containing the cells (~2 × 105 per slide). After solidification, the embedded cells were lysed (2.5 M NaCl, 100 mM Na2EDTA, 10 mM Tris base, pH 10, 1 % sodium N-lauroyl sarcosinate and 1 % Triton X-100 added fresh) at 4 °C for 18–20 h, shielded from light. After lysis, the DNA was allowed to unwind for 20 min in alkaline electrophoresis buffer (300 mM NaOH, 1 mM Na2EDTA, pH 13) and subjected to electrophoresis in the same buffer for 20 min at 0.8 V/cm and 300 mA in a horizontal electrophoresis tank (Cleaver Scientific, Rugby, UK). Finally, the slides were rinsed gently three times with neutralization buffer (0.4 M Tris base-HCl, pH 7.5) to remove excess alkali and detergent. After drying, each slide was stained with ethidium bromide (20 µg/ml) and stored in a humidified container at 4 °C until analysis. The fluorescence signal was detected at 400x magnification using a Zeiss Axioplan epifluorescent microscope equipped with a CCD camera connected to an image analysis system. The Comet Imager v.2.2.1. Software (MetaSystems GmbH, Germany) was used to analyse 2 × 50 randomly captured comets from duplicate slides and compute the DNA damage parameters. Percentage of DNA in the tail (tail DNA%) and tail length in µm (TL) were measured to quantify DNA damage. The results are presented as mean of the median values of DNA damage parameters from repeated experiments. The medians of technical replicates were subjected to statistical analysis.

Data analysis; Experiments were independently performed three times from three different donors. The rate of cell viability and the central values of DNA damage parameters in the comet assay induced by various concentrations of the xenobiotics in repeated experiments were statistically compared to that of untreated cells using ANOVA with Dunnett's post hoc test. To statistically analyse the effect of metabolic activation, DNA damage values were pooled from three repeated experiments to compare results from S9-treated and S9-untreated samples at each exposure concentration by using Mann-Whitney test because pooled data sets followed non-normal distribution. Statistically significant difference was accepted at 5 % significance level.

Results

Cytotoxicity; The viability of HMWB cells treated with glyphosate alone was found to be over 86 % in the absence and presence of S9 over the entire concentration range (please refer to Figure below). Regardless of metabolic activation, GBHs induced a significant decrease in the proportion of living cells from 250 µM of Round Mega and Glyfos whereas from 500 µM of Fozat 480.

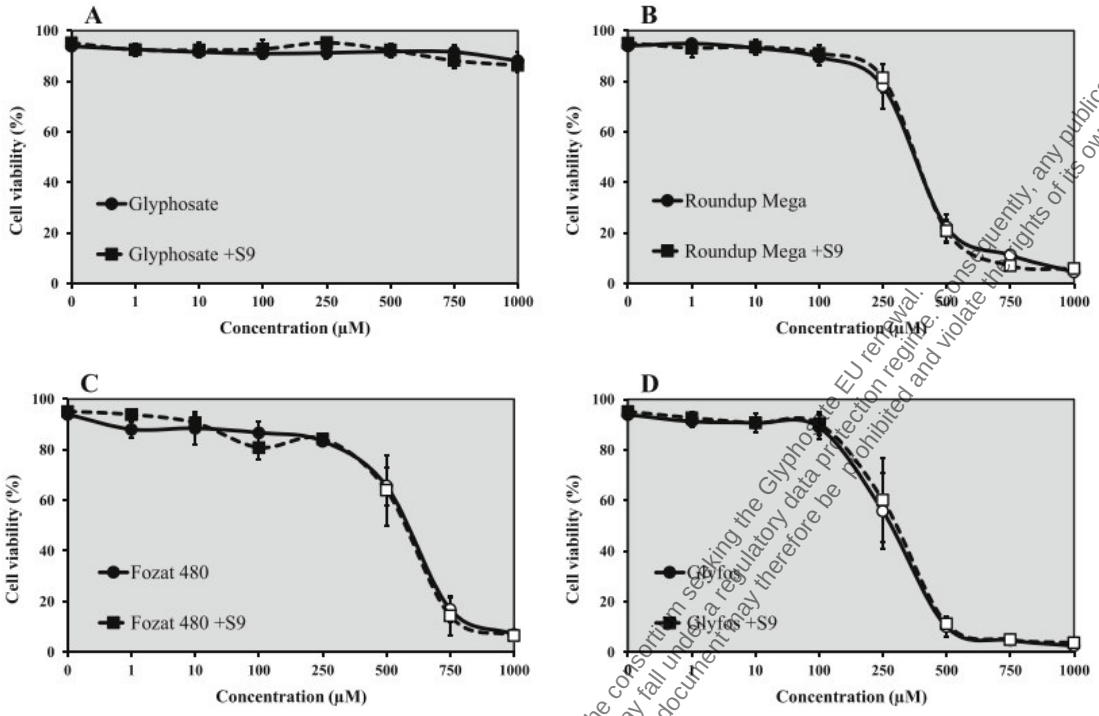


Figure 5.4.3-1: Effect of 4-h exposure to increasing concentrations of glyphosate (A), Roundup Mega (B), Foza 480 (C) and Glyfos (D) on cell viability in the absence and presence (+S9) of metabolic activation system. The data points indicate the means ± standard error of the mean (SEM) and three repeated experiments. Statistically significant decrease of cell viability, indicated by empty data points, was determined by comparing the values induced by various doses of glyphosate or GBHs to the background level of untreated cells by ANOVA with Dunnett's post hoc test. (from: Nagy *et al*., 2019)

DNA damage; Exposure of HMWB cells to glyphosate in the 0–1000 µM concentration range did not result in dose-dependent increase of DNA damage measured by the comet assay parameters. Unlike the active principle, GBHs induced statistically significant increase of both DNA damage parameters from 500 µM (Roundup Mega and Glyfos) and 750 µM (Fozat 480) compared to untreated cells. S9 treatment did not influence the effect of active ingredient glyphosate over the whole concentration range. On the contrary, addition of S9 to the assay modified the effects of GBHs in a non-consistent manner. The presence of metabolic enzymes significantly decreased the DNA damage induced by Roundup Mega and Fozat 480 at 1000 µM and from 10 µM, respectively. Metabolic activation could be observed only in samples exposed to 250 µM or higher concentrations of Glyfos indicated by the statistically significant differences in the tail DNA% and TL values between the S9-treated and S9-untreated cells (Tables 1 and 2).

Table 5.4.3-30: DNA damage induced by 4-hour exposure to glyphosate and GBHs with and without metabolic activation system (S9) in HMWB cells measured as tail DNA% (from: Nagy *et al*., 2019)

Concentration (µM)	Tail DNA% ± SEM							
	- S9				+ S9			
	Glyphosate	Roundup Mega	Fozat 480	Glyfos	Glyphosate	Roundup Mega	Fozat 480	Glyfos
0	1.51 ± 0.05				1.39 ± 0.18			
1	2.81 ± 0.30	2.61 ± 0.39	2.27 ± 0.60	1.51 ± 0.41	2.90 ± 0.49	1.91 ± 0.60	1.60 ± 0.05	3.64 ± 2.33††
10	2.86 ± 0.28	2.97 ± 0.87	8.39 ± 5.37	2.08 ± 0.69	2.03 ± 0.38	1.77 ± 0.82	2.19 ± 1.10†††	0.99 ± 0.10†††
100	2.51 ± 0.52	2.49 ± 0.94	13.09 ± 9.67	1.87 ± 0.80	2.06 ± 0.57	2.23 ± 0.77	1.99 ± 0.76†††	1.24 ± 0.33
250	2.42 ± 0.18	(3.47 ± 0.55)	6.59 ± 1.52	(6.11 ± 2.58)	3.01 ± 0.45	(3.58 ± 0.07)	2.97 ± 0.47†††	(22.84 ± 14.24†††)
500	2.60 ± 0.84	(16.88 ± 3.54***)	(7.47 ± 0.69)	(17.69 ± 2.06*)	2.98 ± 0.24	(13.71 ± 3.09)	(4.59 ± 0.65†††)	(36.81 ± 15.40†††
750	3.64 ± 1.00	(24.67 ± 2.56***)	(28.58 ± 1.93*)	(37.81 ± 5.92***)	3.46 ± 0.67	(25.64 ± 6.53**)	(24.20 ± 1.20***†	(50.47 ± 10.64*†††)
1000	1.57 ± 0.24	(44.75 ± 0.46***)	(45.94 ± 3.74***)	(54.58 ± 2.74***)	1.51 ± 0.16	(31.45 ± 4.14***†††)	(22.28 ± 2.40***†††)	(62.38 ± 2.99**†††)

Data are means of median values of three repeated experiments.
Data in parentheses refer to samples with significant cytotoxic response.
Statistically significant (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) increase in DNA damage was determined by comparing the values of DNA damage induced by various doses of glyphosate and GBHs to the background level of untreated cells by ANOVA with Dunnett's post hoc test.
Statistically significant ($^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$) difference in DNA damage levels between S9-treated and S9-untreated cells induced by the same concentration of glyphosate and GBHs was determined by Mann-Whitney test.

Table 5.4.3-31: DNA damage induced by 4-hour exposure to glyphosate and GBHs with and without metabolic activation system (S9) in HMWB cells measured as tail length (µm) (from: Nagy *et al*., 2019)

Concentration (µM)	Tail length (µm) ± SEM							
	- S9				+ S9			
	Glyphosate	Roundup Mega	Fozat 480	Glyfos	Glyphosate	Roundup Mega	Fozat 480	Glyfos
0	0.47 ± 0.19				0.35 ± 0.06			
1	0.66 ± 0.06	0.52 ± 0.08	0.39 ± 0.08	0.11 ± 0.08	0.60 ± 0.08	0.34 ± 0.14	0.18 ± 0.04	0.56 ± 0.45††
10	0.67 ± 0.07	0.77 ± 0.27	1.77 ± 1.09	0.29 ± 0.15	0.65 ± 0.12	0.40 ± 0.17	0.57 ± 0.26†††	0.05 ± 0.04†††
100	0.64 ± 0.13	0.56 ± 0.27	2.70 ± 1.92	0.27 ± 0.15	0.68 ± 0.15	0.42 ± 0.18	0.41 ± 0.17†††	0.17 ± 0.10
250	0.99 ± 0.22	(1.09 ± 0.14)	1.51 ± 0.12	(1.27 ± 0.38)	0.90 ± 0.25	(1.03 ± 0.25)	1.00 ± 0.32†††	(4.41 ± 2.42†††)
500	0.93 ± 0.35	(3.93 ± 1.41*)	(1.62 ± 0.12)	(5.26 ± 1.37**)	0.81 ± 0.20	(2.87 ± 0.37*)	(1.19 ± 0.23†††)	(7.31 ± 2.27*†††)
750	1.05 ± 0.22	(5.40 ± 0.76**)	(6.57 ± 0.11**)	(8.91 ± 0.97***)	0.99 ± 0.21	(4.51 ± 1.07***)	(4.61 ± 0.19***†††)	(9.55 ± 0.50**†)
1000	0.31 ± 0.08	(6.77 ± 0.41***)	(8.18 ± 0.61**)	(10.09 ± 0.39***)	0.28 ± 0.07	(5.62 ± 0.32***†††)	(3.90 ± 0.31***†††)	(10.41 ± 0.22***†)

Data are means of median values of three repeated experiments.
Data in parentheses refer to samples with significant cytotoxic response.
Statistically significant (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) increase in DNA damage was determined by comparing the values of DNA damage induced by various doses of glyphosate and GBHs to the background level of untreated cells by ANOVA with Dunnett's post hoc test.
Statistically significant ($^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$) difference in DNA damage levels between S9-treated and S9-untreated cells induced by the same concentration of glyphosate and GBHs was determined by Mann-Whitney test.

Discussion

The use of glyphosate containing products both for agricultural and residential purposes continues to rise since their first introduction to the market in 1974. Today, uncountable GBHs are registered in more than 130 countries worldwide. The unrestricted application of GBHs in the past few decades has resulted in the accumulation of glyphosate residues in environmental media exposing – and also impairing the health of – aquatic and terrestrial animals, as well as humans. In this study, we measured potential differences in the cyto- and genotoxicity between the declared active ingredient glyphosate and three marketed GBHs in HMWB cells in vitro with the use of the comet assay. To examine the toxicity of potential metabolites of the selected herbicides, human liver-derived metabolic enzyme system (S9) was also applied. The cytogenetic side effects of glyphosate and GBHs in humans have received pronounced scientific coverage in the last decade, resulting in numerous in vivo and in vitro human studies, which demonstrated that glyphosate alone or GBHs have detectable genotoxic potential, while others have reached opposite conclusions. Our results indicate that glyphosate alone could not induce DNA strand breaks in HMWB cells up to 1000 μM measured with the comet assay even in the presence of metabolic activation. This finding contrasts with previous studies employing isolated human blood mononuclear cells treated with glyphosate concentrations corresponding to ours, but using different experimental settings. A study showed with comet assay that glyphosate at 0.7 μM (in vitro) caused a statistically significant increase of DNA damage in human peripheral blood lymphocytes; however, they applied longer (20 h) exposure time than used in this study, and, unlike in our experiment, cells were incubated on microscope slides embedded in agarose gel at 25 °C. A further study also observed DNA damage in the above cell type from 20.7 μM (3.5 μg/ml) and from 250 μM, respectively. Although they incubated the cell cultures under the same conditions as used in our study (for 4 h at 37 °C), they applied LMA at lower concentration (0.5 %), as well as lower electrophoretic voltage (0.7 V/cm) and time (15 min). The execution of comet assay by them applying 24 h exposure time and lower electrophoretic voltage was also different from the method we used. These discrepancies in the experimental design along with the possible inter individual variability in response to genotoxic insults may explain the contradictory findings. The cytotoxic potential of the active ingredient glyphosate in the 0–1000 μM concentration range was found to be minimal, whereas formulations showed to have pronounced cell-killing activity to HMWB cells in our study. All the three GBHs resulted in substantially decreased cell viability (< 23 %) from 500 μM (Roundup Mega and Glyfos) and from 750 μM (Fozat 480) concentrations. Exposure to GBHs also caused statistically significant ($p < 0.05$) increase of DNA damage from 500 μM (Roundup Mega and Glyfos) and 750 μM (Fozat 480). However, this observation cannot be explained by the direct genotoxic potential of GBHs, rather due to the high level of cytotoxic activity of the formulations, because cell death mechanisms, both apoptosis and necrosis, can produce spontaneous DNA fragmentation that can act as a confounder in the assessment of primary DNA damage in the comet assay. The high cytotoxic potential of GBHs may be attributed to the presence of other ingredients in the formulations, which has been reported in previous publications. POEA, the declared co-formulant in Glyfos, is more than 1000 times more cytotoxic than glyphosate alone, and concerns were also raised for its genotoxic potential at concentrations not causing cytotoxicity. Thus, EFSA concluded that POEA is clearly more toxic than glyphosate when tested in GBHs, and in response to this, Glyfos was withdraw from the Hungarian market in 2017. The ethoxylated etheralkylamine added to Roundup Mega has very similar toxicological properties as POEA, which is supported by the identical dose-response relationships of Roundup Mega and Glyfos, both of them containing surfactants at roughly the same concentration. The adjuvant content of Fozat 480 (< 5 % hygroscopic substances) could not be determined exactly from the MSDS; however, we can suspect from the path of the cell viability dose-response that it may also contain ethoxylated surfactants similarly to the other two GBHs. It is proven that ethoxylated adjuvants can be embedded into the cell membrane disrupting its integrity and permeability and therefore increasing the bioavailability of glyphosate. In this sense, surfactants in GBHs have an indirect synergistic effect with glyphosate. Researchers concluded that DNA damage observed in HMWB cells after exposure to glyphosate, Roundup 360 PLUS or the metabolite of glyphosate (aminomethylphosphonic acid, AMPA) was not due to direct interaction of these compounds with DNA as no DNA adducts formation has been observed, but rather ROS-mediated effects induced by the chemicals leading to cell death and indirect DNA damage. Previous studies have not been able to clearly demonstrate the oxidative DNA damaging potential of glyphosate; however, GBHs produced an increase in reactive oxygen species in both in vitro and in vivo test systems highlighting the role of adjuvants in the cytotoxic effects observed in our experiments. Despite

the definite cytotoxic effect, Roundup Mega and Fozat 480 are still commercially available not only in Hungary, but also in many other European countries under various brand names. In our study, the presence of metabolic activation did not alter the cytotoxic potential of either the active ingredient glyphosate or the GBHs. By contrast, we noted decreased DNA damages in the comet assay with Roundup Mega and Fozat 480, but increased damage with Glyfos as a result of S9 treatment. In humans, the only metabolite of glyphosate is the AMPA. It has been shown that AMPA is not able to induce DNA damage below a relatively high (4.5 mM) concentration, which is in agreement with our data. The diverse response of GBHs to metabolic activation observed in the comet assay may be explained by the presence of variable adjuvants in the formulations that underwent different metabolic modifications. Our results may indicate that the metabolite(s) of POEA in Glyfos can be more toxic than the parent compound in the comet assay, but not in the cell viability assay. There is no evidence in the published literature that POEA or other surfactants, or even products which contain these adjuvants, might undergo metabolic activation, and afterwards, become able to cause cytogenetic effects. The potential metabolic transformation of adjuvants can be hypothesized only from animal experiments; however, these studies have provided contradictory findings so far. This can still be considered as a critical knowledge gap to clarify the genotoxic potential of adjuvants in common commercial formulations of glyphosate under in vivo circumstances. A complex long-term experimental animal study has recently been initiated to assess possible risks resulting from the ubiquitous exposure to GBHs, and has already provided preliminary data on Roundup-induced endocrine effects and altered reproductive developmental parameters in male and female Sprague Dawley rats at a dose level considered as “safe”.

Conclusion

This is the first study that compared toxic effects of various glyphosate-based herbicide formulations to each other and with the declared active ingredient glyphosate in isolated human mononuclear white blood cells. GBHs caused much stronger cytotoxic effect on HMWB cells in comparison to glyphosate that may be attributed to the effect of various surfactants added to the formulations or their interaction with the active ingredient glyphosate and/or with other components of GBHs. Therefore, the GBHs-induced DNA damage observed in the comet assay could be most likely explained by non-genotoxic mechanisms and cannot indicate direct DNA damaging effects of glyphosate-based herbicide formulations. Nevertheless, by applying extended exposure durations and/or other test systems, such as the cytokinesis-block micronucleus cytome assay would allow for the discrimination between the cyto- and genotoxic effects and for the determination of the possible permanent genotoxic effect of these herbicides. Furthermore, this study, for the first time, pointed out the possibility that POEA containing formulation can undergo metabolic activation which draws attention to the need for comprehensive investigation of the toxicity of formulations to confirm our results and to assess the true health risks of environmental and occupational exposures.

3. Assessment and conclusion

Assessment and conclusion by applicant:

This paper describes a well conducted comet assay using human lymphocytes as the test system to examine the genotoxicity and cytotoxicity of glyphosate and 3 commercial products containing glyphosate. No DNA damage was induced by analytical grade glyphosate. The 3 glyphosate products induced an increase in tail intensity in the comet assay only at highly cytotoxic concentrations, non-toxic concentrations induced no DNA damage.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate tested was not sufficiently characterized and no positive control was used.

Assessment and conclusion by RMS:

Reliability criteria for *in vitro* toxicology studies

Publication: Nagy *et al*., 2019	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	N	Purity, content and storage conditions are not reported. Source: VWR International Kft, Debrecen, Hungary.
Only glyphosate acid or one of its salts is the tested substance	N	Also 3 GBH tested: Roundup Mega, Fozat 480, Glyfos.
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	Y	Human mononuclear white blood cells.
Test conditions clearly and completely described	Y	Comet selection criteria were not stated. It is not stated if the slides were coded prior to scoring
Metabolic activation system clearly and completely described	Y	
Test concentrations in physiologically acceptable range (< 1 mM)	Y	1, 10, 100, 250, 500, 750 and 1000 µM.
Cytotoxicity tests reported	Y	
Positive and negative controls	N	No positive control.
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	No HCD so it is unknown what degree of background variation is apparent in this test system. This is exacerbated by the use of a single set of control cultures
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate tested was not sufficiently characterized and no positive control was used.		

1. Information on the study

Data point:	CA 5.4/004
Report author	De Almeida, L.K.S. *et al.*

Report year	2018
Report title	Moderate levels of glyphosate and its formulations vary in their cytotoxicity and genotoxicity in a whole blood model and in human cell lines with different estrogen receptor status
Document No	DOI: 10.1007/s13205-018-1464-z ISSN: 2190-572X
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	Not applicable
GLP/Officially recognised testing facilities	No, not conducted under GLP/ Officially recognised testing facilities.
Acceptability/Reliability:	Yes/Reliable with restrictions

5. Full summary of the study according to OECD format

In vitro studies were conducted to determine the short-term cytotoxic and genotoxic effects of pure glyphosate and two glyphosate formulations (Roundup® and Wipeout®) at concentrations relevant to human exposure using whole blood (cytotoxicity) and various cancer cell lines (cytotoxicity and genotoxicity). Pure glyphosate (pure glyph) and Roundup® (Ro) showed similar non-monotonic toxicological profiles at low dose exposure (from 10 µg/ml), whereas Wipeout® (Wo) demonstrated a monotonic reduction in cell viability from a threshold concentration of 50 µg/ml, when tested in whole blood. We evaluated whether using various cancer cells (the estrogen-E2-responsive HEC1A, MCF7 and the estrogen-insensitive MDA-MB-231) exposed to moderate doses (75–500 µg/ml) would indicate varied toxicity and results indicated significant effects in the HEC1A cancer cells. A non-monotonic reduction in cell viability was observed in HEC1A exposed to pure glyph (75–500 µg/ml) and proliferative effects were observed after exposure to Wo (75, 125 and 250 µg/ml). Genotoxicity assessment (test concentration 500 µg/ml) demonstrated DNA damage in the HEC1A and MDA-MB-231 cells.

Materials and methods

Chemicals - Glyphosate (99.5 % purity) was purchased from Supelco Analytical (USA).

Whole blood cell culture and exposure - Blood from 5 healthy volunteers was collected by venipuncture in heparin-containing tubes. Blood samples were diluted 1:10 in RPMI 1640 media supplemented with 50 µg/ml streptomycin and 50 U/ml penicillin. The 1 mL whole blood samples were exposed to glyphosate at various concentrations up to 500 µg/mL for 18 hours at 37 °C. Lipopolysaccharide (LPS, 5 µg/mL) was used as the positive control and pyrogen-free water as the negative control.

Breast cancer (MCF7 and MDA-MB-231) and endometrial cancer (HEC1A) cell line culture - MCF7 (hormone responsive) and MDA-MB-231 (hormone independent) cell lines were obtained from the American Type Culture Collection (ATCC), USA and HEC1A cells were obtained from Nelson Mandela Metropolitan University, Eastern Cape, South Africa. All cell lines were grown in DMEM supplemented with 5 % heat-inactivated fetal calf serum, 50 U/mL penicillin and 50 µg/mL streptomycin. To maintain a stable estrogen-sensitive phenotype, cells were cultured in phenol-free medium after the removal of phenol red. All data were normalized against untreated controls. Cell lines were routinely maintained under standard cell culture conditions at 37 °C, 5 % CO2 and 90 % humidity. The different cell lines were exposed to glyphosate at varying concentrations up to 500 µg/mL for 24 hours at 37 °C. Camptothecin (100 µM), a DNA topoisomerase inhibitor, was used as the positive control in this study.

Cytotoxicity assay (MTT assay) - Following exposure to glyphosate, samples were incubated in 0.5 mg/mL MTT reagent for 3 hours for the cancer cell lines and for 30 minutes for whole blood at 37 °C. After incubation, the MTT reagent was aspirated and 1 mL of DMSO was added to solubilize the formazan

product formed. Purple color formation was determined spectrophotometrically at 560 nm using a Biotek Powerwave XS microplate reader.

Single-cell gel electrophoresis (comet assay) - The exposure concentrations used for this study were chosen based on results obtained in the cell viability assay and the positive control reference concentration was chosen based on cytotoxicity results reported in the HepG2 human liver cell line. The MCF7, MDA-MB-231 and HEC1A cancer cell lines (100,000 cells/well) were incubated in 24-well plates for 4 hours at 37 °C, in the presence of 500 and 1000 µg/mL glyphosate. Camptothecin was used as a positive control in this study (100 µM). The preparation of the samples and the method used for the comet assay were conducted according to the instructions described in the OxiSelect™ Comet Assay Kit (Cell Biolabs, Inc).
Preparation of cell samples for the comet assay - Trypsinized cells were pooled (three sample wells) and centrifuged at 700×g for 2 minutes. The supernatant was discarded and the pellet washed with ice cold PBS and centrifuged at 700×g. The cells (1×10^5 cells/mL) were then resuspended in ice cold PBS before the assay was conducted.

Comet assay sample slide preparation and cell lysis - Resuspended cells (10 µL) were combined with 100 µL molten comet agarose and the mixture (75 µL/well) was immediately placed onto an OxiSelect™ comet slide. Slides with the cell agarose mixture were incubated at 4 °C in the dark for 15 minutes to allow the agarose to set. Slides were treated in pre-chilled lysis buffer (pH 10) for 60 minutes at 4 °C in the dark followed by treatment in alkaline solution (pH 13) for 30 minutes at 4 °C in the dark.

Alkaline electrophoresis - Slides were subjected to alkaline electrophoresis for 18 minutes at 300 mA, neutralized in pre-chilled deionised water and washed in 70 % cold ethanol for 5 minutes. The slides were then air-dried and incubated with 100 µL of 1 × Vista Green dye prepared in TE buffer (10 mM Tris, pH 7.5, 1 mM EDTA) for 15 minutes at room temperature in the dark.

Comet assay sample visualization and data analysis - Slides were visualized with fluorescence microscopy (5×) using a FITC filter (7 %). Images were obtained using an AxioCam MR3 Camera and stored in the Axio vision Rel.4.8 program. Image J Macro was used to calculate the tail length, % tail moment and % tail DNA. 50 cells were analyzed per slide (replicates of 3) from pooled cell cultures, per experimental treatment (n = 3).

Statistical analysis - All data were presented as mean ± standard error of the mean (SEM). ANOVA single-factor analysis (Microsoft Excel) was used to determine significant differences ($P \leq 0.05$, $P \leq 0.01$). Non-linear least square regression models were applied to cytotoxicity data in human whole blood using the Graphpad Prism 6 software package. Goodness of fit was assessed using R2, R2 adjusted values and by the assessment of upper and lower 95 % confidence limits associated with the model fit. The Wald–Wolfowitz (runs) test was used to determine the deviation of the nonlinear regression model from the experimental data. The models were used to estimate the concentration of glyphosate required to illicit a half maximal response (half maximal effective concentration, EC50). Based on the observed biphasic nature of the curves obtained for glyphosate and Roundup, a model describing a seven-parameter bell-shaped dose (combines two sigmoidal responses)-response curve was selected.

Results

Cell viability in human whole blood – Glyphosate cytotoxicity in human whole blood at concentrations from 0.1 to 500 µg/mL was determined using the MTT reduction assay. A statistically significant reduction in cell viability of whole blood was observed for glyphosate at 10, 50 and 250 µg/mL but not at 500 µg/mL. LPS was a suitable positive control for this study.

Cell viability (MTT assay) in human cancer cell lines - A statistically significant reduction in cell viability was noted for glyphosate in the HEC1A cell line at 75, 125, 250 and 500 µg/mL. No change in viability was seen with the MCF7 and MDA-MB-231 cell lines. The positive control, camptothecin, reduced significantly cell viability in all three cell lines.

Single cell gel electrophoresis (comet assay) - The test concentrations selected for genotoxicity studies were based on the results of the cell viability study and reference concentrations were chosen based on glyphosate concentrations reported in the literature to incur genotoxic damage in human cell lines. Glyphosate was tested in the comet assay at 500 and 1000 µg/mL where a statistically significant increase in tail length and tail moment was observed at both concentrations in the HEC1A and MDA-MB-231 cell lines. The positive control, camptothecin, increased tail length significantly in the HEC1A and MDA-MB-231 cell lines but not in the MCF-7 cell line. The positive control increased tail moment significantly in all 3 cell lines.

Discussion and conclusions

When tested at concentrations ranging from 0.1 to 500 µg/mL, a statistically significant reduction in cell viability was observed in whole blood at glyphosate concentrations of 10, 50 and 250 µg/mL but not at 500 µg/mL. When tested for cytotoxicity glyphosate showed a statistically significant reduction in cell viability in the endometrial cancer cell line HEC1A at 75, 125, 250 and 500 µg/mL. No effect on cell viability was seen on hormone responsive (MCF7) and hormone independent (MDA-MB-231) breast cancer cell lines at concentrations up to 500 µg/mL. When glyphosate was tested for DNA damage in the single cell gel electrophoresis assay (comet assay) a statistically significant increase in tail length and tail moment was observed at 500 and 1000 µg/mL in the endometrial cancer cell line HEC1A and the hormone independent breast cancer cell line MDA-MB-231. No DNA damage was observed in the hormone responsive breast cancer cell line MCF7 up to concentrations of 1000 µg/mL. Cytotoxicity results at concentrations relevant to occupational and residential exposure to glyphosate observed in the three cancer cell lines suggest that toxicity varies depending on cell type, with the most significant results observed in the HEC1A cancer cell line exposed to glyphosate. Moderate concentrations of glyphosate (500 µg/mL) induced genotoxic effects in the HEC1A and MDA-MB-231 cancer cell lines, which suggests that glyphosate may display various mechanisms of toxicity.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The cytotoxicity of glyphosate has been investigated in whole blood, in hormone independent (MDA-MB-231) and in hormone responsive (MCF7) cell lines and in a an endometrial cancer cell line (HEC1A). The capacity of glyphosate to produce DNA damage was investigated in MCF7, MDA-MB-231 and HEC1A cells in the Comet assay. Glyphosate was found to reduce cell viability in whole blood at the intermediate concentrations (10-250 µg/mL) but not at the highest concentration tested (500 µg/L). A significant concentration related reduction in cell viability was seen with glyphosate in HEC1A cells (> 75 µg/mL) but not in the two other cell lines. When glyphosate was tested at 500 and 1000 µg/mL an increase in tail length and tail moment was observed in HEC1A and MDA-MB-231cells but not in the hormone responsive breast cancer cell line MCF7. The *in vitro* concentrations of glyphosate at which DNA damage was observed were 500 and 1,000 µg/mL which are systemic concentrations that cannot be reached in *in vivo* toxicology studies.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the Comet assay was only conducted at concentrations that are physiologically not feasible in *in vivo* toxicology studies (> 1mM).

Assessment and conclusion by RMS:

Reliability criteria for *in vitro* toxicology studies

Publication: De Almeida et al., 2018.	Criteria met? Y/N/?	Comments
Guideline-specific		

Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	?	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity of 99.5 %. Source: Supelco Analytical USA.
Only glyphosate acid or one of its salts is the tested substance	N	Also glyphosate-based formulations were tested.
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	Y	Whole blood from volunteers, breast cancer cells (MCF7 and MDA-MB-231) and endometrial cancer cells (HEC1A).
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	N	
Test concentrations in physiologically acceptable range (< 1 mM)	N	For cytotoxicity testing glyphosate concentrations from 0.1 to 500 µg/mL were used. For comet testing only glyphosate concentrations of 500 and 1000 µg/mL were used (> 1 mM).
Cytotoxicity tests reported	Y	
Positive and negative controls	Y	
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	Y	Was studied but not established.
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the Comet assay was only conducted at concentrations that are physiologically not feasible in *in vivo* toxicology studies (> 1mM).		

I. Information on the study

Data point:	CA 5.4/005
Report author	Ilyushina, N.A. *et al.*

Report year	2018
Report title	Comparative investigation of genotoxic activities of glyphosate technical products in the micronucleus test in vivo
Document No	ISSN: 0869-7922
Guidelines followed in study	OECD Test Guideline 474
Deviations from current test guideline OECD 474 (2016)	Positive control animals were included but the data are not reported. No ratio of PCE to NCE was reported. No evidence of bone marrow exposure. Data have been presented per group rather than per animal
Previous evaluation	None
GLP/Officially recognised testing facilities	No, not conducted under GLP/ Officially recognised testing facilities.
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary of the study according to OECD format

In this study, the induction of micronucleus formation in vivo in polychromatophilic erythrocytes of bone marrow of CD-1 mice was assessed by the action of three different technical glyphosate products on the market in the Russian Federation. It was found that the tested samples of technical products showed different cytogenetic activity, while only one of them caused a statistically significant, dose-dependent increase in the frequency of induction of micronuclei compared to the negative control.

The analysis of the composition of the studied product samples showed that the cytogenetic activity may depend on the content of potentially mutagenic impurities, formaldehyde in particular.

The acquired data provides some additional grounds for lowering the upper limit of formaldehyde content in technical glyphosate products and also indicates the need to assess the genotoxic activity of analogue pesticides entering the market of plant protection products.

Materials and methods

Three glyphosate technical products were used that were manufactured at different factories and that contained the active substance (glyphosate acid) in amounts of 96.6 %, 95.8 % and 95.7 % in technical products I, II and III, respectively.

Chromosomal disturbances were revealed through the use of in vivo micronucleus analysis [OECD Test No. 474: Mammalian Erythrocyte Micronucleus Test. 2016] of the bone marrow erythrocytes in CD-I laboratory mice obtained from the husbandry center at the Andreyevka Branch of the Federal State Budget Research Institution "Biomedical Technology Science Center" of the Russian Federal Medicobiological Agency.

Five mice per group were used. Technical glyphosate was administered intragastrically in 1 % starch in doses of 500, 1,000 and 2,000 mg/kg of body weight (the maximum dose according to OECD Test No. 474) twice, twenty-four hours apart, and the animals were euthanized 22 hours following the second administration. At the same time, an excipient (1 % starch) was given as a negative control following the same pattern as was used for the technical glyphosate. Cyclophosphamide was used as a positive control, given intragastrically in the amount of 40 mg/kg once, at the same time as the second dose of glyphosate. Bone marrow preparations from each specimen were studied microscopically (on a Japan-made Nikon Eclipse Ci-L), counting 4,000 polychromatophilic erythrocytes (PCEs) for measuring micronucleus frequency), and the portion among the erythrocytes as a whole made up by PCEs, counting no fewer than 500 erythrocytes for each animal.

A statistical analysis of the study results was carried out using SPSS Statistics v. 22.0 (IBM, New York, USA). Frequencies of micronuclei in the PCEs were compared by building a generalized log-linear model

of the Poisson Regression ($\alpha = 0.05$). The data were also checked for dependencies between micronucleus frequency and the dose of the technical product being tested using the Mantel-Haenszel method.

Results

All the research was carried out in accordance with OECD Manual No. 474 and Methodological Instructions MU-1.2.3365-16. Each experiment used positive and negative controls simultaneously. The frequency of PCEs containing micronuclei in the case of the positive control was in a range from 1.58 to 2.95 %, while in the case of the negative control it was from 0.06 to 0.12 %. All the values were within the range of the distribution boundaries of historical laboratory controls.

The quantification of the portion of total erythrocytes made up by polychromatophilic erythrocytes show that in none of the analyses was erythropoiesis suppressed, i.e. there were no toxic effects on mouse bone marrow.

Varying effects were revealed during the study of the mutagenic activity of the glyphosate technical products through micronucleus analysis. Technical products II and III did not significantly increase the PCE frequency in mouse bone marrow compared to the simultaneous negative controls (please refer to Table and Figure below).

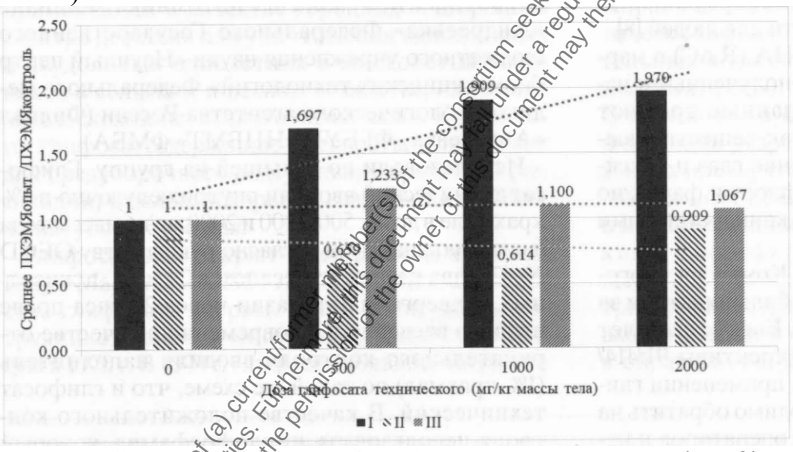


Figure 5.4.3-2: Technical Glyphosate Dose (mg/kg of body weight) Frequencies of increased occurrence of PCEs compared to the negative control depending on the dose of glyphosate technical products. I, II, III – Reference numbers for different technical products. $PCEMN_{exp}$ denotes the frequency of PCEs with micronuclei in groups of mice that received different glyphosate doses. $PCEMN_{control}$ denotes the frequency of PCEs with micronuclei in groups of mice that received an excipient with 1 % starch (negative control) (from: Ilyushina *et al*., 2018)

When administering technical Product I, a low, but statistically significant genotoxic effect was observed at all doses (Table 2). Moreover, a statistically significant linear dependency was observed of the frequency of PCEs with micronuclei upon the dose of the compounds under study (p= 0.002) (please refer to Table below).

A comparison was made between the content of ingredients in the study samples based on the available batch analysis certificates for the technical products. Two ingredients were of special interest from the point of view of genotoxicity: nitroso-glyphosate and formaldehyde. According to the provided certificates, Nitrosoglysophate, was not present in any of the batches of technical products (its content, in accordance with FAO specifications, must not exceed 1 mg/kg). Formaldehyde content in products I, II and III amounted to 0.13 %, 0.024 % and 0.06 %, respectively. A formaldehyde level in the glyphosate product of 0.13 % is the maximum permissible concentration in glyphosate technical products in accordance with the FAO specifications[14]. However, it should be noted that, according to Annex I of Directive 91/414/EEC, as amended in 2017, the partial content of formaldehyde in glyphosate must not exceed 1 mg/kg [10].
A comparison of the results we have obtained on the mutagenic activity and formaldehyde concentrations

in glyphosate technical samples showed that statistically significant genotoxic effects and a linear dependence of the frequency of PCEs with micronuclei upon the dose of the compound under study were only observed in the case of the product with 0.13 % formaldehyde. Lower concentrations of this ingredient did not induce the statistically significant formation of PCEs with micronuclei in bone marrow cells.

Thus, the observed mutagenic effects were most likely not related to the active ingredient, but rather to the formaldehyde contained as an ingredient in the products.

Glysophate dose (mg/kg of body weight)	Sample I		Sample II		Sample III	
	Number of PCEs with MN per 40,000 PCEs	95 % confidence intervals	Number of PCEs with MN per 40,000 PCEs	95 % confidence intervals	Number of PCEs with MN per 40,000 PCEs	95 % confidence intervals
0	33	1	44	1	30	1
500	56	1.111 1.697 2.634	29	0.408 0.659 1.048	37	0.763 1.233 2.009
1000	63	1.263 1.909 2.941	27	0.376 0.614 0.984	33	0.671 1.100 1.812
2000	65	1.306 1.970 3.028	40	0.591 0.909 1.395	32	0.647 1.067 1.762
Assessment of linear trend using the Mantel-Haenszel method	1 (146)		II (189)		III (341)	
	Value	Asymptomatic significance p (one-dimensional)	Value	Asymptomatic significance p (one-dimensional)	Value	Asymptomatic significance p (one-dimensional)
	9.791	0.002	0.28	0.597	0.06	0.938

The results obtained are in agreement with data from the literature. In the majority of studies conducted using the Ames test, glyphosate did not induce gene mutations in bacteria [Li A.P., Long T.J., 1988]. In both *in vivo* and *in vitro* micronucleus analyses carried out on different subjects, contradictory data were obtained. An increased frequency of the appearance of micronuclei was noted in several studies on cultivated human buccal epithelial cells [Koller V.J. et al, 2012], in mouse bone marrow [Manas F., 2009], and in human lymphocytes having undergone been affected by glyphosate-containing preparations during crop spraying [Bolognesi C. eta la, 2009]. However, the majority of the studies gave negative results in terms of micronucleus analyses [Kier L.D., 2013]. However, none of the studies analyzed the content of possible mutagenic ingredients in technical products and glyphosate-containing preparations and their contribution to the observed effects.

Conclusion

The comparative investigation of the genotoxic activity of various glyphosate technical products through the analysis of micronuclei in mouse bone marrow erythrocytes showed that cytogenetic activity may depend on the content in the product of potentially mutagenic ingredients, and in particular of formaldehyde. The contradictory results obtained in different studies of the mutagenic activity of glyphosate and preparations containing it may be connected to the different concentrations of ingredients that may exhibit effects of intracellular genetic structures.

The obtained data comprise additional substantiation for reducing the upper limit for formaldehyde content in glyphosate technical products, and also provide evidence of the need to assess the genotoxic activity of analogous pesticides entering the Russian Federation's market.

3. Assessment and conclusion

4.

Assessment and conclusion by applicant:

This paper describes the results of three different technical batches of glyphosate tested in a mouse micronucleus test. The study followings the recommendations of OECD 474, with some deficiencies, mostly regarding the reporting of data rather than test methodology. However, the reliability of the reported conclusions are unknown due to a lack of clarity and accuracy of the reported data. Individual animal data and group mean±SD frequency of micronucleated (MN) PCE have not been reported. Instead the results per group appear to be presented as the total number of MN PCE found per 40,000 PCE, together with the frequency of MN PCE values, expressed as relative to control, for the group with calculated 95 % upper and lower limits (no explanation for how these data awere derived is provided). The text describes the vehicle control MN PCE frequencies as ranging between 0.06 % and 0.12 % (within HCD) but similar detail is not provided for the treated groups. Consequently, there is no indication of animal variability within the groups and it is unknown if any of the treated animals fall outside of HCD. Furthermore, the total number of MN PCE is described as being per 40,000 PCE, however, only 4000 PCE were scored per animal and with 5 animals per group this would result in total of 20,000 PCE per group.

The authors postulate that the positive results observed for technical batch I are likely to be due to the presence of 0.13 % formaldehyde in the material, although they provide no data to support their hypothesis.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the source of the 3 glyphosate batches was not revealed although the concentrations of the most important impurities were given for each batch. Although reference was made to OECD test guideline 474 too little detail was given on the conduct of the MN assay to conclude to reliability without restrictions and the data are inadequately reported.

Assessment and conclusion by RMS:

Reliability criteria for *in vivo* toxicology studies

Publication: Ilyushina *et al.,* 2018	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	Y	OECD test guideline 474 according to the authors.
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Technical glyphosate from 3 sources with purity of 96.6 %, 95.8 %, and 95.7 %. Sources were not reported but the concentration of impurities (nitroso-

		glyphosate and formaldehyde) was given.
Only glyphosate acid or one of its salts is the tested substance	Y	
AMPA is the tested substance	N	
Study		
Test species clearly and completely described	Y	CD-1 mouse.
Test conditions clearly and completely described	Y	
Route and mode of administration described	Y	Oral by gavage.
Dose levels reported	Y	0, 500, 1,000, and 2,000 mg/kg bw twice, twenty-four hours apart.
Positive control	Y	Cyclophosphamide.
Number of animals used per dose level reported	Y	5/dose group.
Method of analysis described for analysis test media	N	
Validation of the analytical method	N	
Analytical verifications of test media	N	
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical control data of the laboratory reported	Y	Referred to but not presented.
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Reliability not assignable		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the source of the 3 glyphosate batches was not revealed although the concentrations of the most important impurities were given for each batch. Although reference was made to OECD test guideline 474 too little detail was given on the conduct of the MN assay to conclude to reliability without restrictions, and the data are inadequately reported.		

1. Information on the study

Data point:	CA 5.4/006
Report author	Santovito, A. *et al.*
Report year	2018
Report title	In vitro evaluation of genomic damage induced by glyphosate on human lymphocytes
Document No	doi.org/10.1007/s11356-018-3417-9 E-ISSN: 1614-7499
Guidelines followed in study	Some compliance with OECD 473 and OECD 487
Deviations from current test guideline	Only continuous treatments in the absence of S9 were performed and these exceeded the 1.5 cell cycles recommended by both guidelines. No historical control data are reported. Treatment commenced within 24 hours of PHA stimulation rather than 44-48 hours.
Previous evaluation	No

GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary of the study according to OECD format

In this study, the *in vitro* clastogenic and/or aneugenic effects of glyphosate was analysed by chromosomal aberrations and micronucleus assays. Human lymphocytes were exposed to five glyphosate concentrations: 0.500, 0.100, 0.050, 0.025, and 0.0125 µg/mL, where 0.500 µg/mL represents the established acceptable daily intake value, and the other concentrations were tested in order to establish the genotoxicity threshold for this compound.

Materials and Methods
Study population; Peripheral venous blood was collected from six healthy Italian subjects (two males and four females, mean age ± SD, 27.50 ± 12.55), non-smoking, not alcoholics, not under drug therapy, and with no recent history of exposure to mutagens. All subjects signed the informed consent. The study was approved by the University of Turin ethics committee and was performed in accordance with the ethical standards laid down in the 2013 Declaration of Helsinki.

Blood sample collection and lymphocyte cultures; Blood samples were obtained by venipuncture, collected in heparinized tubes, cooled (4 °C), and processed within 2 h after collection. Lymphocyte cultures, fixation, and staining procedures were performed as previously described. Total time of lymphocyte cultures was 52 and 72 h for CA and MNi assays, respectively. After 24 h of incubation, 8.6 µL of glyphosate stock solution (Sigma-Aldrich, Saint Louis, MO, USA, CAS n. 1071-83-6) at the final concentration of 0.5 mg/mL in dimethyl sulfoxide (DMSO) was added to the lymphocyte culture in order to reach a final glyphosate concentration of 0.500 µg/mL. Similarly, 8.6 µL of glyphosate stock solution diluted 5, 10, 20, and 40 times with DMSO were added to the lymphocyte cultures in order to reach the final glyphosate concentrations of 0.100 µg/mL, 0.050 µg/mL, 0.025 µg/mL, and 0.0125 µg/mL, respectively. In particular, 0.500 µg/mL represents the ADI concentration established by EFSA for this compound, whereas 0.100, 0.050, 0.025, and 0.0125 µg/mL concentrations were tested in order to evaluate the genotoxicity threshold. Three control cultures were assessed: (1) positive control, by adding only MMC (final concentration 0.1 µg/mL culture); (2) 0.1 % DMSO solvent control, obtained by adding 8.6 µL of DMSO to the lymphocyte culture; and (3) negative control culture without both glyphosate and DMSO, obtained adding 8.6 µL of RPMI medium to the lymphocyte culture. Only for MNi assay, after 44 h of incubation, cytochalasin-B was added to the cultures at a concentration of 6 µg/mL to block cytokinesis. Similarly, only for CA assay, to arrest cells in mitosis, colchicine was added at the concentration of 0.06 µg/mL during the last 2 h of culture. After 52 h (for CAs assay) and 72 h (for MNi assay) of incubation at 37°C, the cells were collected by centrifugation, treated for 10 min with a pre-warmed hypotonic solution (75 mM KCl). After centrifugation and removal of the supernatant, the cells were fixed with a solution of methanol/acetic acid (3:1 v/v). The treatment with the fixative was repeated three times. Finally, the supernatant was discarded, and the pellet, dissolved in a minimal volume of fixative, was seeded on the slides to detect CAs and MNi by conventional staining with 5 % Giemsa (pH 6.8) prepared in Sörensen buffer.

Cytokinesis-block micronucleus assays; Microscope analysis was performed at ×400 magnification on a light microscope (Dialux 20, Leica, Germany). MNi, nucleoplasmic bridges (NPB), and nuclear buds (NBUD) were scored in 2000 binucleated lymphocytes with well-preserved cytoplasm per subject (total 12,000 binucleated cells per concentration). Cells containing one of more MNi were scored as "micronucleated cell" (MNC). A total of 2000 lymphocytes per donor per concentration were scored to evaluate the cytokinesis-block proliferation index (CBPI), according to the following formula: [1 × N1] + [2 × N2] + [3 × (N3 + N4)] / N, where N1–N4 represents the number of cells with 1–4 nuclei, respectively, and N is the total number of cells scored.

Chromosomal aberration assay; Microscope analysis was performed at ×1000 magnification on a light

microscope (Dialux 20, Leica, Germany). For each subject and glyphosate concentration, 200 complete complete metaphases (for a total of 1200 metaphases for each dose) were analyzed. Cells containing one of more types of CAs were scored as "aberrant cell" (Ab.C). In order to determine cytotoxicity, the mitotic index (MI) was calculated from the number of metaphases in 1000 cells analyzed per subject per concentration (a total of 6000 cells per concentration).

Statistical analysis; Comparison of mean values of the percentage of cells with MNi, MNC, CBPI, NPBs, NBUDs, CAs, Ab.C, and MI between exposure levels and controls was assessed by the nonparametric Mann-Whitney test. Statistical calculations were carried out using the SPSS software package program (version 24.0, Inc., Chicago, IL, USA). All P values were two-tailed, and P values of 5% or less were considered statistically significant for all tests carried out.

Results

Effect of glyphosate on CA formation; **Error! Reference source not found.** shows values of CAs found in the human peripheral lymphocytes cultured in the presence of different glyphosate concentrations. Glyphosate was found to induce the following structural CAs: gaps, chromatid and chromosome breaks, dicentric chromosomes, rings, tri-tetraradials, and acentric fragments. This last, together to chromatid breaks, represent the most frequent observed aberrations (Table below). Gaps were excluded from statistical analysis. Glyphosate was found to significantly increase the CA and Ab.C frequencies at all tested concentrations when compared with the solvent control, including the concentration of 0.025 µg/mL, but with the exception of 0.0125 µg/mL. A dose effect was also observed, since the regression analysis revealed a significant correlation between glyphosate concentrations and the CA and Ab.C frequencies (refer to **Error! Reference source not found.**). Vice versa, no significant differences were found between the DMSO solvent-control and the negative control, whereas the cultures treated with the MMC showed a significant increase of the cytogenetic damage with respect to all concentrations of glyphosate. Finally, no significant differences were found in the MI values between solvent control and all tested concentrations of glyphosate, although at 0.500 µg/mL, the P value resulted to be borderline.

Table 5.4.3-32: Induction of chromosomal aberrations by Glyphosate in human lymphocytes *in vitro* Number if scored metaphases for each concentration = 1200 (from: Santovino *et al*., 2018)

Test substance (µg/mL)	Structural CAs							Total CAs	Total CAs + Gaps	Total Ab.C	Total Ab.C + Gaps	(%) CAs/Cell ± S.E.	(%) Ab.C/Cell ± S.E.	(%) MI ± S.E.
	Gaps	B'	B"	DC	R	TR	AF							
NC	8	8	2	0	0	0	7	17	25	17	25	1.417±0.154	1.417±0.154	5.567±0.042
0.1% DMSO	10	9	4	2	0	6	7	27	37	27	37	2.250±0.335	2.250±0.250	5.433±0.056
MMC (0.100)	41	46	30	4	10	14	36	145	186	127	168	12.083±0.300[a]	10.583±0.473	4.200±0.058[a]
Gly (0.500)	17	41	12	12	3	0	28	96	113	95	112	8.000±0.428[a]	7.917±0.375	5.300±0.026
Gly (0.100)	23	31	10	7	2	2	23	75	98	75	98	6.250±0.359[a]	6.250±0.359	5.333±0.080
Gly (0.050)	9	31	6	7	0	0	16	50	59	50	59	4.167±0.167[a]	4.167±0.167	5.367±0.095
Gly (0.025)	10	15	4	4	3	0	20	46	56	46	56	3.833±0.211[b]	3.833±0.211[b]	5.383±0.040
Gly (0.0125)	8	14	5	1	0	0	14	34	42	34	42	2.833±0.211	2.833±0.211	5.400±0.037

CAs, chromosomal aberrations; *Ab.C*, aberrant cells (cells with 1 ore more aberrations); *MI*, Mitotic Index; *NC*, Negative Control; *MMC*, Mitomycin-C; *B'*, chromatid break; *B"*, chromosome break; *DC*, dicentric; *R*, ring; *TR*, tri-tetraradials; *AF*, acentric fragments; *S.E.*, standard error; *Gly*, Gliphosate

[a] $P = 0.004$; [b] $P < 0.006$ (significantly differs from the DMSO solvent control, Mann-Whitney test)

Table 5.4.3-33: Multiple regression analysis between Glyphosate concentrations (from: Santovino *et al.*, 2018).

Biomarker	β-co	95% CI (Lower) – (Upper)	*P*-value
CAs	0.914	(2.112) – (2.988)	<0.001
Cells with CAs	0.919	(2.099) – (2.935)	<0.001
MI	-0.275	(-0.666) – (0.099)	0.141
MNi	0.908	(4.025) – (5.075)	<0.001
Cells with MNi	0.935	(3.639) – (4.527)	<0.001
CBPI	0.269	(-28.171) – (4.571)	0.151
NPBs	0.674	(0.268) – (0.665)	<0.001
NBUDs	0.395	(0.023) – (0.444)	0.031

CAs, Chromosomal Aberrations; *MI*, Mitotic Index; *MNi*, Micronuclei; *CBPI*, Cytokinesis-block Proliferation Index; *NPBs*, nucleoplasmic bridges; *NBUDs*, nuclear buds

Effect of glyphosate on MNi formation; **Error! Reference source not found.**shows the frequencies of MNi found in the human peripheral lymphocytes cultured in the presence of different glyphosate concentrations. Glyphosate significantly increased the MNi frequency at all tested concentrations when compared to the solvent control, with exception of 0.0125 µg/mL (Table 3). Vice versa, no effect has been observed on the frequencies of NBUD and NPB, with the only exception of 0.500 µg/mL of glyphosate that was found to increase in a significant manner the frequency of NPBs with respect to the solvent control. Also in this case, a relationship between the frequency of MNi and the concentrations of glyphosate was observed (Table 2), as well as the DMSO solvent-control cultures did not show significant differences with respect to the negative controls. MMC was found to significantly increase the MNi, NPB, and NBUD formation compared with the negative control solvent controls and all tested concentrations of glyphosate, with exception of 0.500 µg/mL. After 48-h exposure, a significant reduction of the CBPI value in cultures treated with glyphosate was not observed, indicating that, at the tested concentrations, glyphosate does not seem to produce effects on the proliferation index. Finally, at 0.500 µg/mL, glyphosate significantly induced the NPB formation, whereas no differences were found in the frequency of NBUD between DMSO solvent control and all glyphosate concentrations.

Table 5.4.3-34: Induction of micronuclei by Glyphosate in human lymphocytes in vitro. Number of scored binucleated cells for each concentration of the test substance = 12,000 (from: Santovino *et al*., 2018)

Test substance (µg/mL)	Distribution of BNCs according to the number of MNi				MNi	MNC	MNi/BNCs ± S.E. (%)	MNC/BNCs ± S.E. (%)	CBPI ± S.E	Frequency of BNCs with NPBs (‰)	Frequency of BNCs with NBUDs (‰)
	1	2	3	4							
NC	27	0	0	0	27	27	0.225±0.021	0.225±0.021	1.713±0.003	0.417±0.083	0.833±0.105
0.1% DMSO	33	1	0	0	35	34	0.292±0.024	0.283±0.025	1.589±0.076	0.500±0.129	1.083±0.154
MMC (0.100)	129	9	3	2	164	143	1.367±0.067	1.192±0.015[a]	1.366±0.019	2.333±0.357	3.083±0.473 [a]
Gly (0.500)	132	6	2	1	154	141	1.283±0.017	1.175±0.021[a]	1.545±0.054	1.667±0.211	1.666±0.247
Gly (0.100)	107	7	0	0	121	114	1.008±0.030	0.950±0.029[a]	1.556±0.017	0.883±0.105	1.333±0.167
Gly (0.050)	93	6	1	0	108	100	0.900±0.053	0.833±0.046[a]	1.576±0.015	0.750±0.111	1.250±0.1112
Gly (0.025)	68	5	0	0	78	73	0.650±0.048	0.608±0.035[a]	1.585±0.010	0.667±0.105	1.167±0.105
Gly (0.0125)	39	0	0	0	39	39	0.325±0.021	0.325±0.021	1.589±0.008	0.583±0.083	1.167±0.167

BNCs, Binucleated cells; *MNi*, micronuclei; *MNC*, cells with 1 or more micronuclei; *CBPI*, Cytokinesis-Block Proliferation Index; *NPBs*, nucleoplasmic bridges; *NBUDs*, nuclear buds; *S.E.*, Standard Error; *NC*, Negative Control; *MMC*, Mitomycin-C; *Gly*, Glyphosate

[a] *P* = 0.004 (significantly differs from the DMSO solvent control, Mann-Whitney test)

Discussion

Glyphosate is an active ingredient of most widely used herbicides. Although it is believed to be less toxic than other herbicides, data about its possible genotoxicity are controversial and IARC classified this compound as probably carcinogenic to human (IARC 2015). The genotoxic effects of high concentrations of glyphosate have been documented, although with contradictory results, in a great number of scientific papers, as well as in evaluation reports of different international agencies. On the other hand, the effects of low concentrations of this compound, likely to be encountered in everyday life, were poorly investigated. Results of our study provided information about in vitro clastogenic effects of glyphosate on human lymphocytes at the low ADI concentration of 0.500 µg/mL and its submultiples. Based on the obtained data, it can be concluded that glyphosate significantly increased the CA and MNi levels in human lymphocytes at the ADI concentration of 0.500 µg/mL established by EFSA and at its submultiple concentrations, up to 0.025 µg/mL. The mechanisms underlying genotoxic potential of glyphosate alone or in complex with other compounds are unknown, although the exposure to glyphosate was found to trigger oxidative processes involved in the increase of the genomic damage. NPB frequency was found to increase with increasing glyphosate concentrations, although a statistical significance was found only at the higher glyphosate concentration (Table 3). However, we obtained a significant linear regression (Table 2) due to a steady increase with the dose, indicating a possible effect of the compound inducing this kind of damage, which is consistent with the increased dicentric frequency observed in the chromosomal aberration test. Also, other authors analyzed in vitro the genotoxic potential of glyphosate in lymphocytes, but at exposure levels of higher magnitude orders. For example, in human lymphocytes cultured without S9 and in the presence of glyphosate at concentrations of 3.5, 92.8, and 580 µg/mL, the authors observed a slightly increased frequency of MNi and a significant tail length increase after a comet assay. Other authors evaluated the induction of CAs and MNi in blood cells of other animal models. Positive clastogenic and genotoxic effects of glyphosate on bovine peripheral lymphocytes cultured in vitro with herbicide concentrations ranging from 17 µM (2.874 µg/mL) to 170 µM (28.740 µg/mL) were reported, whereas another study reported no CAs effect of glyphosate at concentrations ranging from 28 (4.734 µg/mL) to 1120 µM (189 µg/mL). Contradictory results were obtained by a further study, in which observed, after 48 h of treatment without S9, a statistically significant increase in MNi frequency at 280 µM (47.34 µg/mL) but not at 560 µM (94.68 µg/mL) of glyphosate in one donor, and the opposite in a second donor (positive at 560 µM but not at 280 µM). Finally, another study in in vitro experiments based on comet assay, showed that 7 mM of glyphosate (1183 µg/mL) caused DNA damage in blood cells of Nile tilapia (Oreochromis niloticus). Concentrations of glyphosate similar to those evaluated in the present paper were tested in HepG2 cells by the MNi assay. Similarly to what we observed in human lymphocytes, these authors found

a significantly higher number of MNi at the ADI value of 0.500 µg/mL, as well as at the residential exposure level of 2.91 µg/mL, after 4 h of treatment. Vice versa, negative results on Hep-2 cells were obtained with CA assay at glyphosate concentrations of 0.20 mM (33.8 µg/mL), 1.20 mM (203 µg/mL), and 6.00 mM (1014 µg/mL). Significant levels of DNA damage were also observed in human buccal epithelial cells exposed to glyphosate concentrations ranging between 10 and 20 mg/L, whereas another study showed that, in peripheral blood mononuclear cells, glyphosate induces DNA damage in the concentration range from 0.5 mM (84.54 µg/mL) to 10 mM (1690 µg/mL), and a significant decrease of global DNA methylation at concentration of 0.25 mM (42.27 µg/mL). Interestingly, the same authors also observed a significantly increased methylation of p53 promoter at concentrations of 0.25 mM and 0.5 mM (42.27 and 84.54 µg/mL). This hypermethylation was found to be able to downregulate the p53 gene expression and to activate proto-oncogenes, with consequent genomic alterations and cancer risk. The possibility of glyphosate causing cancer promotion in skin cells and proliferation in breast cells has been also observed in vivo and in vitro studies by mouse and human models, respectively. In this scenario, the results obtained in the present study require attention. Indeed, increased CA and MNi frequencies in peripheral blood lymphocytes have been positively associated with increased cancer risk and early events in carcinogenesis, respectively. Moreover, it should be emphasized that, beyond the cases of intoxication where glyphosate content in blood was found to range from 0.6 to 150 µg/mL, in subjects who were indirectly exposed to this substance, glyphosate was found in blood at concentrations of 0.074 ± 0.028 µg/mL, a value about seven times lower with respect to the established ADI value, but in the range of concentrations we tested (from 0.5 to 0.0125 µg/mL). At the same time, the genotoxicity of a compound should not be evaluated only after single administrations in in vitro or in vivo systems, but also, and especially, after chronic administration of the same compound, even at lower quantities than those established by the competent agencies. In this sense, the clastogenicity we observed at concentrations of 0.100, 0.050, and 0.025 µg/mL represents an important signal, especially in view of a chronic exposure to these glyphosate concentration levels. Finally, no significant differences in CBPI and MI values were found between all tested concentrations and the solvent control, indicating that, at these concentrations, glyphosate does not influence in a significant manner the replicative capacity of the cells. These data differ from a study which observed a reduction of mitotic and proliferation indices in bovine lymphocytes, but at higher glyphosate concentrations (94.68 µg/mL and 189 µg/mL). Similarly, other authors described decreased levels of MI for other herbicides or insecticides, also in this case, at concentrations much higher than those tested in the present work.

Conclusion

In the present work, there was evidence for cytogenetic effects of glyphosate on cultured human lymphocytes. Despite the limitations of an in vitro study due to the reduced sample size, it is our opinion that the increased cytogenetic damage observed by our group at glyphosate concentrations equal and lower than the established ADI value requires further investigations in order to establish the effective genotoxicity threshold of this extensively used compound. Indeed, the glyphosate concentrations tested in the present work represent more realistic concentrations, likely to be encountered in everyday life, with respect to the higher doses evaluated in other published papers. In this scenario, in order to draw conclusions about the effects associated to the chronic exposure to low doses, in vitro studies are useful tools to investigate the dose response effects, the molecular mechanisms of action of different environmental xenobiotics, and their genotoxicity. This last, compared to other types of toxicity, may result in severe consequences that can be also inherited after long periods following exposure. The same DNA damage that occurs in a single cell, caused by low but chronic exposure to genotoxic compounds, can cause unexpected severe consequences in the long run.

5. Assessment and conclusion

Assessment and conclusion by applicant:

This paper describes human lymphocyte chromosome aberration and micronucleus tests with glyphosate. Although broadly compliant with OECD 473 and 487 there are some critical deficiencies which will have adversely influenced the reliability of the findings. Treatment with glyphosate was initiated 24 hours after lymphocyte cultures were stimulated to divide, instead of the recommended 48

hours, consequently the cultures would not have been asynchronous. This could mean cells in some stages of the cell cycle may have been under-represented, whilst others over-represented. Exposure to glyphosate was continuous for 28 hours in the chromosome aberration assay or 48 hours in the micronucleus assay. In contrast OECD test guidelines recommend maximum exposure of 1.5 cell cycles, equivalent to approximately 24 hours for lymphocyte cultures. For both endpoints the paper does not confirm if the slides were coded prior to analysis. The positive control has been compared statistically to the glyphosate treated cultures rather than the solvent controls.

The authors consider that glyphosate induces tri-tetraradial aberrations (amongst other aberration types) but fails to comment that the frequency of these aberrations observed at a single glyphosate concentration is 3-fold lower than the frequency observed in the solvent control cultures. Furthermore, it is unusual that the only multi-aberrant metaphases observed were a small number of positive control metaphases and gaps did not appear to increase with treatment but chromatid and chromosome breaks did.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate tested was not sufficiently characterized and although the genotoxicity tests conducted were in general in accordance with the OECD test guidelines, significant deficiencies were noted.

Assessment and conclusion by RMS:

Reliability criteria for *in vitro* toxicology studies

Publication: Santovino *et al*., 2018	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	Y	In accordance with OECD TG.
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	N	Purity is not reported. Source: Sigma-Aldrich, Saint Louis, USA.
Only glyphosate acid or one of its salts is the tested substance	Y	
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	Y	Lymphocytes.
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	NA	
Test concentrations in physiologically acceptable range (< 1 mM)	Y	0.500, 0.100, 0.050, 0.025 and 0.0125 µg/mL.
Cytotoxicity tests reported	Y	
Positive and negative controls	Y	
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	

Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate tested was not sufficiently characterized and although the genotoxicity tests conducted were in general in accordance with the OECD test guidelines, significant deficiencies were noted.		

1. Information on the study

Data point:	CA 5.4/007
Report author	Kasuba, V. *et al.*
Report year	2017
Report title	Effects of low doses of glyphosate on DNA damage, cell proliferation and oxidative stress in the HepG2 cell line
Document No	DOI 10.1007/s11356-017-9438-y E-ISSN: 1614-7499
Guidelines followed in study	Not mentioned
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary of the study according to OECD format

The toxic effects of glyphosate in vitro on HepG2 cells exposed for 4 and 24 h to low glyphosate concentrations likely to be encountered in occupational and residential exposures [the acceptable daily intake (ADI; 0.5 μg/mL), residential exposure level (REL; 2.91 μg/mL) and occupational exposure level (OEL; 3.5 μg/mL)] were studied. The assessments were performed using biomarkers of oxidative stress, CCK-8 colorimetric assay for cell proliferation, alkaline comet assay and cytokinesis-block micronucleus (CBMN) cytome assay. The results obtained indicated effects on cell proliferation, both at 4 and 24 h. The levels of primary DNA damage after 4-h exposure were lower in treated vs. control samples, but were not significantly changed after 24 h. Using the CBMN assay, a significantly higher number of MN and nuclear buds at ADI and REL after 4 h and a lower number of MN after 24 h were found. The obtained results revealed significant oxidative damage. Four-hour exposure resulted in significant decrease at ADI [lipid peroxidation and glutathione peroxidase (GSH-Px)] and OEL [lipid peroxidation and level of total antioxidant capacity (TAC)], and 24-h exposure in significant decrease at OEL (TAC and GSH-Px). No significant effects were observed for the level of reactive oxygen species (ROS) and glutathione (GSH) for both treatment, and for 24 h for lipid peroxidation. Taken together, the elevated levels of cytogenetic damage found by the CBMN assay and the mechanisms of primary DNA damage should be further clarified, considering that the comet assay results indicate possible cross-linking or DNA adduct formation.

Materials and Methods

Chemicals - Glyphosate was purchased as analytical standard purity grade (≤100 %) as Pestanal®, a registered trademark of Sigma-Aldrich Laborchemikalien GmbH, Germany.

Cell line - HepG2 cell line (ATCC® HB8065™) was purchased from the American Type Culture Collection (Rockville, MD, USA), cultivated in EMEM supplemented with 10 % foetal bovine serum

(FBS) and 1 % penicillin-streptomycin (10,000 U/mL) and maintained in a humidified atmosphere (95 % relative humidity) with 5 % CO_2 at 37 °C. Testing was performed when the duplication time has been established to be constant, and the passage step was more than 3 but less than 10 cell passages.

Treatment conditions - The cells in culture were exposed to glyphosate concentrations of 0.5 µg/mL, 2.91 µg/mL and 3.5 µg/mL in PBS corresponding with the systemic concentrations at the acceptable daily intake (ADI), the residential exposure level (REL) and the occupational exposure level (OEL). The calculation of the concentrations to be tested was based on the average male human body weight of 60 kg and the total volume of 36 L of extracellular liquid. HepG2 cells were grown until 80 % confluence, trypsinized and transferred in cell culture flasks for the micronucleus and comet assays and in 96 well plates for the cell proliferation assay and the determination of markers of oxidative stress. Prior to treatment, the culture medium was removed, cells were washed with PBS and fresh complete medium with glyphosate was added. Cells were treated for 4 or 24 hours at 37 °C in a humidified atmosphere (95 % relative humidity) with 5 % CO_2. Negative and positive controls were tested in parallel.

Cell proliferation assay - Proliferation of HepG2 cells after 4 and 24 hours of exposure to glyphosate was studied by means of the CCK-8 colorimetric assay, based on the use of Cell Counting Kit-8. This kit uses 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, sodium salt (WST-8) that is bioreduced by cellular dehydrogenases to an orange formazan product, soluble in tissue culture medium in the presence of the electron carrier, 1-methoxy PMS. The amount of formazan produced is directly proportional to the number of living cells. At the end of treatment cells were washed with PBS and 10 µL of WST-8 solution added. After 4 and 24 hours of incubation at 37 °C in a CO_2 incubator, the optical density at 450 nm was determined for each well using a Victor3™ Multilabel Plate Reader. Data were expressed as a percentage absorbance compared to relevant negative controls. The positive control was 10 % DMSO in the complete nutrient medium. The experiment was done twice independently with 4 repeated measurements in each.

Alkaline comet assay - After 4 or 24 hours of treatment, cells were washed twice with PBS, detached by trypsinization, centrifuged and resuspended in complete EMEM. 7 µL of single cell suspension at 10^4 cells/mL was mixed with low melting point agarose and layered on slides. Duplicate slides were prepared for each time period and concentration together with negative and positive controls. Hydrogen peroxide was used as the positive control. Following preparation of all microgels, they were immersed in fresh cold lysis solution for 24 hours. Afterwards, slides were washed with bidistilled water and denatured in freshly prepared cold denaturation/electrophoresis buffer. After 20 min at 4 °C, cells were placed in the electrophoretic chamber and subjected to electrophoresis. Afterwards the slides were neutralized, dehydrated and stained with ethidium bromide (20 µg/mL) for image analysis with the fluorescent microscope Olympus BX51 (×200 magnification) using the Comet Assay IV software. A total of 100 nucleoids (50 per slide) were measured per each experimental point. The level of DNA damage was estimated based on tail intensity (TI), indicating %DNA in the comet tail. The experiment was performed once with two replicates that were compared, and if no statistically significant difference was found they were combined.

Cytochalasin B-blocked micronucleus cytome assay (CBMN cytome assay) - Cells were seeded at 10^4 cells/mL in complete EMEM medium. After 4 or 24 hours of treatment with glyphosate, cells were washed twice with PBS and fresh complete medium was added. At the 44th hour, cytochalasin B (3 µg/mL) was added and the cell cultures harvested 24 hours thereafter. The positive control, cyclophosphamide, was incubated simultaneously. After harvesting, the medium was discarded, and cells were washed twice with PBS, detached by trypsinization, rinsed and resuspended in complete EMEM medium. Cell suspensions in complete EMEM were centrifuged and the pellet resuspended in PBS, centrifuged and the pellet fixed and put on slides for staining with 2 % Giemsa stain and air-dried. The experiment was performed once with two replicates. Micronuclei (MNi), nucleoplasmic bridges (NPBs), nuclear buds (NBUDs) and apoptotic and necrotic cells were scored in binucleated (BN) cells. A total of 2000 BN cells per each experimental point were scored to determine the parameters of the CBMN Cytome assay.

Lipid peroxidation - Malondialdehyde (MDA), and endproduct of lipid peroxidation, was measured using the thiobarbituric acid reactive substance (TBARS) assay with some modification. 0.5 mL of sample was added to 0.5 mL of thiobarbituric-trichloroacetic acid (TBA-TCA) reagent and heated at 90 °C for 30 min and then cooled in an ice bath and centrifuged. Absorbances were measured with a microplate spectrophotometric system (Victor3™ Multilabel Plate Reader) at 530 nm. Two independent experiments were performed and each sample was measured in duplicate. The TBARS concentration in unknown samples was calculated using a standard curve constructed with 1,1,3,3- tetrametoxypropane (0.3–6.07 µM) and expressed as µM.

Total antioxidant capacity - Total antioxidant capacity (TAC) was investigated using the ferric-reducing ability of plasma (FRAP) assay, based on the reduction of Fe3+-TPTZ complex under acidic conditions. The FRAP assay used in this study was slightly modified. 100 µL of cell suspension (10^4 cells in total) was added to 1.0 mL of FRAP reagent. Absorbance was measured after 4 minutes of incubation at 593 nm using the FRAP working solution as a blank. Two independent experiments were performed and each sample was measured in duplicate. The results were calculated on a basis of a standard curve using $Fe_2SO_4 \cdot 7H_2O$ (0.05-4.0 mM).

ROS detection - The amount of intercellular reactive oxygen species (ROS) was measured using 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA). DCFH-DA is deacetylated by cellular esterases to form a non-fluorescent compound which is then oxidized in the presence of hydroxyl, peroxyl and other ROS to the fluorescent 2′,7′-dichlorodihydrofluorescein (DCF). All measurements were performed in quadruplicate in dark-sided 96-well microplates in which each well was added 100 µL of cell suspension containing 10^4 cells. Cells were grown in the same medium and conditions as for the cell proliferation assay and treated with the same concentrations of glyphosate for 4 and 24 hours. After treatment the cells were washed with PBS and 100 µL of 50 µM DCFH-DA dye diluted in PBS was added and kept in contact with the cells for 30 minutes at 37 °C in a CO_2 incubator. The control for dye autofluorescence was prepared without addition of dye. Negative (non-treated) cell controls in EMEM were included in each experiment. The positive control was a solution of 100 mM H_2O_2. Measurements were made using a Victor3™ Multilabel Plate Reader at an excitation wavelength of 485 nm and an emission wavelength of 535 nm. Data were expressed as fluorescence arbitrary units (AUs) and later transformed into percentages compared to control values.

Quantification of glutathione - The fluorogenic bimane probe, monochlorobimane (MBCl), reacts specifically with glutathione (GSH) and forms a fluorescent product equal to the amount of GSH that can be measured fluorometrically. Cell cultures in 96-well plates were prepared with 100 µL of cell suspension containing 10^4 cells and each well was treated with glyphosate for 4 and 24 hours. After washing with PBS, cells were incubated with 100 µL of 50 µM MBCl in PBS for 20 minutes at 37 °C in a CO_2 incubator. Measurements were made using a Victor3™ Multilabel Plate Reader at an excitation wavelength of 355 nm and an emission wavelength of 460 nm. Negative cell controls were included in each experiment. Data were expressed as fluorescence AUs and later transformed into percentages compared to control values. All of the measurements were performed in quadruplicate.

Glutathione peroxidase activity - Glutathione peroxidase (GSH-Px) activity in HepG2 cells was measured according to the European standardized method. To increase assay sensitivity for the measurement of GSH-Px, samples were prediluted 60 times instead of a recommended 110-fold dilution as used for blood samples. The amount of glutathione oxidized by *t*-butyl hydroperoxide was determined by following the decrease in β-NADPH concentration at 340 nm. Two independent experiments were performed and each sample was measured in duplicate. One unit of GSH-Px was expressed as the amount of enzyme that oxidizes 1 µmol β-NADPH/min at 37 °C. Activity of GSH-Px was expressed in U/g total protein. The protein concentration of cell lysates was measured using a Total Protein Kit, Micro. Samples, standards and blanks were tested in triplicate, and absorbance was measured in 96-well plates at 570 nm**.**

Statistical analysis - Statistical analysis was performed using StatSoft Dell Statistica 13 package program. Descriptive statistics were used to determine the basic statistical parameters (mean, standard error and deviation, median and minimum and maximum values). Data gathered with the Comet Assay IV software

were logarithmically transformed prior to statistical evaluation, with the aim of normalizing distributions and equalizing variances. The intra- and intergroup comparisons between samples were performed using one-way analysis of variance (ANOVA) with post hoc Scheffé's test. Comparisons between values obtained with the CBMN Cytome assay and HepG2 proliferation kinetics measured with CBMN were made by Pearson's $\chi 2$ test for two-by-two contingency tables. For the biochemical and cell proliferation assays descriptive statistics and *t* test for comparisons between independent samples were used. The level of statistical significance was set at $p < 0.05$.

Results

Cell proliferation measured by the CCK-8 assay - Although without statistical significance a slight increase of cell proliferation was observed in HepG2 cells after a 4-hour treatment of about 9 % at the ADI and almost 8 % at the REL. The values recorded for the OEL-treated cells were not different from control. After 24 hours of treatment, HepG2 cells showed a not statistically significant increase in cell proliferation of 3 % at the REL and of 1 % at the OEL when compared to the control. Proliferation of ADI-treated cells did not differ from control.

Primary DNA damage measured by the alkaline comet assay - After 4 hours of exposure, the TI was statistically significantly decreased at all glyphosate concentrations. After 24 hours of exposure, the TI at ADI, OEL and REL were not statistically significantly different from control. Throughout comet measurements, apoptotic and necrotic nucleoids were also counted but there were no significant deviations from control.

Cytogenetic damage measured using CBMN cytome assay - After 4 hours of exposure, a not statistically significant increase in MN was found in HepG2 cells treated with glyphosate at all 3 concentrations. In HepG2 cells treated with glyphosate at ADI and REL, a statistically significant increase in nuclear buds (NBUD) frequency was observed. The incidence of nucleoplasmic bridges (NPB) was highest in cells treated with glyphosate at ADI (6 per 2000 BN cells). Only 2 apoptotic cells per 2000 cells were found in the sample treated with glyphosate at OEL. The nuclear division index (NDI) in glyphosate-treated cells was different from that of the control. In the OEL-treated sample, there were statistically significant changes in the distribution of M1–M4 cells and OEL treatment resulted with more cells in the M2 phase. The same effect was observed when ADI-treated and OEL-treated samples were compared, as well as when REL-treated and OEL-treated samples were compared. After 24 hours of exposure, a lower number of micronucleated BN cells and a lower frequency of BN MN was found at all 3 glyphosate concentrations when compared to the control. Also the frequency of BN NBUDS was statistically significantly lower at all 3 concentrations. Control and glyphosate-treated cells did not significantly differ in the number of NPBs. At ADI 7 and at REL 5 apoptotic cells per 2000 BN cells were found. There was no statistically significant difference in NDI values between glyphosate-treated samples and control. A slightly changed incidence of M2 and M3 cells was found in treated samples as compared to control.

Lipid peroxidation - After 4 hours of exposure, TBARS concentrations in samples treated with glyphosate at ADI and OEL were statistically significantly lower than control values. Treatment of HepG2 cells for 24 hours with glyphosate resulted in lower TBARS concentrations at REL, but the difference was not statistically significant.

GSH level - The level of GSH was not statistically significantly different from the control value for all concentrations and exposure times. After 4 hours of treatment, the ADI-treated sample showed a 4 % higher level of GSH than the control whereas REL and OEL-treated cells showed GSH levels similar to control values. The 24-hour ADI-treated sample had the lowest GSH concentration, which was 5 % lower than the levels measured in the control samples, and REL- and OEL-treated cells showed GSH levels similar to control values.

GSH-Px activity - GSH-Px activity was statistically significantly decreased in HepG2 cells exposed to glyphosate at the ADI concentration after 4 hours. After 24 hours of treatment, a statistically significant decrease in the GSH-Px activity was observed at the OEL concentration.

Discussion

The present study evaluated how low-dose exposure for 4 and 24 hours affected cytotoxic, genotoxic and biochemical parameters in the human hepatoma cell line HepG2. Glyphosate increased not statistically significantly cell proliferation in HepG2 cells after 4 and 24 hours of exposure. The effect was more pronounced after 4 hours (8–9 % as compared to control), than after 24 hours (3 % as compared to control). The results of this study have demonstrated that exposure to glyphosate for 4 hours led to a slightly increased cytogenetic damage in terms of MN. Despite the non-significant increase in MN frequencies at ADI and REL, significant increases in nuclear bud frequency were found after 4 hours of exposure. Since nuclear budding represents a mechanism of MN formation, these results indicate that even a low dose of glyphosate as the ADI influences the level of DNA damage and cell stability. After 24 hours of exposure, all of the treated cells showed a lower frequency of micronuclei, nuclear buds and nucleoplasmic bridges than the control. The results obtained suggest that glyphosate exerts the highest DNA-damaging potential after 48 or even after 72 hours of treatment. Considering that the shorter exposure lasted only 4 hours, while the total cell culture lasted for 72 hours before harvesting, there was an opportunity to better examine the effects of low-dose exposure on DNA damage. On the contrary, in the case of an exposure of 24 hours it seems that this effect only started to be slightly visible. Serum in the cell culture can mask the development of DNA damage observed in micronucleus assay results. This can be the explanation, why the standard time period used in this experiment did not demonstrate an effect, while in the comet assay the primary DNA damage demonstrated higher damage levels.

Conclusion

All cytogenetic, molecular and biochemical methods used in this study indicate that glyphosate applied at low concentrations, possesses a toxic potential towards HepG2 cells, which has to be further explained. It seems that the theory of different effects of low-dose vs. high-dose exposure, and more deleterious effects at low doses, is true. Although at ADI, REL and OEL, no drastically different levels of oxidative damage were seen, the elevated level of permanent DNA damage found with the micronucleus assay calls for concern, especially if leading to adduct formation, as shown by comet assay results. Based on the obtained results, it cannot be said without doubt whether glyphosate acts as an aneugen or a clastogen, but there are indications from previous studies that the aneugenic effect plays an important role in the formation of micronuclei. This study did not evaluate the effects of glyphosate on cytoskeleton and proteins, but this would be a direction for future evaluations of glyphosate toxicity, together with the clarification of its effects on cell membrane level, especially in different phases of the cell cycle.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The objective of this study was to examine the effects of acute exposure (4 and 24 hours) of human hepatoma HepG2 cells to glyphosate at concentrations equivalent to the systemic concentrations at the ADI, REL and OEL. The endpoints investigated in this study are cell proliferation, DNA damage, MN formation and oxidative stress. A non-statistically significant increase in cell proliferation was seen in the CCK-8 test with no dose-effect relationship. The comet assay showed a statistically significant decrease in tail intensity after 4 hours with no difference from control after 24 hours. In the CBMN cytome assay a non-statistically significant increase in BN MN frequency was seen after 4 hours without a dose-effect relationship. After 24 hours, a decrease instead of an increase in BN MN frequency was reported. The nuclear bud frequency was statistically significantly elevated after 4 hours of exposure but was statistically significantly lower than control after 24 hours of exposure. The indicator tests for oxidative stress did not show a substance related effect.

Overall, the results of the study do not indicate a genotoxic potential of glyphosate. The lack of statistical significance, reproducible effects as well as the fact that the control values in the Comet assay and micronucleus assay seem to be highly variable limit the reliability of the study.

This publication is considered relevant but reliable with restrictions because the cytogenetic damage

found *in vitro* at a systemic concentration corresponding with the ADI (0.5 µg/mL which should have been 0.17 µg/mL) was not confirmed in *in vivo* regulatory MN studies with doses up to 2000 mg/kg bw corresponding with a systemic concentration of about 50 µg/mL.

Assessment and conclusion by RMS:

Reliability criteria for *in vitro* toxicology studies

Publication: Kasuba *et al.,* 2017	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines		
Study performed according to GLP		
Study completely described and conducted following scientifically acceptable standards		
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity of ≤100 % as Pestanal®. Source: Sigma-Aldrich Laborchemikalien GmbH, Germany.
Only glyphosate acid or one of its salts is the tested substance		As "Pestanal"
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	Y	
Test conditions clearly and completely described	Y	Culture concentration not in correspondence with ADI of 0.5 mg/kg bw.
Metabolic activation system clearly and completely described	N	HepG2 cells used
Test concentrations in physiologically acceptable range (< 1 mM)	Y	
Cytotoxicity tests reported	N	Very low concentrations used
Positive and negative controls	Y	
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	?	No significant effects were observed for the level of ROS and GSH for 4h and 24h incubation, and for 24 h for lipid peroxidation. Elevated levels of cytogenetic damage were found in the CBMN assay and the comet assay results indicate possible cross-linking or DNA adduct formation. These data were obtained at in vitro test concentrations that

		correspond with an external dose of 0.5 mg/kg bw/day (ADI) whereas there are regulatory studies with no effects at doses up to 2,000 mg/kg bw/day
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Not reliable		
This publication is considered relevant but reliable with restrictions because the cytogenetic damage found in vitro at a systemic concentration corresponding with the ADI (0.5 µg/mL, which should have been 0.17 µg/mL) was not confirmed in *in vivo* regulatory MN studies with doses up to 2000 mg/kg bw corresponding with a systemic concentration of about 50 µg/mL.		

1. Information on the study

Data point:	CA 5.4/008
Report author	Kwiatkowska, M. *et al.*
Report year	2017
Report title	DNA damage and methylation induced by glyphosate in human peripheral blood mononuclear cells (in vitro study)
Document No	doi.org/10.1016/j.fct.2017.03.051 ISSN: 0278-6915
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No, not conducted under GLP/ Officially recognised testing facilities.
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary of the study according to OECD format

The purpose of this study was to assess DNA damage (determination of single and double strand-breaks by the comet assay) as well as to evaluate DNA methylation (global DNA methylation and methylation of p16 (CDKN2A) and p53 (TP53) promoter regions) in human peripheral blood mononuclear cells (PBMCs) exposed to glyphosate. PBMCs were incubated with the compound studied at concentrations ranging from 0.1 to 10 mM for 24 h. The study has shown that glyphosate induced DNA lesions, which were effectively repaired. However, PBMCs were unable to repair completely DNA damage induced by glyphosate. A decrease in global DNA methylation level at 0.25 mM of glyphosate was also observed. Glyphosate at 0.25 mM and 0.5 mM increased p53 promoter methylation, while it did not induce statistically significant changes in methylation of p16 promoter. To sum up, it was shown for the first time that glyphosate (at high concentrations from 0.5 to 10 mM) may induce DNA damage in leucocytes such as PBMCs and cause DNA methylation in human cells.

Materials and methods

Chemicals – Glyphosate (95 % purity) commercially obtained from Sigma-Aldrich, USA.

Isolation of PBMCs - PBMCs were isolated from leucocyte-buffy coat from blood collected from 9 healthy volunteers (aged 18-55 years) with no symptoms of infectious disease. For each parameter, 3 leucocyte-buffy coats were taken from 3 blood donors. After dilution with PBS (1:4) PBMCs were isolated from the

buffy coat by centrifugation using lymphocyte separation medium (LSM). The PBMCs were collected, suspended in erythrocyte lysis buffer and incubated. Afterwards PBS was added and the cells centrifuged. The supernatant was decanted and the cells were washed twice with RPMI with L-glutamine and 10 % fetal bovine serum (FBS). The cells were resuspended in RPMI medium with L-glutamine, 10 % FBS and penicillin-streptomycin (0.5 %) and counted in the hemocytometer. The final PBMCs density used in the experiments after addition of glyphosate was $1x10^6$ cells/mL. After incubation, PBMCs were diluted to a density of 5 x 10^4 cells/mL for the comet assay and condensed to a density of 5 x 10^6 cells/mL for the conduct of epigenetic methods. The viability of the cells was over 94 %.

Treatment of PBMCs - Glyphosate was dissolved in PBS and the final concentrations of glyphosate in the comet assay were in the range of 0.25 to 10 mM, while epigenetic changes were assessed after exposure to glyphosate at 0.25 and 0.5 mM. For the determination of DNA damage the cells were incubated with glyphosate for 24 hours and repair of the DNA lesions was assessed after a recovery period of 120 minutes following incubation. In the comet assay, 0.25 mM was chosen as the lowest concentration of glyphosate which produced any statistically significant changes in DNA damage. Epigenetic changes were assessed at 0.25 mM which did not induce DNA damage and at 0.5 mM which induced statistically significant DNA damage. For the assessment of cell viability PBMCs were incubated for 24 hours with different concentrations of glyphosate. Afterwards, the cells were centrifuged and the test solution discarded. The cells were then resuspended in RPMI medium and incubated for an additional 2 hours. Cell viability was 90.0 %, 90.2 %, 90.4 %, 88.5 %, and 87.8 % for control, 0.5, 5, 7.5 and 10 mM of glyphosate, respectively. Each DNA damage experiment included hydrogen peroxide as the positive control.

Alkaline comet assay - The comet assay was performed under alkaline conditions with some modifications. A freshly prepared cell suspension in 0.75 % low melting point agarose dissolved in PBS was layered onto microscope slides pre-coated with 0.5 % normal melting point agarose. The cells were lysed for 1 hour at 4 ºC in an alkaline buffer and DNA was allowed to unwind for 20 minutes in an alkaline solution. Electrophoretic separation was performed in an alkaline solution at 4 ºC for 20 minutes at an electric field strength of 0.73 V/cm (28 mA). Then, the slides were washed in water and stained with 2 mg/mL 4',6-diamidino-2-phenylindole (DAPI) and covered with cover slips. The comets were observed at 200 x magnification in an Eclipse fluorescence microscope. Fifty images (comets) were randomly selected from each sample and the mean value of DNA in the comet tail was taken as an index of DNA damage (expressed in percent). For one blood donor, 3 parallel tests with aliquots of the sample of the cells were performed for a total number of 150 comets. A total number of 450 comets from 3 blood donors was recorded to calculate mean ± SEM.

DNA repair - The control samples and the PBMCs treated with glyphosate at 0.5, 5, 7.5 and 10 mM were washed and resuspended in fresh RPMI 1640 medium with L-glutamine preheated to 37 ^{0}C. Aliquots of the suspension were taken immediately after incubation at time "zero" and 120 minutes later. The samples were placed in an ice bath to stop DNA repair. The repair was assessed as a decrease in the extent of DNA damage measured after 120 minutes of post-incubation using the alkaline version of the comet assay.

Methylation of p16 (CDKN2A) and p53 (TP53) promoter regions - Chemical modification of 500 ng of genomic DNA was performed with the Cells-to-CpG™ Bisulfite Conversion Kit. For methylation analysis, a quantitative methylation-specific real-time PCR assay (qMSP) was conducted in 3 independent experiments including 3 blood donors with FastStart SYBR Green Master. All samples were amplified in triplicate. To determine the methylation status of a particular gene expressed as the methylation index (MI) in percentage, the Ct values of the methylated gene of interest were compared with the Ct values of the unmethylated gene of interest.

Global DNA methylation - Global DNA methylation was determined by means of DNA quantification using 5-methyl cytosine (5-mC) monoclonal antibodies in an ELISA-like reaction using the Methylflash Methylated DNA Quantification Kit. DNA (100 ng) isolated from whole blood PBMCs was used for analysis. Each sample was analyzed in duplicate and the determination was repeated whenever there was a failure in detection. The calculation of the amount of 5-mC was done with the use of a standard curve.

Methylation levels were calculated relative to the methylated control DNA and expressed as a percentage of methylated DNA.

Statistical analysis - The mean value was calculated for three independent experiments (3 blood donors), whereas for each individual an experimental point was a mean value of at least 2 (methylation analysis) or 3 replications. Statistical analysis was conducted using the Mann-Whitney test, the Student's t-test and one-way analysis of variance (ANOVA) with a post hoc multiple comparisons procedure. The differences were considered to be statistically significant when $p<0.05$.Data analysis was performed using STATISTICA software.

Results

Analysis of DNA strand-breaks and DNA repair - DNA damage (single and double strand-breaks and alkali-labile sites formation) was statistically significantly increased from a glyphosate concentration of 0.5 mM on. At 10 mM the statistically significant increase in DNA damage exceeded 13 times the control value. After 120 minutes of recovery significant repair was observed of the DNA lesions induced by glyphosate (5.61 % vs 2.93 %, 10.51 % vs 5.07 %, , 14.91 % vs 7.76 %, and 27.49 % vs 11.05 % at 0.5 mM, 5 mM, 7.5 mM, and 10 mM of glyphosate, respectively.

DNA methylation status - As compared to control cells, the percentage of the global DNA methylation level was statistically significantly decreased by glyphosate at 0.25 mM, but not at 0.5 mM. On the contrary, p53 promoter methylation was statistically significantly increased at both concentrations of glyphosate. Methylation of the p16 gene promotor was increased after treatment with glyphosate but this change was not statistically significant.

Discussion and Conclusions

This study revealed that glyphosate decreased global DNA methylation in PBMCs statistically significantly at a concentration of 0.25 mM. Surprisingly, the effect of glyphosate at 0.5 mM on global DNA methylation was not statistically significant although the mean value from three independent experiments was still decreased as compared to control. Along with the decreased global DNA methylation, a statistically significant increase in methylation of the p53 promoter region was observed at 0.25 and 0.5 mM. Altered p53 promoter hypermethylation is an epigenetic pattern frequently observed in human cancers. Thus, the results of this study suggest that glyphosate at high concentrations (≥ 0.25 mM) may cause a down-regulation of p53 gene expression and activate proto-oncogenes or retrotransposable sequences which may induce genomic alterations by insertion and/or homologous recombination. This study showed for the first time that glyphosate may induce DNA damage in human leucocytes and cause epigenetic alterations in animal cells.

3. Assessment and conclusion

<u>Assessment and conclusion by applicant</u>:

It was the objective of this study to investigate the effect of high glyphosate concentrations on DNA integrity and DNA methylation in PBMCs *in vitro*. It was demonstrated that glyphosate increased statistically significantly DNA damage (single and double strand-breaks and alkali-labile sites formation) from 0.5 mM up to 10 mM. Repair of the DNA lesions was significant at all concentrations tested after 120 minutes of recovery. The percentage of the global DNA methylation level was statistically significantly decreased by glyphosate at 0.25 mM but not at 0.5 mM. On the contrary, p53 promoter region methylation was statistically significantly increased as compared to control cells at 0.25 and 0.5 mM.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the lowest concentration at which DNA damage was observed (0.5 mM) is higher than the blood concentrations in rats (0.3 mM) obtained after dosing at the limit dose of 2000 mg/kg bw where no MN effects were seen.

Assessment and conclusion by RMS:

Reliability criteria for *in vitro* toxicology studies

Publication: Kwiatkowska *et al.,* 2017.	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity of 95 %. Source: Sigma-Aldrich, St Louis, USA.
Only glyphosate acid or one of its salts is the tested substance	Y	Salt not mentioned
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	Y	
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described		
Test concentrations in physiologically acceptable range (< 1 mM)	Y/N	0.25 to 10 mM
Cytotoxicity tests reported	Y	
Positive and negative controls	Y	
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	Y	For DNA damage. The concentration at which DNA damage was observed is higher than the blood concentrations in rats obtained after dosing at the limit dose of 2000 mg/kg bw for the detection of MN in vivo. The results obtained are not corroborated by regulatory in vivo genotoxicity studies.
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the lowest concentration at which DNA damage was observed (0.5 mM) is higher than the blood concentrations in rats (0.3 mM) obtained after dosing at the limit dose of 2000 mg/kg		

bw where no MN effects were seen.

1. Information on the study

Data point:	CA 5.4/009
Report author	Suárez-Larios, K. *et al.*
Report year	2017
Report title	Screening of Pesticides with the Potential of Inducing DSB and Successive Recombinational Repair
Document No	DOI: 10.1155/2017/3574840
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No, not conducted under GLP. Officially recognised testing facilities.
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary of the study according to OECD format

A study was realized to ascertain whether glyphosate and other selected pesticides would induce double strand breaks (DSB) in lymphocyte cultures and whether this damage would induce greater levels of proteins Rad51 participating in homologous recombination or of p-Ku80 participating in nonhomologous end joining. Only five pesticides were found to induce DSB of which only glyphosate and another one pesticide induced a significant increase of p-Ku80 protein, indicating that nonhomologous end joining recombinational DNA repair system would be activated. The type of gamma-H2AX foci observed was comparable to that induced by etoposide at similar concentrations. These results are of importance since these effects occurred at low concentrations in the micromolar range, in acute treatments to the cells. Effects over longer exposures in actual environmental settings are expected to produce cumulative damage if repeated events of recombination take place over time.

Materials and Methods

Chemicals - Endosulfan, glyphosate, pentachlorophenol, permethrin, propoxur, paraoxon, AMPA (glyphosate metabolite), endosulfan lactone (endosulfan metabolite), and etoposide (positive control) were obtained from Sigma-Aldrich, Mexico. Purity was not reported.

Evaluation of DNA double strand breaks (DSB)- Three mL of blood were obtained from fully informed healthy young male donors of 21 to 35 years old. The volunteers were nonsmokers, did not consume alcohol and had not taken medication or were not subjected to radiation for medical purposes. The concentrations of glyphosate tested were 0, 0.4, 2, 10, and 50 µM, and those of AMPA were 0, 40, 200, 1,000 and 5,000 µM. Whole blood was diluted in RPMI-1640 medium and treated with the test compound 1.5 hours at 37 ºC, after which 0.075M KCl was added and the incubation continued for 30 minutes. Lymphocytes were recovered by centrifugation and formaldehyde added to reach a final concentration of 4 %. Then PBS and Triton X-100 were added followed by an incubation of 30 minutes. Thereafter the cells were washed, supplemented with 4 % fetal bovine serum and isolated by centrifugation and 1mL of cold 50 % methanol added. After one night at −20 ºC the samples were centrifuged at 0 ºC, and cold methanol added to the cells which were kept at −20ºC until analysis. Treatments were done in duplicate. DNA double strand breaks were detected by means of immunofluorescence of phosphorylated histone H2AX foci. Staining of lymphocyte nuclei was done as follows: the slides were washed and blocked with KCMT buffer for 1 hour at room temperature. Primary antiphospho-histone H2AX (Ser139) in blocking solution was added and left to incubate overnight followed by washes with KCMT buffer. Then the cells were incubated with the

secondary antibody Alexa-Fluor 555 goat anti-mouse for 1 hour at room temperature. The slides rinsed in deionized water before mounting in DAPI mounting medium and subsequently analyzed for γ-H2AX foci under a fluorescence microscope. Evaluation of foci was done in 2 slides per concentration and 50 cells were evaluated in 3 different regions per slide. In total, 300 cells were evaluated per treatment. When a nucleus presented one or more foci, it was considered positive. The extent of DNA damage was classified in 3 categories: percentage of cells without γ-H2AX foci (no DNA damage), with less than 10 γ-H2AX foci (moderate DNA damage) and with more than 10 γ-H2AX foci (severe DNA damage). Additionally, the damage was expressed as mean percentage of γ-H2AX positive nuclei.

Cytotoxicity - Cytotoxicity was tested using The CellTiter 96 AQueous One Solution Reagent from PROMEGA and following the manufacturer's instructions. Mononuclear cells were isolated from blood using Histopaque-1077. The cells were plated in well plates at 100,000 cells per well, and treated with the test compounds in triplicate for each concentration. Absorbance at 490 nm was recorded using a 96-well plate reader. The percentage of survival was calculated as (Absorbance at 490 nm of treatment/Absorbance at 490 nm of the negative control) × 100.

Western blot analysis of proteins participating in DNA recombination - Mononuclear cells were isolated with Histopaque-1077 and treatments were applied to cells resuspended in RPMI-1640 at 500,000 cells per tube. Glyphosate was tested in duplicate at 1.25, 2.5, and 5 µM. The cells were then centrifuged, the supernatant discarded and 0.5 M sodium azide added. After centrifugation, the cell pellets were kept at −70 ºC until used for Western blot analysis. Two separate experiments were performed per test compound, with two donors each time. RIPA lysis buffer solution containing phosphatase and protease inhibitors was added to each cell pellet. The samples were then sonicated, incubated in ice, and centrifuged. The supernatant was recovered and 5 µL of each sample was placed in a 96-well plate for protein quantification. The Lowry assay was performed with the DC Protein Assay kit and the concentration determined in a plate reader at 750 nm. The samples were then stored at −70∘C until use.
For the determination of phosphorylated Ku80 (phospho-T714) and Rad51 by Western blotting 35 µg of total protein was separated in a 10 % SDS-polyacrylamide gel and transferred to a nitrocellulose membrane with a Trans-Blot SD Semi-Dry Transfer Cell. After incubation with blocking solution and rinsing the appropriate membrane zones were isolated and incubated with rabbit anti-Rad51 polyclonal antibody or rabbit anti- phosphorylated Ku80 (phosphoT714) polyclonal antibody (p-Ku80). Incubation with primary goat anti-Actin polyclonal antibody was done for the determination of β-actin as the internal control. The membranes were then rinsed and incubated with secondary antibody goat anti-rabbit IgG-HRP for Rad51 and p-Ku80 detection and donkey anti-goat IgG-HRP for actin detection. After rinsing the protein fractions were quantified with a luminescence kit. Optical densities were measured with Quantity One software, version 4.1.1. The values obtained for each protein (Rad51 or p-Ku80) were normalized with respect to β-actin and the mean of the normalized negative controls. Results are presented as the % with respect to normalized negative controls. Two membranes were prepared per separate experiment with each test compound .

Statistical Analysis - Statistical calculations were made with the GraphPad Prism 6 software package and the results for γ-H2AX foci and optical density from Western blot analysis were evaluated with the Kruskal-Wallis test and Dunn's multiple comparison as a post hoc test. The value of etoposide as a positive control at the concentration of 50 µM was analyzed with the Mann-Whitney *U* test with respect to the negative control. Cytotoxicity was analyzed with linear regressions. The results were considered statistically significant if $p<0.05$.

Results

Identification of DNA double strand breaks(DSB) - For glyphosate, the mean of the % cells with more than 10 γ-H2AX foci was 0.33, 1.67, 9.33, 8.83, and 3.17 for 0, 0.4, 2, 10, and 50 µM (linear regression, $R^2 = 0.2$, $p = 0.02$). No effect was seen with AMPA. The positive control, etoposide, showed a significant correlation with dose (, $R^2 = 0.82$, $p < 0.0001$) for cells with more than 10 foci.

Cytotoxicity - The concentrations used for the determination of DSB were also used to assess the

cytotoxicity of the test compounds that produced DSB. The survival range for glyphosate was 100 % to 70 % viability for concentrations ranging from 1.25 to 5 µM.

Quantification of p-Ku80 and Rad51 Proteins
The test compounds that showed positive for DSB were further tested to determine whether DNA recombination would be induced. Glyphosate was found to induce statistically significantly p-Ku80 protein in a dose-dependent manner, whereas Rad51 was not significantly affected. Etoposide consistently induced p-Ku80, although with a wide variation between tests at 10 µM.

Discussion and Conclusions
Pesticides amongst which glyphosate were evaluated for their capacity to induce DNA double strand breaks, a lesion related to the formation of chromosomal rearrangements and leukemia risk. Glyphosate exhibited an ability to induce this kind of DNA damage in the form of phosphorylated γ-H2AX foci in the nuclei of human lymphocytes in at least two of the concentrations tested. AMPA tested negative. It is noticeable that the positive control, etoposide, induced more than 85 % of cells with more than 10 foci at 50 *µ*M although it induced foci in a manner comparable to glyphosate at lower concentrations. This extent of DNA damage seems to be of relevance to the increase of repair proteins like p-Ku80, since glyphosate also produced a significant increase in this protein, whereas the rest of the test compounds that induced a lower percentage of cells with γ-H2AX foci did not. To evaluate proteins participating in DNA recombination, the highest concentration of glyphosate used was 5 µM to avoid cytotoxicity. Exposure of cells in a non-proliferative state to glyphosate not only induced the breakage of DNA, but also the phosphorylation of Ku80, a protein that participates in the c-NHEJ repair pathway. This pathway is known for being prone to error, introducing micro deletions or micro insertions, which could be mutagenic and alter cell behavior if they occur in coding or regulatory sequences. This is one possible outcome of the DSB in non-proliferating cells. Etoposide, the positive control, is well known as a topo II inhibitor capable of producing complex DSB. The DNA damage induced by etoposide in this study was comparable to the damage induced by paraoxon and glyphosate (DSB and pKu80 induction), so the question emerging from these results is whether the outcome for cells damaged by glyphosate would be similar to the outcome of cells damaged by etoposide and whether they would also induce chromosomal rearrangements.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The objective of this study was to assess whether glyphosate and its metabolite AMPA produced DNA double strand breaks in human peripheral lymphocytes and whether proteins involved in DNA repair were induced. The results show that glyphosate, but not AMPA, increased the mean of the percent cells with more than 10 γ-H2AX foci, however, without a clear dose-effect relationship. Glyphosate was found to induce statistically significantly a protein involved in DNA repair, p-Ku80, at 5 µM without a dose-effect relationship (when measured as median OD).

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because glyphosate as a test chemical was not sufficiently characterized and the effect found on an indicator of DNA double strand breaks was not concentration related and occurred at concentrations that were much lower than the systemic concentrations (approx. 300 µM) of regulatory *in vivo* MN tests at 2000 mg/kg bw which were negative.

Assessment and conclusion by RMS:

Reliability criteria for *in vitro* toxicology studies

Publication: Suárez -Larios *et al.*, 2017	**Criteria**	**Comments**

	met? Y/N/?	
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y?	No guideline study for genotoxicity
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	N	Only source reported: Sigma-Aldrich Mexico.
Only glyphosate acid or one of its salts is the tested substance	Y	
AMPA is the tested substance	Y	
Study		
Test system clearly and completely described	Y	
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	N	Not applied
Test concentrations in physiologically acceptable range (< 1 mM)	Y	Up to 50 µM
Cytotoxicity tests reported	Y	
Positive and negative controls	Y	
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	Y	SS increase at 2 concentrations but no dose effect relationship for γ-H2AX foci. SS increase of P-Ku80 at 5 µM but no dose-effect relationship in treated cultures (measured as median OD)
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	Results of this study are not corroborated by in vivo MN studies with much higher blood concentrations (approx. 300µM).
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because glyphosate as a test chemical was not sufficiently characterized and the effect found on an indicator of DNA double strand breaks was not concentration related and occurred at concentrations that were much lower than the systemic concentrations (approx. 300 µM) of regulatory *in vivo* MN tests at 2000 mg/kg bw which were negative.		

1. Information on the study

Data point:	CA 5.4/010
Report author	Townsend, M. *et al.*

Report year	2017
Report title	Evaluation of various glyphosate concentrations on DNA damage in human Raji cells and its impact on cytotoxicity
Document No	doi.org/10.1016/j.yrtph.2017.02.002 E-ISSN: 1096-0295
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No, not conducted under GLP/ Officially recognised testing facilities.
Acceptability/Reliability:	Yes/Reliable without restrictions

2. Full summary of the study according to OECD format

he DNA damage and cytotoxicity of various glyphosate concentrations on human cells was studied to evaluate DNA damaging potential. Utilizing human Raji cells, DNA damage was quantified using the comet assay, while cytotoxicity was further analyzed using MTT viability assays. Several glyphosate concentrations were assessed, ranging from 15 mM to 0.1 μM. It was found that glyphosate treatment is lethal to Raji cells at concentrations above 10 mM, yet has no cytotoxic effects at concentrations at or below 100 μM. Treatment concentrations of 1 mM and 5 mM induce statistically significant DNA damage to Raji cells following 30-60 min of treatment, however, cells show a slow recovery from initial damage and cell viability is unaffected after 2 h. At these same concentrations, cells treated with additional compound did not recover and maintained high levels of DNA damage. While the cytotoxicity of glyphosate appears to be minimal for physiologically relevant concentrations, the compound has a definitive cytotoxic nature in human cells at high concentrations.

Materials and Methods

Chemicals and reagents - Glyphosate (95 % w/w purity) was obtained from Sigma-Aldrich, Inc. (Milwaukee, WI).

Cell culture - Burkitt's Lymphoma (Raji) cells (ATCC CCL-86) were obtained from American Type Culture Collection (ATCC) and cultured according to ATCC recommendations. Cells were cultured in RPMI 1640 and supplemented with 10 % FBS and 2 mM L-glutamine. Media were replaced every 48 hours and the cells used for testing were placed in exponential growth with a minimum viability of 95 %. Raji cells were selected for this study because the replication time is 18 hours allowing the assays to cover the entire cell cycle.

Cell viability assay - For use in the MTT viability assay, glyphosate was diluted in cell culture RPMI media to the final test concentrations and stored at 4° C. Glyphosate stock solutions were diluted in RPMI media to their final test concentrations. Raji cells were incubated for 24 hours in a 96-well plate at 37° C and 5 % CO2. Afterwards, MTT reagent was added to each well. Following 3 hours of incubation, 100 μL of DMSO detergent was added to each well and incubated for 2 hours at 4 oC, and cytotoxicity evaluated at 570 nm absorbance.

Alkaline comet assay - Raji cells were incubated with either hydrogen peroxide as the positive control, PBS as the negative control, or glyphosate. The concentration and time points varied depending on the experimental run. The time intervals tested included 10, 20, 30, 40, 50, 60, 75, 90, 105, and 120 minutes. The concentrations of glyphosate used at each of the time points were 0.1, 1, 10, and 100 μM, and 1, 5, 10, and 15 mM. 200,000 cells per 100 μL were incubated with positive and negative controls and several concentrations of glyphosate at 37 ºC. Once treated, the cells were washed with PBS and suspended at 200,000 cells per 100 μL of PBS for the conduct of the comet assay. The test samples were mixed with

low melting point agarose and layered on double frosted microscope slides and placed in alkaline lysis buffer for 60 minutes, rinsed with purified water and then placed in alkaline electrophoresis buffer for 20 minutes and submitted to electrophoresis. After fixation and drying, the slides were stained with propidium iodide and imaged using a Zeiss Axioscope fluorescence microscope. All comets were scored using TriTek CometScore Freeware v1.5. Every experimental run tested a single concentration for multiple time points. Each time point contained a minimum of two slides as replicas. Approximately 50 comets were analyzed per slide, totaling 100 comets per time point and per treatment concentration. Each concentration was replicated multiple times to ensure consistency. Comet assay results are reported as tail moment which is defined as the product of the tail length and the percentage of DNA in the tail. A similar protocol was followed to test the effects of secondary glyphosate exposure at 1 mM and 5 mM by adding 200 µL glyphosate solution to the cells after 60 minutes of initial treatment, while 200 µL of PBS was added to the negative control.

Statistical analysis - Relationships between exposure time and tail moment were modeled statistically using a natural spline to account for nonlinearity. The number of knots was selected based on Akaike Information Criteria (AIC) and parameters were estimated using least squares. P-values of <0.05 were considered statistically significant.

Results

MTT analysis of Raji cells exposed to various glyphosate concentrations for 24 hours indicated a significant loss of cell viability at 10 and 15 mM. Glyphosate concentrations of 5 mM and lower did not have a significant effect on viability when compared to the negative control.

Comet assay analysis of Raji cells exposed to 10 and 15 mM glyphosate indicated severe DNA damage and cell death soon after exposure. Within 30 min of treatment, all cells had adopted an apoptotic profile which is characterized by a loss of a defined comet head and a large, fragmented DNA tail. Tail moments were significant after just 10 minutes of glyphosate exposure. Raji cells exposed to glyphosate concentrations of 1 and 5 mM produced statistically significant DNA damage after 40 minutes of treatment. Tail moments reached a maximum after 60 and 80 minutes of treatment at 5 mM and 1 mM, respectively. A steady decrease in tail moment was observed in later time points and the cells were able to recover to full viability after 120 minutes of treatment. Cells exposed to 10 and 100 µM of glyphosate did not show statistically significant DNA damage, and the cells retained full viability throughout 120 minutes of treatment. Raji cells exposed to physiologically relevant concentrations of glyphosate for 120 minutes did not experience any significant DNA damage. Following treatment with 10 µM glyphosate, Raji cells showed no signs of DNA damage and the 'head' of the cells stayed intact throughout the 2-hour exposure time. To further investigate the comet results at 1 and 5 mM, cells were treated again with glyphosate at these concentrations one hour after initial treatment. A significant difference could be observed between cells receiving only primary treatment and cells receiving the additional treatment. Raji cells exposed twice to glyphosate did not show the same pattern of recovery, with tail moments reaching levels above 20 for 1 mM and 25 for 5 mM. Cells with only primary exposure to glyphosate showed a decrease in DNA damage, with tail moments dropping from 15 to 5.8 for 1 mM and 23.67 to 6.74 for 5 mM.

Discussion

The results show that the DNA damaging and cytotoxic potential of glyphosate is related to exposure length and treatment concentration. Glyphosate only induced significant DNA damage at concentrations several orders of magnitude larger than those attainable *in vivo*. The data support the established evidence that glyphosate is not genotoxic in human cells at physiologically relevant concentrations. While these data ultimately support glyphosate's classification as a potential carcinogen, they suggest that its effects are negligible when exposure is minimal. Furthermore, study results obtained at 1 mM and 5 mM suggest that cells initially damaged by glyphosate may have the ability to repair and regain viability after single exposure. The results show also that the extent of DNA damage changed drastically across different incubation time points. For example, incubation at 1 mM for one hour produced severe DNA damage whereas no DNA damage was evident after 2 hours at the same concentration. If cells had only been evaluated at this time point, results would suggest that there was no cytotoxic activity and the initial DNA

damaging event would be missed. Cytotoxic activity might also be underestimated by standard viability assays in which the DNA damage is insufficient to induce cell death. Considering multiple time points made it possible to observe both DNA damage as well as the ensuing recovery.

Conclusion
Exposure of human cells to glyphosate produces minimal cytotoxicity and DNA damage at concentrations at or above 1 mM.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The purpose of this study was to investigate the concentration and time dependent DNA damaging potential of glyphosate in Burkitt's B Cell Lymphoma (Raji) cells using the comet assay and MTT viability assay. The cells were exposed to glyphosate concentrations ranging from 0.1 µM to 15 mM and resulting DNA damage and loss of cell viability were measured after various lengths of exposure. DNA damage could only be observed at 1mM and higher which are concentrations that cannot be attained in vivo. The DNA damage seen at 1 and 5 mM reached its maximum between 60 and 80 minutes of incubation which returned to control values thereafter. To reach 1 mM of systemic concentration *in vivo* experimental animals have to be treated orally with glyphosate at dose levels that are much higher than those used in long term carcinogenicity studies which showed no carcinogenic effect of glyphosate.

This publication is considered relevant for the risk assessment of glyphosate and reliable without restrictions because it complies with most of the reliability criteria for *in vitro* testing although no historical control data were reported. The significance for the risk assessment of glyphosate is limited because DNA damage has only been demonstrated at concentrations of glyphosate that cannot be attained in *in vivo* test systems.

Assessment and conclusion by RMS:

Reliability criteria for *in vitro* toxicology studies

Publication: Townsend *et al.*, 2017	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity of 95 %. Source: not reported.
Only glyphosate acid or one of its salts is the tested substance	Y	
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	Y	
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	N	
Test concentrations in physiologically acceptable range (< 1 mM)	Y/N	0.1 µM to 15 mM for alkaline comet assay
Cytotoxicity tests reported	Y	

Positive and negative controls	Y	
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	Y	Human cell exposure to glyphosate has minimal cytotoxicity and DNA damage at concentrations at or below 100 µM. Only effects found beyond 1 mM.
Overall assessment		
Reliable without restrictions	Y	
Reliable with restrictions		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate and reliable without restrictions because it complies with most of the reliability criteria for in vitro testing although no historical control data were reported.		

1. Information on the study

Data point:	CA 5.4/011
Report author	Roustan, A. *et al.*
Report year	2014
Report title	Genotoxicity of mixtures of glyphosate and atrazine and their environmental transformation products before and after photoactivation
Document No	E-ISSN: 1879-1298
Guidelines followed in study	OECD Test Guideline 487
Deviations from current test guideline (OECD 487 (2016))	None
GLP/Officially recognised testing facilities	No, not conducted under GLP/ Officially recognised testing facilities.
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary of the study according to OECD format

Abstract
The photo-inducible cytogenetic toxicity of glyphosate, aminomethyl phosphoric acid (AMPA), and their various mixtures was assessed by the in vitro micronucleus assay on CHO-K1 cells. Results demonstrated that the cytogenetic potentials of pesticides greatly depended on their physico-chemical environment. The mixture made with the four pesticides exhibited the most potent cytogenetic toxicity, which was 20-fold higher than those of the most active compound AMPA, and 100-fold increased after light-irradiation. Intracellular ROS assessment suggested the involvement of oxidative stress in the genotoxic impact of pesticides and pesticide mixtures.

Materials and methods
Chemicals - Glyphosate and AMPA were purchased from Sigma-Aldrich Chemical Company, St Quentin-

Fallavier, France.

Cell culture - Experiments were performed in CHO-K1 cells (ATCC-LGC Standards Sarl, Molsheim, France). CHO-K1 cells were maintained in McCoy's 5A medium supplemented with 10 % bovine calf serum, 1 mM glutamine, and 100 U/mL-10 µg/mL penicillin-streptomycin. Cells were incubated at 37 °C in a humidified atmosphere containing 5 % CO_2.

Metabolic activation mixture (S9-mix) - The metabolic activation system used was a 9000g centrifuged supernatant (S9) of a 10 % liver homogenate prepared from male Sprague-Dawley rats treated with a single injection of Aroclor 1254 (500 mg/kg bw), 5 days before sacrifice. The protein concentration in the S9 homogenate was 26 mg/mL. For the micronucleus assay, the S9-mix contained 10 % S9, 5 mM glucose-6-phosphate, 4 mM NADP, 33 mM KCl and 8 mM $MgCl_2$ diluted in PBS.

Photoactivation - To reproduce photoinduction during exposure to glyphosate and AMPA in the environment, the micronucleus assay was performed with a photoactivation procedure. Irradiation of cell cultures was carried out 1 hour after the addition of test compound using a solar simulator Suntest CPS+ apparatus equipped with a xenon arc lamp (1,100 W), a special glass filter restricting transmission of light below 290 nm and a near IR-blocking filter. The irradiance for the photoactivation was fixed at 750 W/m^2 throughout testing. The combined light dose was 4.5 J/cm^2 for one minute irradiation (0.03 J/cm^2 of UVB, 0.41 J/cm^2 of UVA and 4.06 J/cm^2 of visible light). This irradiation dose is representative of a 1-3 minute period of solar exposure during a clear summer day in the United Kingdom. The temperature of the samples was kept at 4 °C. UVA-visible light (320-800 nm) was obtained using the solar ID65 filter plus a window glass filter.

Micronucleus assay - A total of 50,000 CHO-K1 cells was plated in chamber slides and incubated for 24 hours at 37 °C in a humidified atmosphere containing 5 % CO_2. Various concentrations of glyphosate (5, 10, 50, and 100 µg/mL) and AMPA (0.005, 0.01, 0.05, and 0.1 µg/mL without S9-mix; 0.1, 0.5, 1, and 5 µg/mL with S9-mix; 0.00005, 0.0001, 0.0005, and 0.001 µg/mL with light irradiation) were incorporated into duplicate CHO-K1 cell cultures. To determine the background DNA-damage levels in CHO cells in the dark and with light irradiation 2 negative controls were added for glyphosate and AMPA: culture medium and PBS. Mitomycine C (0.06 µg/mL) without S9-mix and benzo[a]pyrene (5 µg/mL) with S9-mix were selected as positive controls. After 3 hours of exposure, cells were rinsed with PBS and incubated in fresh medium containing cytochalasin B (3 µg/mL) for an additional 24 hours to stop cytokinesis. At the end of the incubation period, cells were rinsed twice with PBS and fixed with methanol. The slides were air dried and stained with 5 % Giemsa stain in Milli-Q water for 15 minutes. The Cytokinesis Blocked Proliferation Index (CBPI) was used to select adequate concentrations for the assessment of micronuclei. CBPI was determined by scoring the number of mononucleated (M1), binucleated (M2), and trinucleated (M3) cells among 500 Giemsa-stained cells with well-preserved cytoplasm: CBPI = [(M1) + (2 x M2) + (3 x M3)]/500. When a cytotoxic effect was observed, micronucleated cell rates were determined for concentrations inducing less than a 50 % decrease of CBPI. When no cytotoxic effect was observed, the maximal concentration assessed was 100 µg/mL. When a clastogenic/aneugenic activity was observed, 4 relevant concentrations were selected to obtain a dose-response relationship. A total of 2000 binucleated cells were examined for each concentration, and micronuclei were identified according to the morphological criteria previously defined.

Intracellular ROS analysis - Intracellular ROS was determined with the cell-based OxiSelect™ Intracellular ROS Assay Kit using 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA). After diffusion into the cell, this fluorescent probe is deacetylated by cellular esterases to non-fluorescent 2',7'-dichlorodihydrofluorescein (DCFH), which is rapidly oxidized to the highly fluorescent 2',7'-dichlorofluorescein (DCF) by ROS. A total of 50,000 cells were seeded in a black 96-well cell culture plate and incubated at 37 °C for 24 hours. Cells were rinsed 3 times with PBS and incubated in 100 µL of DCFH-DA/media solution at 37 °C for 60 minutes. Cells were subsequently rinsed with PBS and treated with glyphosate or AMPA. Experiments were performed in triplicate. The concentrations tested were the same as those assessed for the micronucleus assay. H_2O_2 (100 µM) was used as the positive control. After a 30-

minute incubation period, fluorescence was measured with a fluorometric plate reader at 480 nm and 530 nm.

Statistical analysis – Statistically significant differences between negative controls and treated samples were determined using the ^{2}test. The standard micronucleus assay was considered positive when a dose-response relationship could be established between the numbers of micronucleated cells and the concentrations of pesticide solutions, and when at least one concentration induced a significant increase of micronucleated cells as compared to the medium-only control culture. The dose-response relationships were calculated by nonlinear regression analysis with TableCurve2D®. Model significance was based on three criteria: (i) correlation coefficient r^2 being > 0.62; (ii) model probability (P) being < 0.05; (iii) error probability (PE) being > 0.05. The Minimal Clastogenic Concentration (MCC) was defined as the lowest concentration of test item (µg/mL) that induced a significant increase of micronucleated cells. The Cytogenetic Potency (CP) was defined as the slope of the dose–response curves. It was calculated by non-linear regression analysis with TableCurve2D®.
For the determination of ROS, the dose-response relationships were calculated by linear regression analysis with TableCurve2D®. Model significance was based on three criteria: (i) correlation coefficient r^2 being > 0.62; (ii) model probability (P) being < 0.05; (iii) error probability (PE) being > 0.05. The Oxidative Potency was defined as the slope of the dose-response curves and was calculated according to a standard curve obtained with various concentrations of fluorescent DCF.

Results
No statistically significant increase in the incidence of bi-micronucleated cells (BMC) was observed with glyphosate in the dark and without S9-mix at concentrations up to 100 µg/mL. In the presence of S9-mix a statistically significant and dose-related increase was noted from 10 µg/mL. The calculated minimal clastogenic concentration (MCC) was 5.8 µg/mL. With light irradiation a statistically significant increase in BMC was noted at the highest concentration of glyphosate tested (100 µg/mL) with a MCC of 93.4 µg/mL. Without S9-mix a statistically significant and dose-related increase in BMC was produced by AMPA from a concentration of 0.01 µg/mL with a MCC of 0.006 µg/mL. In the presence of S9-mix a statistically significant increase in BMC was seen with AMPA from 1 µg/mL with a MCC of 0.78 µg/mL. With light irradiation, the lowest test concentration of AMPA with a statistically significant increase in BMC was 0.0005 µg/mL with a MCC of 0.0004 µg/mL. The oxidative potency of H_2O_2, used as a positive control, was 333.3 nM DCF/µM^{-1} (data not shown). Only AMPA exerted an elevated oxidative effect (5.9 nM DCF/µg mL^{-1}), whereas the oxidative potency of glyphosate was very low.

Discussion
The results obtained in the present study confirmed the cytogenetic toxicity of glyphosate and AMPA *in vitro*. In the dark, glyphosate was not directly active in the absence of S9-mix, but it induced micronuclei in the presence of S9-mix. On the contrary, without S9-mix, AMPA displayed a direct cytogenetic effect which was 1,000 fold higher than that of its parent compound. The weak mutagenic activity of glyphosate with S9-mix could be explained by its weak metabolization yield *in vivo*. After sunlight irradiation, glyphosate was weakly active, whereas the cytogenetic effect of AMPA was about 20-fold increased. Since the photodegradation of glyphosate by UV light has been shown to produce AMPA, the photoinducible genotoxic potential of glyphosate could be partially explained by the formation of its metabolite AMPA in the intracellular environment. In the present study, cells were subjected to a very low irradiation that did not induce intrinsic DNA damage. The results of the present study implied more probably the involvement of a photoinduced oxidative stress, which transformed glyphosate and AMPA into photoactivated intermediates favoring interactions with cellular targets.

Conclusion
The results of this study demonstrated that light-irradiation, corresponding to a few minutes of solar exposure, greatly potentiated the cytogenetic impact of AMPA. *In vitro* experiments showed that the genotoxic impact of pesticides greatly depend on their physico-chemical environment,

3. Assessment and conclusion

Assessment and conclusion by applicant:

The cytogenetic effect of two herbicides (glyphosate and atrazine), their metabolites (AMPA and DEA), and mixtures thereof was investigated in CHO-K1 cells in the *in vitro* micronucleus test. Only the results of glyphosate and AMPA tested alone are reported and discussed in the summary. Glyphosate and AMPA were tested with and without metabolic activation and with light irradiation. Also the potency of glyphosate and AMPA to produce ROS was investigated. The concentrations tested ranged from 5 to 100 µg/mL for glyphosate and from 0.00005 to 5 µg/mL for AMPA. No statistically significant increase in the incidence of bi-micronucleated cells (BMC) was observed with glyphosate at concentrations up to 100 µg/mL in the dark and without metabolic activation. However, a statistically significant and dose-related increase in BMC was noted from 10 µg/mL in the presence of metabolic activation. With light irradiation a statistically significant increase in BMC was noted for glyphosate at a concentration of 100 µg/mL. AMPA produced a statistically significant and dose-related increase in BMC from a concentration of 0.01 µg/mL in the dark and without metabolic activation. With metabolic activation a statistically significant increase in BMC was seen with AMPA from 1 µg/mL. With light irradiation the lowest test concentration of AMPA with a statistically significant increase in BMC was 0.0005 µg/mL. Only AMPA was found to produce an elevated oxidative effect, whereas the oxidative potency of glyphosate was very low. The results of glyphosate in the *in vitro* micronucleus test with metabolic activation reported in this study are surprising since glyphosate is essentially unmetabolized *in vitro* in the presence of a rat liver S9 homogenate. Moreover, these results are not corroborated by regulatory *in vivo* micronucleus tests in the mouse dosed up to more than 2,000 mg/kg bw.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate and AMPA tested were not sufficiently characterized and no positive and control historical data were reported. The *in vitro* micronucleus test carried out was in compliance with OECD TG 487.

Assessment and conclusion by RMS:

Reliability criteria for *in vitro* toxicology studies

Publication: Roustan *et al*., 2014	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	Y	In vitro MN test compliant with OECD TG 487.
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity not reported. Source: Sigma-Aldrich Chemical Company, St Quentin-Fallavier, France.
Only glyphosate acid or one of its salts is the tested substance	N	Other pesticides (atrazine, desethyl atrazine (DEA)) were tested and mixtures thereof.
AMPA is the tested substance	Y	Tested alone and as mixtures with glyphosate, atrazine and DEA.

Test system clearly and completely described	Y	CHO-K1 cells.
Test conditions clearly and completely described	Y	CHO-K1 cell MN test.
Metabolic activation system clearly and completely described	Y	S9-mix.
Test concentrations in physiologically acceptable range (< 1 mM)	Y	From 5 to 100 µg/mL for glyphosate and from 0.00005 to 5 µg/mL for AMPA.
Cytotoxicity tests reported	Y	Cytokinesis Blocked Proliferation Index (CBPI) and incidence of bi-micronucleated cells (BMC).
Positive and negative controls	Y	
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Reliability not assignable		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate and AMPA tested were not sufficiently characterized and no positive and control historical data were reported. The *in vitro* micronucleus test carried out was in compliance with OECD TG 487.		

1. Information on the study

Data point:	CA 5.4/012
Report author	Mañas, F. *et al.*
Report year	2013
Report title	Oxidative stress and comet assay in tissues of mice administered glyphosate and AMPA in drinking water for 14 days
Document No	ISSN: 1666-0390
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No, not conducted under GLP/ Officially recognised testing facilities.
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary of the study according to OECD format

In this study the authors determined the levels of thiobarbituric acid reactive substances (TBARs); quantified superoxide dismutase (SOD) and catalase (CAT) activity in liver, kidney, lung and heart, and performed the comet assay in blood and liver of mice administered glyphosate (40 or 400 mg/kg/day) or AMPA (100 mg/kg/day) in drinking water for 14 days. Exposure to glyphosate 400 mg/kg induced a

statistically significant ($p < 0.05$) decrease of SOD activity in heart and an increase in CAT activity in kidney. In the comet assay there were statistically significant differences in all the treatments and tissues studied in comparison to control animals ($p \leq 0.01$). The major results of this study were that mice administered glyphosate or AMPA in drinking water for 14 days induced a significant increase in DNA damage in liver and blood but minor effects on oxidative stress parameters. DNA effects on liver and blood indicate that these compounds could be of concern in terms of their potential to damage the genetic material, and that oxidative stress does not seem to be the mechanism causing that effect.

Materials and Methods

Chemicals - Analytical grade glyphosate (96 % purity), and analytical grade AMPA, (99 % purity) were obtained from Sigma-Aldrich, Argentina.

Animals - Twenty-four Balb C mice of approximately 45 days of age were used in this study. Four groups of six animals each (a control group, 2 groups treated with glyphosate and a group treated with AMPA) were used for the determination of oxidative stress (TBARs, SOD and CAT) and DNA damage in the comet assay. During 7 days prior to the beginning of the study, the volume of water ingested per day was measured in every 2 animals per cage to determine the concentration of glyphosate and AMPA in the drinking water to be supplied to each group. Mice received approximately 40 or 400 mg/kg bw/day of glyphosate and 100 mg/kg bw/day of AMPA. The control group received drinking water without test compound. After 14 days of exposure, before sacrifice, peripheral blood was drawn from the tail vein to perform the comet assay. At necropsy, heart, lungs, liver, and kidneys were removed for the determination of TBARs, SOD and CAT and frozen at -80 ºC pending analysis. A part of the liver was homogenized in phosphate buffer (pH 7.4) immediately after excision for the conduct of the comet assay. All determinations were performed in triplicate.

Single cell gel electrophoresis assay in mouse blood and liver - The protocol followed the general guidelines proposed by Singh et al. (Exp. Cell Res., 175, 184-191, 1988) with minor modifications. The slides were fixed in absolute ethanol, stained with ethidium bromide and scored using fluorescence microscopy. Images of 100 "nucleoids" counted for each animal were captured with a camera attached to the fluorescence microscope and linked to the Comet Score 1.5 software. Tail moment (TM), percentage of DNA in tail (% of DNA) and tail length (TL) were used to estimate DNA damage (in arbitrary units).

TBARs, SOD and CAT determinations - Tissue homogenates (10 %) were prepared in chilled 0.05 M potassium phosphate buffer at pH 7.4. TBARs concentrations, expressed as nmol of malondialdehyde MDA/g of tissue, were measured spectrophotometrically at 532 nm in liver and kidney homogenates. TBARs concentrations were determined using a standard curve at different concentrations of MDA versus optical density, individually prepared for each tissue. Superoxide dismutase (SOD) activity was assessed spectrophotometrically in the supernatant of liver homogenates. One unit of enzymatic activity has been defined as the amount of enzyme capable of causing 50 % inhibition of auto-oxidation of epinephrine. Catalase (CAT) activity was measured at 240 nm by the decomposition of the H_2O_2.

Statistical analysis

Statistical analysis was performed using Prism software (PRISM, 1997). The Kolmogorov-Smirnov test was performed to verify if the results follow a normal distribution. ANOVA followed by the Dunnett's test, or the Kruskal-Wallis test followed by the Dunn's test were performed for data with and without normal distribution respectively.

Results

The volume of water ingested per animal/day did not show any statistically significant differences among the experimental groups throughout treatment. No statistically significant differences were found in liver, kidney, lung and heart for all oxidative stress parameters measured with the exception of a decrease in SOD in the heart and an increase in CAT in the kidney at a daily glyphosate dose of 400 mg/kg bw. A non-statistically significant increase in CAT was observed in the lung. There were no statistically significant changes in the concentrations of MDA/g of tissue at 40 and 400 mg/kg bw/day of glyphosate and at 100

mg/kg bw/day of AMPA. Tail intensity, tail length and tail moment were statistically significantly elevated in blood and liver of all dosed groups with the exception of tail intensity in liver at 40 mg/kg bw/day glyphosate.

Discussion and Conclusion

The presence of glyphosate or AMPA in drinking water did not affect water consumption. Statistically significant differences in oxidative stress (decrease in SOD activity in the heart and increase in CAT activity in the kidney) were only observed in mice treated with glyphosate at 400 mg/kg bw for 14 days. The increase in CAT activity in the lungs was not statistically significant. A non-statistically significant decrease in SOD activity was observed in all tissues of animals treated with 100 mg/kg bw AMPA but no effect of AMPA was found on CAT activity. Liver was the only tissue where no effects were recorded, neither in TBA reactive substances nor in SOD and CAT activity. In the comet assay of blood statistically significant differences from control were seen for glyphosate and AMPA in tail intensity, tail length and tail moment. Tail moment values in the comet assay were similar for both glyphosate and AMPA treatments. In this study, genotoxic changes were observed with glyphosate at 40 and 400 mg/kg bw/day and AMPA at 100 mg/kg bw/day. However, statistically significant changes in the levels of SOD and CAT especially in heart and kidney were only seen with glyphosate at 400 mg/kg bw/day. This suggests that the genotoxic effects of glyphosate and AMPA are much more important than the indicators of oxidative stress.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The objective of this study was to investigate the effect of glyphosate and AMPA on indicators of oxidative stress and DNA integrity in mice after oral exposure for 14 days via the drinking water. The results of this study indicate that no statistically significant differences have been found in liver, kidney, lung and heart for all oxidative stress parameters measured with the exception of a decrease in SOD activity in the heart and an increase in CAT activity in the kidney at a daily glyphosate dose of 400 mg/kg bw. There was an increase in CAT activity in the lung but this was not statistically significant and did not show a dose-effect relationship. A statistically significant increase in DNA damage parameters was observed for glyphosate and AMPA with the exception of tail intensity in the liver for glyphosate at 40 mg/kg bw/day. No clear dose-effect relationship was evident for DNA damage parameters in blood after treatment with glyphosate. A dose-effect relationship was present for tail length and tail moment in the liver.

This publication is considered relevant for glyphosate risk assessment but reliable with restrictions because the increased DNA damage seen (only 2 dose levels tested for glyphosate with too few animals) didn't show a dose-effect relationship in blood and occurred at dose levels (40 and 400 mg/kg bw/day) that are much lower than the 2000 mg/kg bw used in regulatory *in vivo* MN tests in the mouse with negative results.

Assessment and conclusion by RMS:

Reliability criteria for *in vivo* toxicology studies

Publication: Manas et al., 2013	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	2 dose groups for glyphosate, one dose group for AMPA
Study performed according to GLP	N	

Study completely described and conducted following scientifically acceptable standards	Y?	Very old determination methods applied
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity of 96 %. Source: Sigma-Aldrich, Argentina.
Only glyphosate acid or one of its salts is the tested substance	Y	
AMPA is the tested substance	Y	Purity of 99 %. Source: Sigma-Aldrich, Argentina.
Study		
Test species clearly and completely described	Y	
Test conditions clearly and completely described	Y	
Route and mode of administration described	Y	Exposure via drinking water
Dose levels reported	Y	
Number of animals used per dose level reported	Y	
Method of analysis described for analysis test media	N	
Validation of the analytical method	N	
Analytical verifications of test media	N	
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical control data of the laboratory reported	N	
Dose-effect relationship reported		Only 2 dose levels used. DNA damage (comet) at 40 and 400 mg/kg bw/day (no dose-effect relationship) not confirmed by regulatory genotoxicity (MN) studies up to 2000 mg/kg bw/day
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Not reliable		
This publication is considered relevant for glyphosate risk assessment but reliable with restrictions because the increased DNA damage seen (only 2 dose levels tested for glyphosate with too few animals) didn't show a dose-effect relationship in blood and occurred at dose levels (40 and 400 mg/kg bw/day) that are much lower than the 2000 mg/kg bw used in regulatory in vivo MN tests in the mouse with negative results.		

1. Information on the study

Data point:	CA 5.4/013
Report author	Koller, V. J. *et al.*
Report year	2012
Report title	Cytotoxic and DNA-damaging properties of glyphosate and Roundup in human-derived buccal epithelial cells

Document No	DOI 10.1007/s00204-012-0804-8 E-ISSN: 1432-0738
Guidelines followed in study	SCGE assays were performed according to the guidelines described by Tice et al. (2000) and the CBMN Cytome assay according to Fenech (2007). The corresponding Guidance Documents are OECD 489 and OECD 487
Deviations from current test guideline	Cell type (buccal cells)
GLP/Officially recognised testing facilities	No, not conducted under GLP/ Officially recognised testing facilities.
Acceptability/Reliability:	Yes/Reliable without restrictions

2. Full summary of the study according to OECD format

Aim of this study was to investigate the cytotoxic and genotoxic properties of glyphosate (G) and Roundup (R) (UltraMax) in a buccal epithelial cell line (TR146), as workers are exposed via inhalation to the herbicide. R induced acute cytotoxic effects at concentrations > 40 mg/l after 20 min, which were due to membrane damage and impairment of mitochondrial functions. With G, increased release of extracellular lactate dehydrogenase indicative for membrane damage was observed at doses > 80 mg/l. Both G and R induced DNA migration in single-cell gel electrophoresis assays at doses > 20 mg/l. Furthermore, an increase of nuclear aberrations that reflect DNA damage was observed. The frequencies of micronuclei and nuclear buds were elevated after 20-min exposure to 10 -20 mg/l, while nucleoplasmatic bridges were only enhanced by R at the highest dose (20 mg/l). R was under all conditions more active than its active principle (G).

Materials and Methods

Chemicals - Glyphosate (purity 95 % w/w) and Roundup (Roundup Ultra Max, 450 g/L glyphosate acid) were obtained from Monsanto Europe S.A.

Storage and cultivation of the indicator cells - The human cell line TR146 was cultured under standard conditions in DMEM supplemented with 10 % heat-inactivated fetal calf serum. The medium was changed every 2–3 days. When the cultures reached confluency, the cells were washed with Dulbecco's PBS, detached with TrypLE Express, centrifuged, and sub-cultured. TR146 cells express ultrastructural characteristics of normal human buccal epithelial cells, e.g. intermediate filaments, microvilli-like processes, and lack of complete keratinization.

Cytotoxicity assays - For cytotoxicity experiments, $5x10^4$ indicator cells were seeded in 96-well plates and exposed to concentrations of glyphosate and Roundup ranging from 0 to 200 mg glyphosate equivalents/L for 20 minutes. Toxicity due to damage of the cell membrane was determined with the extracellular LDHe assay that is based on the measurement of the oxidation of NADH to NAD. Alterations of mitochondrial functions were studied in XTT (2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenyl-amino)carbonyl]-2H-tetrazolium hydroxide) assays that measure succinate dehydrogenase activity of viable cells. The SRB test was used to monitor total protein synthesis as a marker of cell proliferation. SRB binds to cellular proteins and can be quantified after solubilization. The neutral red assay was conducted to evaluate membrane integrity and lysosomal activity of the cells. All experiments are based on spectrophotometric measurements and were evaluated with an automated microplate reader. In all experiments, three measurements were performed per dose and repeated at least once.

Single-cell gel electrophoresis (SCGE) assay - SCGE assays were performed to determine the effect of glyphosate and Roundup on DNA stability. The concentrations of glyphosate and Roundup used in this assay ranged from 0 to 2000 mg glyphosate equivalents/L. TR146 cells were seeded in 24-well plates and allowed to attach. Thereafter, the culture medium was replaced by 400 µL of different concentrations of glyphosate dissolved in serum-free medium. After incubation in the dark at 37ºC and 5 % CO_2 for 20 minutes, the medium was discarded and the cells washed twice with DPBS and detached. After two washing

steps with DMEM and centrifugation, the pellets were resuspended in low melting point agarose, spread onto slides precoated with normal melting point agarose and lysed in the dark at 4°C for at least 1 hour. After 20 minutes unwinding in an alkaline electrophoresis solution electrophoresis was carried out. Air-dried slides were stained with ethidium bromide and the percentage of tail DNA was measured using a computer-aided image analysis system (Comet IV, Perceptive Instruments Ltd., Haverhill, UK). For each experimental point, 3 cultures were made in parallel and from each culture, one slide was prepared and 50 randomly distributed cells evaluated.

Cytokinesis-block micronucleus (CBMN) cytome assay - The CBMN assay using the cytochalasin B technique was performed to determine the effect of glyphosate and Roundup on chromosome integrity. The concentrations of glyphosate and Roundup used in this assay ranged from 0 to 20 mg glyphosate eq./L. Into 6-well plates, 4.5 x 10^5 cells were seeded and allowed to attach overnight. After treatment for 20 minutes with the test compounds or 100 µg/mL methyl methanesulfonate as the positive control, the cells were washed twice with PBS and cultured in DMEM containing 10 % FCS and cytochalasin B for 48 hours. Subsequently, the cells were washed twice with DPBS and harvested. The slides were made by cytocentrifugation and subsequently air-dried, fixed, and stained with Diff Quick stain. The total number of micronuclei (MNi) in binucleated cells (BN) as well as the number of binucleated cells with micronuclei (BN-MNi), nuclear buds (NB), nucleoplasmic bridges (NPB), and apoptotic and
necrotic cells was determined. Cells that divided after addition of cytochalasin B were recognized as binucleated. The nuclear division index (NDI) was determined in 500 cells. For each experimental point, TR146 cultures were prepared in triplicate. From each culture, >1,000 binucleated cells were evaluated under 400-fold magnification (Nikon Photophot-FXA, Tokyo, Japan).

Statistical analysis - Data analyses of the cytotoxicity and SCGE assays were performed with the GraphPad Prism 5 Project software system. Results are reported as means ± standard deviations (SD). The results were analyzed using one-way ANOVA and Dunnett's test and p values ≤0.05 were considered as statistically significant. The chi-square test with Yate's correction was used for the evaluation of the CBMN experiments and p values ≤0.05 were considered statistically significant.

Results

Cytotoxicity - Clear differences have been found between the effects of glyphosate and Roundup. Roundup was in all cases more cytotoxic than the active ingredient. In the LDHe test Roundup NADH consumption was statistically significantly increased from 10 mg glyphosate eq./L on whereas this was the case with glyphosate from 80 mg/L. In the XTT test, a statistically significant decrease in cellular integrity was seen with Roundup from concentrations of 40 mg glyphosate eq./L on while there was no effect with glyphosate up to 200 mg/L. In the SRB test there was a statistically significant decrease in cellular integrity from 100 mg glyphosate eq./L on whereas there was no effect with glyphosate up to 200 mg/L. The same difference in cytotoxicity was evident in the NR assay where Roundup produced a statistically significant decrease in cell integrity from 100 mg glyphosate eq./L on whereas there was no effect with glyphosate. The LC_{50} values for the cytotoxicity of Roundup were about 100 mg glyphosate eq./L for the XXT test, 140 mg/L for the NR assay and 150 mg/L for the SRB assay.

SCGE assay - In the SCGE assay, glyphosate produced at statistically significant increase in tail intensity from 20 mg/L on or without a dose-effect relationship from 40 to 2,000 mg/L. A statistically significant and dose dependent increase in tail intensity was observed with Roundup from 20 mg glyphosate eq./L up to 200 mg glyphosate eq./L, a dose level with 0 % cell integrity.

CBMN assay - The endpoints recorded in this assay were the frequency of binucleated (BN) cells with micronuclei (MNi), the total number of MNi, nuclear buds (NB) and nucleoplasmic bridges (NPB). After addition of cytochalasin B for 48 h, more than 75 % of the cells were binucleated (BN) and the nuclear division index (NDI) in untreated cultures was 1.89 ± 0.09. Treatment of the cells with 100 µg/mL of the positive control MMS for 20 minutes produced a significant induction of BN cells with MNi, total number of MNi, NB and NPB. Exposure of the cells to glyphosate and Roundup at 10, 15 and 20 mg glyphosate eq./L for 20 minutes led to a statistically significant and dose-dependent increase of the frequency of MN,

BN-MN, NB and NPB. The number of necrotic cells was statistically significantly increased for glyphosate and Roundup at 20 mg/L. The number of apoptotic cells was statistically significantly increased with glyphosate at 20 mg/L but not with Roundup.

Discussion

The results show that Roundup but not glyphosate causes pronounced cytotoxic effects in human-derived buccal epithelial cells. The genotoxicity tests show that glyphosate as well as its formulation induce DNA strand breaks as well as nuclear anomalies that reflect DNA instability including chromosomal damage. Glyphosate did not induce effects in the NR, SRB, and in the XTT assay up to concentrations of 200 mg/L, while a clear effect was seen in the LHDe assay from 80 mg/L on. On the contrary, statistically significant cytotoxic effects were observed with Roundup in all four assays, and significant changes were seen in the LDHe assay from 10 mg glyphosate eq./L and in the XTT test from 40 mg glyphosate eq./L on. Comparisons of the sensitivity of the different toxicity tests indicate that Roundup causes membrane damage at lower concentrations than the inhibition of mitochondrial functions in embryonic and placental-derived cells (JEG3), while umbilical vein cord endothelial cells (HUVEC) were equally sensitive to both endpoints. High concentrations of glyphosate affected in these experiments primarily mitochondrial functions while no effects were seen in an assay detecting changes of cell membrane functions.

The results of the SCGE assays show that glyphosate as well as Roundup induce comet formation under alkaline conditions. The effects increased as a function of the exposure and DNA migration was also observed under conditions that did not affect the cellular integrity. Also in earlier investigations with human-derived cells, SCGE assays were used to monitor the DNA-damaging effects of glyphosate. Significant induction of DNA migration was seen in human hepatoma cells (HepG2) with Roundup 400 with glyphosate levels of more than 5 mg/L after 24 hours of treatment. Other cell lines such as normal human fibroblasts (GM38) and the human fibrosarcoma line HT1080 were less sensitive in long-term (72 h) exposure studies. Taken together, these effects show that distinct differences exist in the sensitivity of cells from different organs and indicate that drug-metabolizing enzymes, which are represented in the enzyme activation mix and in HepG2 cells, increase the DNA-damaging properties of glyphosate. The most relevant finding of the present study is the observation of a statistically significant, dose-dependent induction of MNi, BN-MNi, and NB reflecting the genomic damage by glyphosate and its formulation. The most sensitive endpoint was MNi induction. Treatment of the cells with highest level (20 mg glyphosate eq./L) of Roundup caused a 3-fold increase over the background. A weaker effect was seen with the corresponding concentration of glyphosate. NPB was a less responsive endpoint with a statistically significant increase with glyphosate and Roundup at all dose levels. These results indicate that the damage seen in the SCGE assays is not completely repaired but leads to persisting alterations of the genetic material. MNi reflect numerical as well as structural chromosomal aberrations, while NB are formed as a consequence of gene amplification or expulsion of intact chromosomes or fragments; NPB are caused by formation of dicentric chromosomes.

The findings of the present study suggest that buccal epithelial cells are more sensitive toward the cytotoxic and DNA-damaging effects of glyphosate and Roundup than cells from the hematopoietic system. The Roundup formulation that was tested contains 450 g/L of glyphosate and should be diluted according to the instructions of the manufacturer to 1–3 % before use (final concentration 4,500–13,500 mg/L). The fact that we found significant acute and genotoxic effects at levels between 10 and 20 mg/L after 20 min indicates that short contact with a 225–1,350-fold dilution of the formulation may cause adverse effects in cells from the oral cavity (and possibly also in other respiratory epithelia).

3. Assessment and conclusion

Assessment and conclusion by applicant:

The objective of the present study was to find out whether exposure of human-derived buccal epithelial TR146 cells to glyphosate and Roundup causes adverse effects. In cytotoxicity experiments, four different endpoints were used, which reflect different modes of action. To assess the effect of glyphosate and Roundup on DNA stability, single cell gel electrophoresis assays (SCGE) were conducted under

standard alkaline conditions reflecting the formation of single- and double-strand breaks. Additionally cytokinesis-block MN cytome assays have been conducted in which different nuclear anomalies were measured. This study demonstrated that there is a big difference in cytotoxicity between glyphosate and Roundup. This is not surprising since the surfactants present in glyphosate formulations decrease the integrity of cell and mitochondrial membranes causing toxicity and ensuing DNA instability. Glyphosate was found to significantly increase tail intensity as of 20 mg/L but without any further increase with dose from 40 to 2000 mg/L. Roundup increased in a dose dependent manner the tail intensity from 20 mg glyphosate eq./L up to 200 mg glyphosate eq./L with increasing cytotoxicity and 0 % cell integrity at 200 glyphosate eq. mg/L. This indicates that there is a relationship between the cytoxicity of Roundup and DNA instability. This study has demonstrated a greater sensitivity of buccal epithelial cells for glyphosate and its formulations than hematopoietic cells where no effects have been noted in *in vivo* MN tests with doses up to 2,000 mg/kg bw. Since there is no direct exposure of the buccal epithelium with the Roundup formulation (unless it is swallowed) during application and the inhalation of aerosol of the spray dilution during application is negligible (Jauhiainen A *et al*. (1991) Am. Ind. Hyg. Assoc. J. 52, 61–64) the likelihood of DNA damage in epithelial cells of the GI and the respiratory tract remains very low.

This publication is considered relevant for the risk assessment of glyphosate and reliable with restrictions. Although it complies with most of the reliability criteria of an *in vitro* toxicology study, no blinded scoring of coded slides is reported. Also, concentrations with positive findings (20-2000 mg/L) noted significant effects on necrosis and apoptosis markers in parallel experiments at the low dose of 20 mg/mL.

Assessment and conclusion by RMS:

Reliability criteria for *in vitro* toxicology studies

Publication: Koller *et al.*, 2012.	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity of 95 %. Source: Monsanto Europe S.A.
Only glyphosate acid or one of its salts is the tested substance	Y	Also formulation (Roundup Ultra Max) was tested
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	Y	
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	NA	
Test concentrations in physiologically acceptable range (< 1 mM)	Y	10-1000 µg/mL
Cytotoxicity tests reported	Y	
Positive and negative controls	Y	

Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	Y	Roundup, but not glyphosate causes pronounced cytotoxic effects in human-derived buccal epithelial cells. The alkaline comet test results show that glyphosate as well as Roundup induce comet formation that reflect strand breaks and apurinic sites. 20 µg/mL glyphosate in Roundup caused a 3-fold increase over the background, with glyphosate alone a weaker effect was seen.
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate and reliable without restrictions because it complies with most of the reliability criteria of an in vitro toxicology study. Although it complies with most of the reliability criteria of an in vitro toxicology study, no blinded scoring of coded slides is reported. Also, concentrations with positive findings (20-2000 mg/L) noted significant effects on necrosis and apoptosis markers in parallel experiments at the low dose of 20 mg/mL.		

CA 5.5 Long-term toxicity and carcinogenicity

The long-term toxicity and carcinogenic potential of glyphosate has been assessed in rats and mice. The study results are summarised in Table 5.5-1 and Table 5.5-91. In long-term studies in rats and mice there was no evidence of carcinogenicity. Furthermore, in rats, there was no adverse effects on survival or clinical signs. Reductions in body weight gain, increases in alkaline phosphatase and liver weight changes, an increase in incidence of cataracts, inflammation of the gastric mucosa and histopathological changes in the salivary glands were observed sporadically across the studies previously reviewed. In the mouse, non-neoplastic treatment related effects were limited to high dose males in the [redacted] (1983) study and comprised of a reduction in body weight gain, hepatocyte hypertrophy and bladder epithelial hyperplasia.

In conclusion, there was no evidence that glyphosate acid is carcinogenic in any of the studies submitted.

The majority of the *in vitro* studies in the literature performed with glyphosate did not indicate a potential effect of glyphosate to induce tumorigenesis. In two out of five *in vitro* publications, glyphosate showed some activity in the underlying models, but without relevance for the risk assessment. Hypomethylation by

glyphosate was observed in MCF10A cells, but did not lead to tumour development as seen in other demethylating agents. In a study with Vk*MYC mice (multiple myeloma mouse model) and C57Bl/6 mice (wild-type, WT), glyphosate was reported to induce benign monoclonal gammopathy in WT mice as well as promoting myeloma progression in Vk*MYC mice. Due to several restrictions with regards guideline study requirements the results are considered supplementary only, as the data do not allow a direct risk assessment.

Long-term and carcinogenicity studies in the rat

The most recent rat dietary carcinogenicity study was conducted in 2009 by [redacted] (CA 5.5/001), there were no adverse treatment related effects at the highest dose tested. No indication of a carcinogenic effect was observed. The NOAEL for systemic toxicity was 1077 and 1382 mg/kg bw/day for males and females, respectively. Minor changes as reduced body weight gain (-9 %) in males only, transient increase in ALP activity or fatty infiltration of the bone marrow are not considered to fulfil the criteria for an adverse effect. Adverse effects include consistent effects with toxicologically significant changes affecting the function or morphology of a organ or producing serious changes to haematology or biochemistry parameters of the organism. Isolated elevation of alkaline phosphatase activity is considered to be related to an adaptation of the metabolism rather than to damage of (liver) cells. Liver damage is not to be considered the reason for the ALP increase, as no other plasma enzyme activities commonly linked to damage of this organ were changed nor was any pathological and histopathological finding reported. ALP is not only changed based on cell damage but also due to adaptive changes. The isolated increase of ALP activity of up to 160 % of the control values is not considered adverse against a normal adaptive background showing marked fluctuation. Okamura[19] *et al*. (2011) reported background levels of ALP in RccHan™:WIST rats at 8 to 32 weeks of age from 195 to 608 IU/L for males and 63 to 322 IU/L for females. In humans the reference range is between 70-175 U/I in males and 55-147 in females[20]. The conclusion on liver enzyme fluctuation is that, in the absence of correlating histopathological changes are considered adaptive and reversible rather than adverse.

In another combined chronic toxicity and carcinogenicity study ([redacted] 2001, CA 5.5/002), which was performed with glyphosate technical in rats receiving diets providing 0, 2000, 6000 or 20000 ppm glyphosate acid, the NOAEL was set at 6000 ppm equivalent to 361 and 437 mg/kg bw/day for males and females, respectively. The limiting effects were based on liver and kidney, prostatitis, periodontal inflammation, urinary acidosis and haematuria, which may be attributed to the acidity of the test substance. The salivary gland findings reported by [redacted] (1996, CA 5.5/006) were not confirmed although the study was run in the same laboratory employing rats of the same strain. It is concluded that Glyphosate technical is not carcinogenic. In a further study, by [redacted] (1997, CA 5.5/004), rats received diets providing 0, 3000, 10000 or 30000 ppm glyphosate. The NOAEL for toxicity is 3000 ppm equivalent to 104 and 115 mg/kg bw/day for males and females, respectively, based on histopathological and clinical effects of the caecum together with follicular hyperkeratosis and/or folliculitis/follicular abscess in the mid and high dose groups. No carcinogenic potential was observed.

A further 2-year study from [redacted] (1996, CA 5.5/005) in Wistar rats with up to 741 mg/kg bw/day glyphosate concluded that glyphosate was not carcinogenic to rats. A NOAEL of 741 mg/kg bw/day (combined sexes) was also established for systemic toxicity based on the lack of toxicity treatment related. Single and inconsistent changes of clinical chemistry parameters were considered not treatment-related. Histopathology did not reveal any treatment-related changes in any organ further supporting the conclusion that clinical chemistry parameters changes were not related to treatment-related adverse effects.

A 1-year toxicity study ([redacted] 1996, CA 5.5/006) was performed in rats with dietary doses of 0, 2000, 8000 and 20000 ppm glyphosate acid. Based on body weight and salivary gland effects at 20000 ppm, the

[19] Okamura T, Suzuki S, Ogawa T, *et al*. Background Data for General Toxicology Parameters in RccHan:WIST Rats at 8, 10, 19 and 32 Weeks of Age. *J Toxicol Pathol*. 2011;24(4):195-205. doi:10.1293/tox.24.195

[20] Thomas, L.: Labor und Diagnose. TH Books Verlagsgesellschaft 5. Auflage 2005: page 38

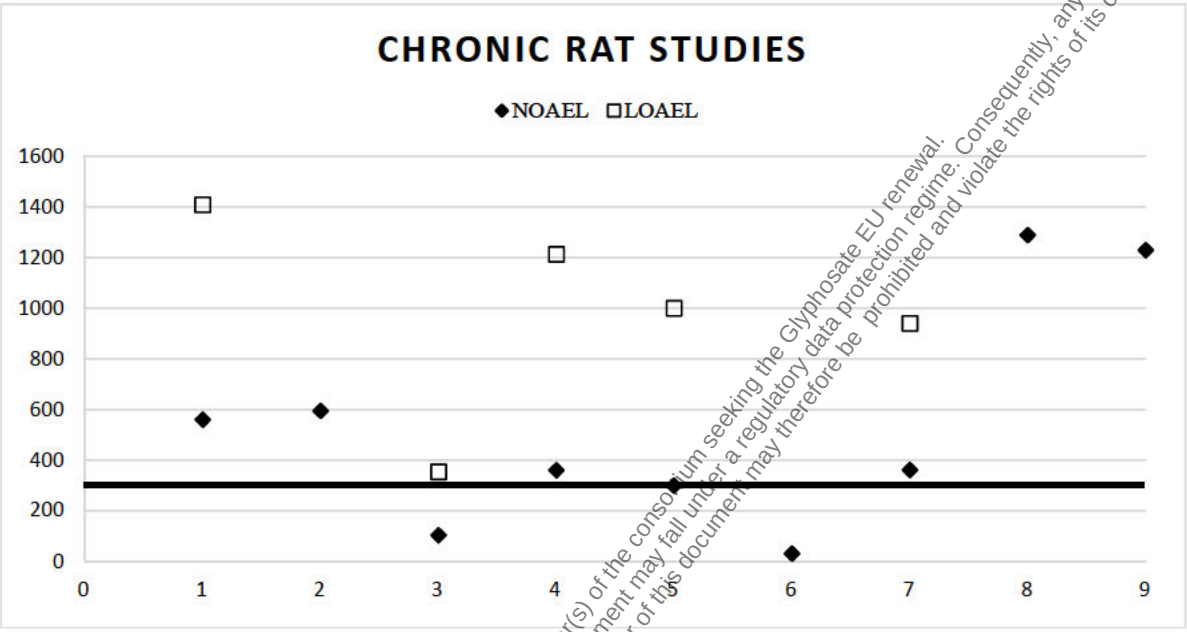
NOAEL for toxicity for glyphosate acid was 8000 ppm equivalent to 560 mg/kg bw/day in males and 671 mg/kg bw/day in females. There was no evidence of carcinogenicity.

In a 2-year oral diet study where Sprague-Dawley rats were continuously exposed to diets of up to 1000 mg/kg bw/day glyphosate ([redacted], 1993, CA 5.5/007-009) no carcinogenic potential was observed. Based on the study results and the lack of toxicological significance of the salivary gland findings, as well as a slight increase of plasma ALP observed at 300 mg/kg bw/day, the NOAEL in rats after chronic exposure to glyphosate technical for 104 weeks is considered to be 300 mg/kg bw/day. Cellular alteration of the parotid/mandibular salivary gland was considered an adaptive response based on the addition of glyphosate acid to the diet. This effect is in concordance with findings in other studies with glyphosate acid as well as with citric acid (see CA 5.8.2). Furthermore, the effect on the salivary glands was not clearly dose related but may be following a threshold effect potentially related to pH changes. This assumption is also supported by the fact that the effects on the salivary glands were already observed after 52-weeks and did not increase in incidence or severity. Increased ALP activity is not considered an adverse effect as the increase was slight, not accompanied by other liver enzymes or treatment-related histopathological changes in the liver. Furthermore, the liver weight was comparable between control and treatment groups.

[redacted] (1990, CA 5.5/010) concluded that glyphosate was not carcinogenic in Sprague-Dawley rats following continuous dietary exposure of up to 20000 ppm for 24 months (corresponding to 940 mg/kg bw/day in males and 1183 mg/kg bw/day in females). The NOAEL for toxicity is 8000 ppm (corresponding to 362 mg/kg bw/day in males and 437 mg/kg bw/day in females), based on reduced body weights in females and cataract lens changes in males at 20000 ppm.

The 2-year dietary rat study conducted by [redacted] (1997, CA 5.5/003) concluded that there were no adverse treatment related effects and the NOAEL was 30000 ppm equivalent to 1290/1740 mg/kg bw/day in males and females respectively. However, this study is considered invalid because no core information on the test substance was recorded and there were further deficiencies compared to the guideline. A further study was considered to be not acceptable due to too low dose levels and serious reporting deficiencies ([redacted] 1981, CA 5.5/011). In this study, glyphosate was not carcinogenic in Sprague-Dawley rats following continuous dietary exposure of up to 300 ppm for 26 months (corresponding to 31.49 mg/kg bw/day in males and 34.02 mg/kg bw/day in females). The NOAEL for toxicity is 300 ppm (corresponding to 31.49 mg/kg bw/day in males and 34.02 mg/kg bw/day in females), based on the absence of findings in body weight changes, haematology, clinical chemistry, organ weight data or histopathological examinations.

Figure 5.5.1-1: Chronic rat NOAEL and LOAEL values, showing an overall NOAEL of 300 mg/kg bw/day [1. [redacted] (1996); 2. [redacted] (1996); 3. [redacted] (1997); 4. [redacted] (2001); 5. [redacted] (1993); 5. [redacted] (1981); 7. [redacted] (1990); 8. [redacted] (1997); 9. [redacted] (2009)]



The overall chronic NOAEL in rats is 300 mg/kg bw/day. No test substance related carcinogenic potential in rats was concluded in all studies.

Salivary gland

Histological changes described as "cellular alteration" in the parotid and mandibular salivary glands and a higher organ weight of these glands were noted at 100 mg/kg bw/day and higher in the study from [redacted] (1993, CA 5.5/007-009). In addition similar changes have been observed in subchronic rat studies (see CA 5.3). In contrast, there are several chronic studies where no effects on the salivary glands were reported. Furthermore, in the more recent performed studies by [redacted] (2001) (same laboratory and rat strain as [redacted] 1996) and [redacted] (2009) salivary gland findings were not observed. These differences may be more or less pronounced depending on the rodent strain used or methodological differences. Additional studies were conducted to examine species sensitivity, reversibility of the effects and the hypothesis previously suggested in the WHO/FAO 2004 evaluation of glyphosate; that local irritation of the oral cavity by the organic acid mixed into diet may result in an adaptive salivary gland response (CA 5.8.2/002 and CA 5.8.2/003). Based on the outcome of these examinations the treatment-related pathological findings (increased salivary gland size and flow) can be considered as adaptive responses due to oral irritation from the ingestion of glyphosate acid in the diet.

Table 5.5-1 Summary of long-term toxicity and carcinogenicity studies in rats

Annex Point	Study	Study type	Substance(s)	Reference list-related category§	Result NOAEL (NOAEL)* (mg/kg bw/day)
CA 5.5/001	[redacted] 2009	2-year study, oral diet Rat, Wistar	Glyphosate technical (Batch: H05H016A,	Valid, Category 2a	1230 (1230)

			Purity: 95,7 %)		
CA 5.5/002	[redacted] 2001	2-year, oral diet Rat, Wistar Alpk: APfSD	Glyphosate acid (Batch: P30, Purity: 97.3 %)	Valid, Category 2a	361/437 ♂/♀ (1214/1498)
CA 5.5/003	[redacted] 1997	2-year, oral diet Rat, Sprague-Dawley	Glyphosate technical (Batch, Purity: not provided)	Invalid, Category 3b	1290/1740 ♂/♀ (1290/1740)
CA 5.5/004	[redacted], 1997	2-year, oral diet Rat, Sprague-Dawley	Glyphosate technical (Batch: T-941209, T-950308, Purity: 97.56 %, 94.61 %	Valid, Category 2a	104/115 ♂/♀ (1127/1247)
CA 5.5/005	[redacted] 1996	2-year, oral diet Rat, Wistar	Glyphosate technical (Batch: 60;046, Purity: 96.8 %; 96 %)	Valid, Category 2a	595.2/886.0 ♂/♀ 741 ♂+♀ (741 ♂+♀)
CA 5.5/006	[redacted], 1996	1-year, oral diet Rat, Wistar Alpk: APfSD	Glyphosate acid (Batch: P24, Purity: 95.6 %)	Valid, Category 2a	560/671 ♂/♀ (1409/1664)
CA 5.5/007-009	[redacted] 1993	2-year, oral diet Rat, Sprague-Dawley	Glyphosate technical (Batch: 229-JaK-5-1; 229-Jak-142-6, Purity: 98.9 %; 98.7 %)	Valid, Category 2a	300 (1000)
CA 5.5/010	[redacted], 1990	2-year, oral diet Rat, Sprague-Dawley	Glyphosate (Batch: XLH-264, Purity: 96.5 %)	Valid, Category 2a	362/457 ♂/♀ (940/1183)
CA 5.5/011	[redacted] 1981	26-month, oral diet Rat, Sprague-Dawley	Glyphosate technical (Batch: XHJ-64, Purity: 98.7 %)	Invalid, Category 3b	31/34 ♂/♀ 31/34

* NOAEL for carcinogenicity

§ The category describes the acceptability of the study within the AIR 5 submission

1. Information on the study

Data point:	CA 5.5/001
Report author	[redacted]
Report year	2009
Report title	Glyphosate Technical: Dietary Combined Chronic Toxicity/Carcinogenicity in the Rat
Report No	2060-0012
Document No	NA
Guidelines followed in study	OECD 453 (1981), JMAFF Guideline 2-1-16 (2005), US OPTTS 870.4300 (1996)

Deviations from current test guideline (OECD 453, 2018)	Yes, the appearance of the urine was not investigated; the organ weights of the epididymides, thyroid/parathyroids and the uterus were not determined; histopathological observations of the cervix, coagulating gland; lacrimal gland and the upper respiratory tract were not performed.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The chronic toxicity and carcinogenic potential of Glyphosate technical was assessed in a 24-month feeding study in 51 male and 51 female Wistar rats at dietary concentrations of 0, 1500, 5000 and 15000 ppm (equivalent to mean achieved dose levels of 0, 95.0, 316.9 and 1229.7 mg/kg bw/day) Glyphosate technical. To ensure that a received dose of 1000 mg/kg bw/day overall was achieved, the highest dose level was progressively increased to 24000 ppm. In addition, three satellite groups with 15 rats per sex each were included for interim sacrifice at the 12th month to study non-neoplastic histopathological changes.

Observations covered clinical signs, behavioural assessment, functional observations, body weight, food consumption, ophthalmology, haematology, clinical chemistry and urinalysis as well as organ weights, necropsy and histopathological examination.

The treatment-related findings of this study were elevations in plasma electrolyte values for both sexes at 18 months. Elevations in alkaline phosphatase activity were seen at 6, 12 and 18 months in males only. Histopathological examinations revealed at 15000 ppm a significant difference in the site of mineral deposition within the kidneys compared with controls. There was a lower incidence of pelvic/papillary deposition and an increase in the corticomedullary deposition. At the same time there was a reduction in the incidence of renal pelvic hyperplasia; which is considered a consequence of decreased mineral deposition. An increase in severity of adipose infiltration into the bone marrow of males only was observed. This finding was not seen amongst high dose females but was seen at both 5000 and 1500 ppm group females.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate Technical
Description:	White crystalline solid
Lot/Batch #:	H05H016A
Purity:	95.7 % w/w
Stability of test compound:	No data
2. Vehicle and/ or positive control:	Diet

3. Test animals:

Species:	Rat

Strain:	Wistar Han Crl:WI (GLx/BRL/Han) IGS BR
Source:	[redacted]
Age:	5 - 6 weeks
Sex:	Males and females
Weight at dosing:	Males: 112 – 183 g, females: 98 – 150 g
Acclimation period:	At least ten days
Diet/Food:	Rat and Mouse SQC Ground Diet No.1 (BCM IPS Ltd., London, UK), *ad libitum*
Water:	Mains drinking water, *ad libitum*
Housing:	Initially in groups of three per sex in polypropylene solid-floor cages.
Environmental conditions:	Temperature: 21 ± 2 °C Humidity: 55 ± 15 % Air changes: at least 15/hour 12 hours light/dark cycle

B: Study design and methods

In life dates: 2005-09-01 to 2007-08-31

Animal assignment and treatment:
In a combined chronic toxicity and carcinogenicity study groups of 51 Wistar rats per sex received daily dietary doses of 0, 1500, 5000 and 15000 ppm (equivalent to mean achieved dose levels of 0, 95.0, 316.9 and 1229.7 mg/kg bw/day) Glyphosate technical. To ensure that a received dose of 1000 mg/kg bw/day overall was achieved, the highest dose level was progressively increased to 24000 ppm.

In addition, three satellite groups with 15 rats per sex each were included for interim sacrifice at the 12th month to study non-neoplastic histopathological changes. The satellite control group with 12 rats per sex served as veterinary control. The animals were to be used for investigations should any health problems have developed with study animals. No such problems occurred and therefore the observations of these animals have not been included in the report.

Test diets were prepared weekly by mixing a known amount of the test substance with a small amount of basal diet for 19 minutes at a constant speed. This pre-mix was then added to larger amount of basal diet and blended for further 30 minutes.

The stability and homogeneity of the test substance in the diet was determined in an in-house stability study. The homogeneity and achieved concentrations of the test substance preparations was determined at monthly intervals until Week 26, and in 3-month intervals thereafter.

Clinical observations
Rats were examined for toxic signs, ill-health or behavioural changes once and for pre-terminal deaths twice a day. A routine clinical observation session including veterinary examination was made weekly, including palpation for new or existing masses. Ophthalmic examination was done at the start of the study in all satellite animals and at Week 50 in ten satellite animals per sex of the control and high dose group. Prior to treatment and at weekly intervals thereafter all satellite animals were observed for behavioural toxicity.

Body weight
Individual body weights were recorded prior to start of treatment, at weekly intervals from Week 1 to 13

and every four weeks thereafter until termination as well at terminal kill.

Food consumption and compound intake
Food consumption was recorded once weekly for each cage group from Week 1 to Week 13 and subsequently for one week in each four weeks until termination.

Water consumption
Water intake was observed daily, for each cage group, by visual inspection.

Haematology and clinical chemistry
Haematological examinations were performed on ten animals per sex from the satellite and main groups at 3, 6 and 12 months. Further haematological investigations were performed on 20 animals per sex from the main groups at 18 and 24 months. The following parameters were measured: haematocrit, haemoglobin, erythrocyte count, MCV, MCH, MCHC, platelet count, total leukocyte count, differential leukocyte count, reticulocyte count, prothrombin time, and activated partial thromboplastin time.

Blood chemical investigations were performed on ten animals per sex from the satellite groups at 6 and 12 months and from the main groups at 18 and 24 months. The following parameters were determined: urea, glucose, total protein, albumin, albumin/globulin ratio, sodium, potassium, chloride, calcium, inorganic phosphorus, ASAT, ALAT, alkaline phosphatase, creatinine, total cholesterol, total bilirubin, and cholinesterase.

Urinalysis
Urinalytical investigations were performed on ten animals per sex from satellite groups at 3, 6 and 12 months and from main groups at 18 and 24 months. The following measurements were made: specific gravity, volume, pH, protein, glucose, ketones, blood, urobilinogen, reducing substances and microscopic examination of sediment.

Sacrifice and pathology
Necropsy was conducted for all animals surviving until study termination (main groups: 104 weeks; satellite groups: 52 weeks) as well for all animals found dead or killed in extremis.

The following organ weights were determined from 10 rats per sex and main group and from all satellite animals: adrenals, brain, gonads, heart, kidneys, liver, spleen and thymus.

Tissue samples were taken from the following organs: adrenals, aorta (thoracic), bone & bone marrow (sternum and femur incl. joint), brain (cerebrum, cerebellum, pons), caecum, colon, duodenum, epididymides, eyes (with optic nerve), gross lesions including palpable masses, head (pharynx, nasopharynx, paranasal sinuses), heart, Harderian gland, ileum (incl. Peyer's patches), jejunum, kidneys, liver, lungs (with bronchi), lymph nodes (cervical and mesenteric), mammary gland, muscle (skeletal), oesophagus, ovaries, pancreas, pituitary, prostrate, rectum, salivary glands (submaxillary), sciatic nerve, seminal vesicles, skin (hind limb), spinal cord (cervical, mid-thoracic and lumbar), spleen, stomach, testes, thymus, thyroid/parathyroid, tongue, trachea, urinary bladder, uterus and vagina. A detailed histopathological examination was performed on all sampled tissues of the control and high-dose animals. In addition, gross lesions and masses from low and intermediate dose groups at termination were examined microscopically.

Histopathological examination was initially carried out on all tissues collected from control and high dose groups; all pre-terminally dead and moribund sacrificed rats and on all lesions and palpable masses of the terminally sacrificed rats from the low and mid dose groups.
Since there were no indications of treatment-related bone marrow changes, examination was subsequently extended to the remaining treatment groups.

Statistics

Where appropriate quantitative data was analysed by the ProvantisTM Tables and Statistics Module. For each variable, the most suitable transformation of the data was found; the use of possible covariates checked and the homogeneity of means assessed using ANOVA or ANCOVA and Bartletts's test. The transformed data was analysed to find the lowest treatment level that shows a significant effect, using the Williams Test for parametric data or the Shirley Test for non-parametric data. If no dose response is found, but the data shows non-homogeneity of means, the data will be analysed by a stepwise Dunnett (parametric) or Steel (non-parametric) test to determine significant differences from the control group. Finally, if required, pair-wise tests are performed using the Student t-test (parametric) or the Mann-Whitney U test (non-parametric). Histopathology data were analysed using the following methods to determine significant differences between control and treatment groups for the individual sexes.

1. Chi squared analysis for differences in the incidence of lesions occurring with an overall frequency of I or greater.
2. Kruskal-Wallis one way non-parametric analysis of variance for the comparison of severity grades for the more frequently observed graded conditions.

II: RESULTS

A. ANALYSIS OF DOSE FORMULATIONS

Stability assessment demonstrated that the test material preparations in the diet were stable for at least six weeks.

Analyses for achieved concentrations showed that the diet preparations were within an acceptable range. On one occasion the achieved concentrations of the low, mid and high-dose group were 79 %, 83 %, and 87 %, respectively. At week 2 the concentration in the mid dose group was 112 %. However, these isolated deviations from the nominal range were still considered to be acceptable.

B. MORTALITY

No significant treatment-related effects on mortality were observed during the study.
The numbers of pre-terminal deaths in the main group are displayed in the table below:

Table 5.5-2 Glyphosate Technical: Dietary Combined Chronic Toxicity/Carcinogenicity in the Rat ([redacted], 2009): Cumulated mortalities after 104-week dietary exposure to Glyphosate technical

	Dose group (ppm)			
Sex	**0**	**1500**	**5000**	**15000-24000**
Male	12	14	13	6
Female	14	17	15	12

C. CLINICAL OBSERVATIONS

No significant treatment-related clinical observations occurred during the study.

There were no treatment-related effects on behavioural assessments, functional performance tests or sensory reactivity assessments observed.

D. BODY WEIGHT

Body weights were not statistically significantly changed in any dose group. At the end of the dosing period the body weight of males/females of the high dose group was reduced by 7 %/3 % compared to the control without being statistically significant. Body weight gains were partly changed in dose groups compared to the control. However, the variation of body weight changes was generally very high and increases as well as decreases were observed comparing control and dose groups. In conclusion, the changes in body weight and body weight gains was not considered an adverse effect.

Table 5.5-3 Glyphosate Technical: Dietary Combined Chronic Toxicity/Carcinogenicity in the Rat ([redacted], 2009): Intergroup comparison of body weights

	Dose group (ppm)							
	0		1500		5000		15000	
	♂	♀	♂	♀	♂	♀	♂	♀
Body weight [g]								
Week 1	141 ± 17	123 ± 11	142 ± 17	123 ± 10	141 ± 17	122 ± 10	141 ± 16	123 ± 10
Week 13	393 ± 40	228 ± 18	401 ± 42	233 ± 18	367 ± 41	229 ± 17	379 ± 34	227 ± 15
Week 52	544 ± 55	289 ± 38	554 ± 62	294 ± 33	554 ± 55	290 ± 35	511 ± 51	281 ± 26
Week 104	618 ± 83	362 ± 67	641 ± 102	375 ± 61	648 ± 81	378 ± 54	577 ± 66	354 ± 49

Table 5.5-4 Glyphosate Technical: Dietary Combined Chronic Toxicity/Carcinogenicity in the Rat ([redacted], 2009): Intergroup comparison of body weight gains

	Dose group (ppm)							
	0		1500		5000		15000	
	♂	♀	♂	♀	♂	♀	♂	♀
Body weight gain [g]								
Week 1	39.6 ± 5	19 ± 7	40.6 ± 4	21 ± 4	39.5 ± 8	20 ± 4	37.2 ± 4** (91)	19 ± 6
Week 13	7.8 ± 3.4	1.2 ± 3.9	10.7 ± 16.5* (137)	2.1 ± 4.1	8.5 ± 3.1* (109)	2.5 ± 3.8	9.2 ± 3.4* (118)	2.6 ± 4.5
Week 52	-1.32 ± 3.5	-2.2 ± 7.1	-0.48 ± 4.3	-0.29 ± 5.7	-0.20 ± 6.9	1.2 ± 7.0	-1.5 ± 5.0	-1.3 ± 6.5
Week 104	3.0 ± 11.6	-1.8 ± 12	-4.4 ± 28.7	1.4 ± 12	-1.1 ± 23.7	2.5 ± 9	1.22 ± 14.1	1.5 ± 7

** Statistically significant difference from the control group mean at the 1 % level (Student's t-test, two-sided)
* Statistically significant difference from the control group mean at the 5 % level (Student's t-test, two-sided)
() percent of control (only indicated when statistically significant)

E. FOOD CONSUMPTION AND COMPOUND INTAKE

There were no treatment-related effects on food consumption or food efficiency for either sex noted during the study.

The group mean achieved doses are summarised below.

Table 5.5-5 Glyphosate Technical: Dietary Combined Chronic Toxicity/Carcinogenicity in the Rat ([redacted], 2009): Group mean achieved dose levels

Dose group	Dietary concentration (ppm)		Mean achieved dose level (mg/kg bw/day)		
			Males	Females	Overall mean
1 (control)	0				
2 (low)	1500		85.5	104.5	95.0
3 (mid)	5000		285.2	348.6	316.9
4 (high)	15000	Week 1-11	1077.4	1381.9	1229.7
	17000	Week 12-15			
	19000	Week 16-26			
	21000	Week 27-39			
	24000	Week 40-104			

The results show a higher test material intake for females when compared to males for each dose level. The mean intake for each dose group is 95.0, 316.9 and 1229.7 mg/kg bw/day for 1500, 5000, and 15000-24000 ppm, respectively. The mean intake values represent the combination of satellite and main group values.

F. WATER CONSUMPTION

There were no treatment-related effects on water consumption during the study.

G. OPHTHALMOSCOPY

There were no treatment-related effects observed.

H. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

All variations were considered to be incidental and unrelated to treatment because of the lack of either a true dose response, a consistent change throughout the study, a lack of progression of change with time and/or lack of concomitant effect in both sexes.

Clinical chemistry

At the highest dose level there was an increase in alkaline phosphatase activity for satellite group males and females compared with controls at 6 and 12 months. Main group males were also affected at 18 months. Values for all alkaline phosphatase activity values are presented as follows:

Table 5.5-6 Glyphosate Technical: Dietary Combined Chronic Toxicity/Carcinogenicity in the Rat ([redacted], 2009): Alkaline phosphatase activity (IU/L)

	Dose level							
	Control		Low		Intermediate		High	
Timepoint	♂	♀	♂	♀	♂	♀	♂	♀
Month 6 (Satellite)	87.8	49.6	↑94.5	↑62.9	↑103.4	↑62.0	↑128.5**	↑91.9**
Month 12 (Satellite)	87.7	46.1	↑96.5	↑59.7	↑116.3*	↑58.1	↑140.2**	↑91.3**
Month 18 (Main)	93.3	65.7	↑110.5	↓55.8	↑110.9	↑70.9	↑125.0*	↑92.7
Month 24 (Main)	107.2	66.0	↓98.8	↓58.5	↓101.0	↑81.7	↑111.9	↑86.8

* $p < 0.05$; ** $p < 0.01$

The magnitude of the effect does not appear to increase with age plus the lack of a consistent effect for females and the absence of any histopathological correlation does suggest this to be of limited toxicological importance.

At the 18 month evaluation there was an increase in plasma electrolytes for both sexes. Sodium and chloride values for males and females and potassium values for males only were increased compared with controls. Female calcium levels were lower than controls. These elevations/decrements were also observed at lower dose levels but were not seen in a dose related trend. In addition at the 12 month evaluation for satellite females a lower sodium value was seen for females. Values for all calcium and chloride values are presented as follows:

Table 5.5-7 Glyphosate Technical: Dietary Combined Chronic Toxicity/Carcinogenicity in the Rat ([redacted], 2009): Calcium and chloride values (mmol/L)

	Dose level							
	Control		Low		Intermediate		High	
Timepoint	♂	♀	♂	♀	♂	♀	♂	♀
Calcium								
Month 6 (Satellite)	2.587	3.693	↑2.701	↑3.752	↑2.617	↓3.637	↓2.508	↓3.604*

Table 5.5-7 Glyphosate Technical: Dietary Combined Chronic Toxicity/Carcinogenicity in the Rat ([redacted], 2009): Calcium and chloride values (mmol/L)

	Dose level							
	Control		Low		Intermediate		High	
Timepoint	♂	♀	♂	♀	♂	♀	♂	♀
Month 12 (Satellite)	2.530	2.602	↑2.543	↓2.587	↓2.458	↓2.475	↓2.514	↓2.483
Month 18 (Main)	2.231	2.775	↑2.523	↓2.645*	↑2.656	↓2.554**	↑2.598	↓2.468**
Month 24 (Main)	2.431	2.293	↑2.487	↑2.396	↑2.511	↓2.288	↓2.297	↑2.347
Chloride								
Month 6 (Satellite)	107.7	105.8	↓107.1	↑106.1	↓107.0	↑106.1	↑108.5	↑106.7
Month 12 (Satellite)	105.6	103.9	↓105.1	↑104.8	↓104.3	↑104.7	↑105.9	↑104.2
Month 18 (Main)	103.3	101.8	↑105.8**	↑104.2**	↑105.8**	↑106.4**	↑107.6**	↑107.8**
Month 24 (Main)	104.5	103.4	↓104.4	↓103.1	↓104.3	↓102.2	↑105.4	↓102.8

* $p < 0.05$; ** $p < 0.01$

At intermediate level similar findings to the highest dose level were seen for plasma electrolytes at the 18 month evaluation. A slight increase in alkaline phosphatase activity was seen for satellite group males at 12 months. At the low dose level there was a similar effect on the plasma electrolytes for both sexes at the 18 month evaluation of main group animals. Whilst these observations were seen at the highest dose level, the lack of dose response or the effect being limited to one sex does make the toxicological significance questionable.

All other differences were isolated in their finding and are therefore not toxicologically relevant.

I. URINALYSIS

There were no treatment-related effects observed.

J. NECROPSY

Gross pathology

There were no treatment-related macroscopic findings observed during the study period.

Organ weights

No effects on organ weight values were observed.

Histopathology

Adipose infiltration of the bone marrow was seen for the majority of animals examined, with both sexes being more or less equally affected in terms of incidence and severity. However, greater effects were seen among male rats dosed at the highest level and this attained statistical significance for terminal kill animals. This data indicates the possibility of myeloid hypoplasia as a consequence of treatment. However, given the normal variability of this condition and the influence of other pathological conditions upon marrow cellularity in ageing rats, the effect was not altogether convincing but cannot be dismissed. A similar effect was not seen among male rats in the remaining treatment groups but among premature deaths for animals of both sexes at the intermediate level and only low-dosed females. However, the variable duration of exposure and significant background pathology for premature death animals further negates this as an effect of treatment upon marrow cellularity for female rats.

Moreover, at the highest dose level there was a significant difference in the site of mineral deposition within the kidneys compared with controls. Pelvic mineralisation was commonly seen in both sexes and was more prevalent among female rats; however corticomedullary mineralisation was seen in female rats only. Nephrocalcinosis in rats is generally considered to be related to diet and hormonal status. There was a lower incidence of pelvic/papillary deposition and an increase in the corticomedullary deposition. At the same time there was a reduction in the incidence of renal pelvic hyperplasia in both sexes; which is considered

to be a consequence of the decreased mineral deposition.

The effects on pelvic and corticomedullary mineralisation, and hyperplasia of the pelvic/papillary epithelium were confined to high dose animals with no indication of a similar effect at any other treatment level for either sex.

No other treatment-related changes were observed.

Neoplastic changes
No significant effects associated with tumour development were observed.

3. Assessment and conclusion

Assessment and conclusion by applicant:
Based on the study results the NOAEL in rats after chronic exposure to Glyphosate technical for 24 month is 24000 ppm (corresponding to 1229.7 mg/kg bw/day for combined sexes). It is concluded that Glyphosate technical is not carcinogenic in rats.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.5/002
Report author	[redacted]
Report year	2001
Report title	Glyphosate Acid: Two Year Dietary Toxicity and Oncogenicity Study in Rats
Report No	[redacted]/PR1111
Document No	NA
Guidelines followed in study	OECD 453 (1981), EEC B.33 (1988), MITI (1992), US OPTTS 870.4300 (1998)
Deviations from current test guideline (OECD 453, 2018)	Yes, organ weights of epididymides, thyroid/parathyroid and uterus were not determined; histopathological examination of coagulating glands, gall bladder, upper respiratory tract, peripheral nerve and vagina were not performed.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The chronic toxicity and carcinogenic potential of glyphosate acid was assessed in a 24-month feeding study in 52 male and 52 female Wistar rats with 0, 2000, 6000 and 20000 ppm (equivalent to mean achieved dose levels of 0, 121, 361 and 1214 mg/kg bw/day for males and 0, 145, 437 and 1498 mg/kg bw/day for

females). In addition, three satellite groups with 12 rats per sex each were included for interim sacrifice at the 12th month to study non-neoplastic histopathological changes.

Observations covered clinical signs, body weight, food consumption, haematology, clinical chemistry and urinalysis as well as organ weights, necropsy and histopathological examination.

Treatment related findings in this study were found in the liver and kidney and were confined to animals (predominantly males) fed 20000 ppm glyphosate acid. There were a number of changes in males and females fed 20000 ppm, notably renal papillary necrosis, prostatitis, periodontal inflammation, urinary acidosis and haematuria, which may be attributed to the acidity of the test substance. Despite the findings at 20000 ppm, survival was better in males fed 20000 ppm than in the controls and lower dose groups. This improved survival was associated with lower food consumption, lower bodyweights and a decreased severity of renal glomerular nephropathy.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate acid (technical material)
Description:	White solid
Lot/Batch #:	P30
Purity:	97.6 % w/w
Stability of test compound:	At least 2 years when stored at -20 °C

2. Vehicle and/or positive control: Diet

3. Test animals:

Species:	Rat
Strain:	Wistar (Alpk:APfSD)
Source:	[redacted]
Age:	3 weeks (on delivery)
Sex:	Males and females
Weight at dosing:	Males: 155.0 – 156.6 g (mean values); females: 136.0 – 138.4 g (mean values)
Acclimation period:	At least 10 days
Diet/Food:	CT1 diet (Special Diet services Ltd., Essex, UK), *ad libitum*
Water:	Mains drinking water, *ad libitum*
Housing:	Initially in litters, sexes separately, after assignment to experimental groups in group of four rats per sex per cage
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 30 - 70 % Air changes: at least 15/hour 12 hours light/dark cycle

B: Study design and methods

In life dates: 1998-04-07 to 2000-05-07

Animal assignment and treatment:
In a combined chronic toxicity and carcinogenicity study groups of 52 Wistar-derived rats per sex received daily dietary doses of 0, 2000, 6000 and 20000 ppm glyphosate acid (equivalent to mean achieved dose levels of 0, 121, 361 and 1214 mg/kg bw/day for males and 0, 145, 437 and 1498 mg/kg bw/day for females).
A further twelve animals per sex were added to each group and were designated for interim kill after one year to study chronic toxicity and non-neoplastic histopathological changes.

Test diets were prepared in 60 kg batches by mixing a known amount of the test substance with 1 kg of basal diet. This pre-mix was then added to the remainder of the 60 kg batch of basal diet and mixed thoroughly. The stability and homogeneity of the test substance in the diet was determined in an in-house stability study at 2000 and 20000 ppm.

Clinical observations
Rats were examined for toxic signs, ill-health or behavioural changes and pre-terminal deaths prior to the start of the study and once a day afterwards. Detailed clinical observations were conducted weekly. Ophthalmic examination was done in all animals at the start of the study, at Week 52 and prior to termination. A functional observational battery including motor activity was conducted in Week 52 in animals allocated to the chronic toxicity assessment of the study.

Body weight
Individual body weights were recorded prior to start of treatment, at weekly intervals from Week 1 to 15 and every two weeks thereafter until termination.

Food consumption and compound intake
Food consumption was recorded once weekly for each cage group from Week 1 to Week 14, once in week 16 and every fourth week thereafter.

Haematology and clinical chemistry
Blood was collected from 13 animals per sex and group at Week 14, 27, 53, 79 and at termination. Different animals were used for the tail vein haematology and clinical chemistry samples.

The following blood parameters were measured: haematocrit, haemoglobin, erythrocyte count, MCV, MCH, MCHC, blood cell morphology, platelet count, total leukocyte count, differential leukocyte count, reticulocyte count, red blood cell distribution width, prothrombin time, activated partial thromboplastin time. The following clinical chemistry parameters were measured: alkaline phosphatase, aspartate amino transferase (AST), alanine aminotransferase (ALT), γ-glutamyl-transferase, creatine kinase, creatinine, urea, total protein, glucose, albumin, globulin, albumin/globulin ratio, total bilirubin, triglycerides, total cholesterol, inorganic phosphorus, calcium, sodium, potassium, and chloride.

Urinalysis
Individual urine samples were collected from the same animals as those used for haematology analyses at Week 13, 26, 52, 78 and prior to termination. The following parameters were determined: volume, abnormal colour and appearance, specific gravity, pH, glucose, ketones, protein, bilirubin, and blood.

Sacrifice and pathology
Necropsy was conducted on all animals. The following organ weights were determined from all animals surviving to scheduled termination: adrenals, brain, gonads, heart, kidneys, liver and spleen.

Tissue samples were taken from the following organs: adrenals, aorta, bone and bone marrow (femur incl. joint), brain (cerebrum, cerebellum, brainstem), caecum, cervix, colon, duodenum, epididymides, eyes (retina, optic nerve), gross lesions including palpable masses, Harderian gland, heart, ileum, jejunum, kidneys, lachrymal gland, larynx, liver, lung, lymph nodes (cervical and mesenteric), mammary gland, muscle, oesophagus, ovary, pancreas, pharynx, pituitary, prostrate, rectum, salivary glands (submandibular,

parotid), seminal vesicles, skin, spinal cord (cervical, thoracic, lumbar), spleen, sternum, stomach, testes, thymus, thyroid/parathyroid, trachea, urinary bladder and uterus.

Statistics
All data were evaluated using analysis of variance and/or analysis of covariance for each specified parameter using the MIXED procedure in SAS (1996). Kaplan-Meier survival estimates (Kaplan and Meier, 1958) were calculated separately for each sex and treatment group.
The overall incidence of each tumour type was considered by comparing each treated group and the control group using Fisher's Exact Test. In addition, a test for trend with group number was performed using the Cochran-Armitage Test described in Gart *et al*. (1986). Analyses were carried out for all animals, intercurrent deaths and at terminal kill.

II. RESULTS

A. ANALYSIS OF DOSE FORMULATIONS
The mean achieved concentrations of glyphosate acid in each dietary preparation were within 10 % of the nominal concentration and the overall mean concentrations were within 1 % of nominal.

The homogeneity of glyphosate acid in diet at concentrations of 2000 and 20000 ppm was satisfactory; percentage deviations were within 2 % of the overall mean for the 20000 ppm group and within 4-9 % of the overall mean for the 2000 ppm group.

The stability tests determined at 2000 and 20000 ppm showed that the test substance stability was satisfactory at room temperature and when stored at -20 °C for at least 45 days which covered the period of use in the current study.

B. MORTALITY
The male groups were terminated in Week 100 because survival in the control, low and mid dose groups was approaching 25 % (criteria for termination of the study). Statistically significantly better survival was observed in males fed 20000 ppm than in the other groups ($p = 0.02$). A statistically significant overall trend was also observed for males ($p = 0.03$).

The female groups survived to scheduled termination and there were no significant differences in mortality between the groups.

The survival rates are displayed in the table below.

Table 5.5-8 Glyphosate Acid: Two Year Dietary Toxicity and Oncogenicity Study in Rats ([redacted], 2001): Survival rates during up to 104-week dietary exposure to glyphosate technical

	Dose group (ppm)							
	0		2000		6000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
Week 1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Week 13	0.98	1.00	1.00	1.00	↓0.98	↓0.98	1.00	1.00
Week 26	0.95	1.00	1.00	1.00	0.98	0.98	1.00	1.00
Week 39	0.94	1.00	1.00	1.00	↓0.97	0.98	1.00	1.00
Week 52	0.91	1.00	↓0.97	1.00	0.97	0.98	↓0.98	↓0.98
Week 56	0.89	1.00	↓0.93	1.00	↓0.93	0.98	0.98	0.98
Week 60	0.87	1.00	↓0.92	1.00	↓0.91	↓0.97	0.98	↓0.97
Week 64	0.87	1.00	↓0.90	↓0.98	0.91	↓0.95	0.98	0.97
Week 68	0.87	0.94	↓0.88	↓0.96	↓0.87	0.95	0.98	↓0.95
Week 72	0.85	0.94	↓0.84	0.96	↓0.85	↓0.93	↓0.97	↓0.91
Week 76	0.81	0.94	↓0.80	↓0.92	↓0.82	↓0.89	0.97	0.91

Table 5.5-8 Glyphosate Acid: Two Year Dietary Toxicity and Oncogenicity Study in Rats ([redacted], 2001): Survival rates during up to 104-week dietary exposure to glyphosate technical

	Dose group (ppm)							
	0		2000		6000		20000	
Week 80	0.73	0.88	↓0.78	↓0.87	↓0.72	0.89	↓0.89	↓0.83
Week 84	0.69	0.85	↓0.67	↓0.83	↓0.63	0.89	↓0.85	0.83
Week 88	0.64	0.81	↓0.57	↓0.81	↓0.59	↓0.83	↓0.77	↓0.81
Week 92	0.56	0.79	↓0.50	0.81	↓0.53	↓0.81	↓0.71	↓0.80
Week 96	0.50	0.73	↓0.46	↓0.73	0.53	↓0.77	↓0.66	↓0.72
Week 100	0.40	0.69	↓0.44	↓0.63	↓0.42	0.77	↓0.56	↓0.66
Week 104	—*	0.62	—*	↓0.56	—*	0.77	—*	↓0.57

* Terminated in Week 100 because survival in the control, low and mid dose groups was approaching 25 % (criteria for termination of the study).

C. CLINICAL OBSERVATIONS

At 20000 ppm there was a treatment related increase in the incidence of red-brown staining of tray papers, particularly in males.

There were no other treatment-related clinical observations.

There were also no treatment-related effects noted in the functional observational battery.

D. BODY WEIGHT

The bodyweights of the animals fed 20000 ppm glyphosate acid were statistically significantly lower than controls throughout the study. The maximum reduction from control values was approximately 5 % for males and 8 % for females.

There were no treatment related effects in animals fed 2000 or 6000 ppm glyphosate acid.

Table 5.5-9 Glyphosate Acid: Two Year Dietary Toxicity and Oncogenicity Study in Rats ([redacted], 2001): Intergroup comparison of body weights

	Dose group (ppm)							
	0		2000		6000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
Body weight [g]								
Week 1	155.5 ± 14.8 (N = 64)	138.4 ± 12.3 (N = 64)	↑156.3 ± 16.0 (N = 64)	138.4 ± 11.5 (N = 64)	↑156.6 ± 16.2 (N = 64)	↓137.5 ± 11.6 (N = 64)	↓155.0 ± 14.0 (N = 64)	↓136.0 ± 13.0 (N =)
Week 2 adjusted mean	206.7 ± 17.2 (N = 64) 206.8	163.9 ± 13.0 (N = 64) 163.0	↑207.1 ± 18.1 (N = 64) 206.6	163.9 ± 11.9 (N = 64) 163.0	↓206.1 ± 19.1 (N = 63) 205.3	↓162.2 ± 12.9 (N = 64) 162.3	↓201.5 ± 16.2 (N = 64) 202.6**	↓160.7 ± 14.8 (N = 64) 162.3
Week 3 adjusted mean	260.7 ± 20.1 (N = 64) 206.8	188.8 ± 15.8 (N = 64) 187.9	↓260.0 ± 22.1 (N = 64) 259.4	↓187.7 ± 13.7 (N = 64) 186.8	↑261.0 ± 21.7 (N = 63) 206.2	↓186.6 ± 14.3 (N = 64) 186.6	↓252.4 ± 19.7 (N = 64) 253.7**	↓182.4 ± 16.4 (N = 184.2**)
Week 4 adjusted mean	308.4 ± 23.4 (N = 64) 308.5	207.3 ± 17.1 (N = 64) 206.4	↓308.3 ± 26.1 (N = 64) 307.6	↓205.9 ± 15.8 (N = 64) 204.9	↑309.2 ± 25.6 (N = 63) 308.4	↓204.7 ± 15.2 (N = 64) 204.8	↓296.3 ± 22.7 (N = 64) 297.7**	↓198.8 ± 18.6 (N = 64) 200.7**
Week 5 adjusted mean	339.4 ± 25.9 (N = 64) 339.5	219.0 ± 18.1 (N = 64) 218.1	↑342.0 ± 30.2 (N = 64) 341.3	↑219.9 ± 15.5 (N = 64) 219.0	↑342.0 ± 28.9 (N = 63) 341.1	↓216.5 ± 14.8 (N = 64) 216.5	↓324.9 ± 27.4 (N = 64) 326.5**	↓208.8 ± 19.8 (N = 210.6**)

Table 5.5-9 Glyphosate Acid: Two Year Dietary Toxicity and Oncogenicity Study in Rats ([redacted], 2001): Intergroup comparison of body weights

	Dose group (ppm)							
	0		2000		6000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
Week 6	365.2 ± 29.4	227.3 ± 19.2	↑365.7 ± 33.9	↑260.2 ± 16.0	↑367.8 ± 32.5	↓223.7 ± 16.6	↓348.9 ± 30.4	↓218.7 ± 19.7
adjusted mean	(N = 64) 365.3	(N = 64) 226.4	(N = 64) 364.9	(N = 64) 229.3	(N = 63) 366.9	(N = 63) 223.6	(N = 350.5**	(N = 64) 220.4**
Week 7	388.3 ± 30.0	236.5 ± 18.9	↑389.7 ± 36.0	↑236.9 ± 15.4	↑390.8 ± 35.1	↓234.1 ± 16.9	↓369.3 ± 33.2	↓227.1 ± 20.5
adjusted mean	(N = 64) 388.2	(N = 64) 235.6	(N = 64) 388.7	(N = 64) 236.0	(N = 63) 389.7	(N = 64) 234.1	(N = 63) 370.7**	(N = 64) 228.8**
Week 13	489.4 ± 35.6	266.8 ± 19.5	↑495.1 ± 44.1	↑269.3 ± 17.3	↑494.7 ± 46.8	↑268.2 ± 17.4	↓464.9 ± 38.6	↓258.6 ± 21.8
adjusted mean	(N = 63) 488.9	(N = 64) 265.9	(N = 64) 494.4	(N = 64) 268.4	(N = 63) 493.6	(N = 63) 268.2	(N = 64) 466.8**	(N = 64) 206.4**
Week 51	669.0 ± 54.7	350.9 ± 35.1	↑676.0 ± 55.9	↑352.5 ± 31.5	↑680.7 ± 76.1	↓350.9 ± 35.4	↓636.3 ± 53.8	↓324.2 ± 37.0
adjusted mean	(N = 58) 667.8	(N = 64) 349.6	(N = 62) 677.3	(N = 64) 351.2	(N = 62) 678.1	(N = 63) 350.7	(N = 63) 640.2**	(N = 63) 326.8**
Week 91	627.0 ± 74.2	408.7 ± 36.5	↑642.7 ± 85.6	↑420.1 ± 53.5	↑620.2 ± 90.0	↑418.2 ± 42.7	↓626.2 ± 66.3	↓377.0 ± 43.5
adjusted mean	(N = 30) 623.9	(N = 41) 410.3	(N = 27) 641.2	(N = 42) 422.7	(N = 28) 617.7	(N = 43) 419.9	(N = 37) 628.6	(N = 42) 378.8**
Week 104 (males); week 105 (females)	590.6 ± 59.4	390.9 ± 31.8	↓578.6 ± 85.8	↑397.2 ± 43.7	↓572.2 ± 70.6	↓384.0 ± 48.3	↓569.6 ± 83.6	↓363.7 ± 35.7
adjusted mean	(N = 16) 592.1	(N = 32) 391.0	(N = 17) 569.9	(28) 398.7	(N = 18) 565.0	(N = 39) 384.7	(N = 26) 560.0	(N = 30) 374.7

** Statistically significant difference from the control group mean at the 1 % level (Student's t-test, two-sided)
* Statistically significant difference from the control group mean at the 5 % level (Student's t-test, two-sided)

E. FOOD CONSUMPTION AND COMPOUND INTAKE

Food consumption was lower throughout the first year of the study in animals fed 20000 ppm glyphosate acid. In females the difference was statistically significant over the first 11 weeks (with a maximum reduction of approximately 5 %) and again in weeks 40-56 (with a maximum reduction of 6 %). In males, the difference was statistically significant over most of the first 6 months with a maximum reduction of 6 %.

The group mean achieved doses are summarised below.

Table 5.5.-5.5-10 Glyphosate Acid: Two Year Dietary Toxicity and Oncogenicity Study in Rats ([redacted], 2001): Group mean achieved dose levels

Dose group	Dietary concentration (ppm)	Mean achieved dose level (mg/kg bw/day)	
		Males	Females
1 (control)	0		
2 (low)	2000	121	145
3 (mid)	6000	361	437
4 (high)	20000	1214	1498

The results show a higher test material intake for females when compared to males for each dose level. The mean intake for each dose group is 0, 121, 361 and 1214 mg/kg bw/day for males and 0, 145, 437 and 1498 mg/kg bw/day for females for 0, 2000, 6000 and 20000 ppm, respectively.

F. OPHTHALMOSCOPY

There were no treatment-related effects observed.

G. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

Minor variations from control values were obtained for most parameters but showed no consistency and were confined to intermediate time points and/or dose groups and were considered not to be treatment-related. An increased haemoglobin concentration and decreased platelet count was seen in all female treated groups at the interim kill but, in the absence of any apparent dose-response or effects at other time points, these variations from mean control values are considered not to be treatment-related (see table below).

Table 5.5-11 Glyphosate Acid: Two Year Dietary Toxicity and Oncogenicity Study in Rats ([redacted], 2001): Haemoglobin and platelet count

	Dose group (ppm)							
	0		2000		6000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
Haemoglobin (g/dL)								
Week 14	15.9	15.7	↑16.0	↓15.5	↑16.0	↑15.9	↓15.8	↓15.0*
Week 27	15.5	15.7	↑15.8	↑15.8	↑15.8	15.7	↑15.7	↓15.6
Interim Kill	14.7	14.4	↓14.4	↑15.1**	↓14.3	↑14.9*	↓14.4	↑15.0*
Week 53	16.1	15.9	↓15.7*	15.9	↓15.5**	15.9	↓15.9	↓15.8
Week 79	15.9	15.9	↓15.2	↓15.8	↓15.5	↑16.0	↓15.4	↓15.5
Week 105	13.3	14.3	↓12.9	↓14.1	↓13.1	↓13.8	↑13.6	↓14.2
Platelet count (× 109/L)								
Week 14	885	911	↑897	↓877	↑892	↓910	↓847	↑948
Week 27	903	909	↓871	↓868	↑917	↓858	↓880	↓830*
Interim Kill	889	821	↑895	↓761*	↓888	↓740**	↓860	↓764*
Week 53	911	842	↑977	↓794	911	↓754	↓865	↓814
Week 79	963	854	↑993	↓796	↓950	↓817	↓935	↑855
Week 105	1015	780	↓980	↑783	↓988	↓750	↓877	↑846

* $p < 0.05$; ** $p < 0.01$

Clinical chemistry

In rats fed 20000 ppm glyphosate acid, increases in plasma alkaline phosphatase were present until Week 79 (**Table 5.5-12**). Increases in alanine aminotransferase activities were present consistently in males until Week 79 and in females in Weeks 14, 79 and 105. Increased total bilirubin was also present in these males throughout the study and increased plasma aspartate aminotransferase activity was present in males at the interim kill. Plasma triglycerides and cholesterol levels were reduced (from Weeks 14-53 and Weeks 53 onwards, respectively) in males.

In animals fed 6000 ppm, there were small increases in alkaline phosphatase activity over the first year of the study and variable increases in plasma alanine aminotransferase activity at intermediate time points throughout the study.

Plasma creatinine values were lower in all treated female groups at Week 27 and in females receiving 6000 and 20000 ppm at Week 14, but in the absence of any effects later in the study, this is considered to be of no toxicological significance.

Other minor variations from mean control values were confined to intermediate dose groups or time points and/or showed no dose response, and so were considered not to be treatment-related.

Table 5.5-12 Glyphosate Acid: Two Year Dietary Toxicity and Oncogenicity Study in Rats ([redacted], 2001): Clinical chemical findings

	Dose group (ppm)							
	0		2000		6000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
Alkaline Phosphatase (IU/L)								
Week 14	234	156	↑246	↑177	↑284**	↑245**	↑387**	↑266**
Week 27	196	121	↑219	↑136	↑239**	↑166**	↑327**	↑203**
Interim Kill	230	82	↑244	↑102	↑269	↑123*	↑306**	↑144**
Week 53	231	92	↑249	↑117*	↑277**	↑152**	↑357**	↑172**
Week 79	208	114	↑254*	↑131	↑244	↑181**	↑353**	↑178**
Week 105	184	144	↑205	↑129	↑218	↑158	↑280	↑173
Alanine Aminotransferase (IU/L)								
Week 14	94.9	81.9	↑103.5	↑92.5	↑121.8**	↑103.9*	↑143.4**	↑104.7*
Week 27	91.8	99.5	↑95.9	↑113.8	↑116.8	↑132.7*	↑125.9*	↑101.8
Interim Kill	77.6	83.4	↑84.0	↑82.8	↑97.7	↑113.2*	↑123.3**	↑95.9
Week 53	84.2	90.1	↑99.8	↑108.2	↑103.5	↑121.5*	↑133.8*	↑114.0
Week 79	69.2	90.0	↑81.2	↑ 97.2	↑102.4**	↑110.6	↑105.9**	↑116.0*
Week 105	64.1	83.5	↓58.6	↓78.6	↓63.9	↓78.9	↑82.7	↑108.2**
Total Bilirubin (µmol/L)								
Week 14	1.23	2.00	1.23	↓1.92	↑1.46	2.00	↑1.85**	↑2.46*
Week 27	2.08	2.31	↑2.31	↓2.08	↑2.31	↓2.08	↑2.62**	↓2.23
Interim Kill	2.09	2.50	↓1.91	↓2.42	↑2.18	↑2.58	↑2.67**	↑2.64
Week 53	2.62	2.54	↓2.46	↓2.31	↑2.92	↓2.46	↑3.46**	↑3.15**
Week 79	2.46	2.92	↑2.92	↓2.31	↑2.85	↓2.38	↑3.15**	↑3.08
Week 105	1.75	1.19	↑2.29	↓1.04	↓1.67	↑1.77	↑2.54	↑1.40
Aspartate Aminotransferase (IU/L)								
Week 14	107.9	104.5	↑113.5	↑112.6	↑129.2	↑124.0	↑148.0*	↑114.3
Week 27	110.5	156.8	↑114.8	↑185.5	↑138.0	↑208.4	↑141.3	↓148.3
Interim Kill	90.0	117.8	↑91.5	↓109.0	↑110.4	↑149.3	↑132.0*	↑131.5
Week 53	111.8	151.9	↑124.8	↑194.4	↑130.2	↑219.1*	↑160.7	↑214.8*
Week 79	88.2	156.0	↑102.7	↓129.2	↑130.0	↑177.7	↑112.2	↑197.0
Week 105	75.8	130.7	↑81.4	↓102.8	↑78.4	↓121.8	↑92.8	↑168.5
Plasma Triglycerides (mmol/L)								
Week 14	1.33	1.03	↑1.48	↓0.96	↑1.43	↓0.96	↓1.11*	↓0.94
Week 27	1.40	1.18	↑1.42	↑1.22	↓1.38	↓0.95*	↓1.14*	↓1.09
Interim Kill	1.65	1.00	↑2.07	↑1.13	↑2.09	↑1.07	↓1.45	↓0.99
Week 53	1.53	1.62	↑1.55	↑1.75	↓1.50	↓1.39	↓1.15*	↓1.39
Week 79	1.90	2.15	↑1.96	↑2.77	↓1.67	↑2.26	↓1.42	↑2.31
Week 105	1.83	3.26	↓1.81	↑3.58	↑1.94	↓3.02	↓1.67	↓2.82
Cholesterol (mmol/L)								
Week 14	2.40	2.66	↑2.51	↓2.62	↑2.48	↑2.80	↑2.54	↑2.71
Week 27	2.92	3.19	↑3.02	↑3.24	↑3.18	↓3.13	↑2.98	↓3.15
Interim Kill	4.74	2.69	↑5.05	↑2.95	↑4.83	↑2.98	↓3.89*	↑3.01
Week 53	5.03	3.56	↓4.57	↓3.49	↑5.15	↓3.45	↓4.06**	↑3.66
Week 79	6.87	4.26	↓6.30	↑4.64	↓5.81*	↓3.92	↓5.20**	↓3.96
Week 105	6.76	4.44	↑7.22	↑4.54	↑7.79	↓4.13	↓5.72*	↓4.11
Plasma Creatinine (µmol/L)								
Week 14	58.5	61.4	↑59.9	↓59.6	↓57.2	↓59.0*	↓56.8	↓58.6**

Table 5.5-12 Glyphosate Acid: Two Year Dietary Toxicity and Oncogenicity Study in Rats ([redacted], 2001): Clinical chemical findings

	Dose group (ppm)							
	0		2000		6000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
Week 27	60.8	62.7	↑61.2	↑60.3*	↓59.4	↓60.5*	↓58.4*	↓58.2**
Interim Kill	55.8	53.6	↑58.0	↓51.8	↑56.5	↓52.3	↑56.6	↓50.9
Week 53	61.0	58.8	↑61.5	↑59.5	↑62.5	↓58.1	↓60.5	↓58.2
Week 79	80.7	62.7	↑85.9	↓59.2	↑86.2	↑62.8	↓66.4	↓61.8
Week 105	79.1	50.9	↑80.8	↑51.4	↑79.2	↑53.5	↓66.2	↓50.7

* $p < 0.05$; ** $p < 0.01$

H. URINALYSIS

Urinary pH was lower throughout the study in males fed 20000 ppm glyphosate acid (**Table 5.5-13**). Moreover, in the same dose group an increased incidence and severity of blood/red blood cells was present in males and, to a lesser extent, in females.

There were no other treatment related findings in the urinalysis.

Table 5.5-13 Glyphosate Acid: Two Year Dietary Toxicity and Oncogenicity Study in Rats ([redacted], 2001): Urine-analytical findings

	Dose group (ppm)							
	0		2000		6000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
Urine pH								
Week 13	6.85	6.00	↓6.77	6.00	↓6.92	↑6.08	↓6.31**	↓5.85
Week 26	6.77	5.77	↓6.69	↑5.85	↓6.69	↑6.00	↓6.15**	5.77
Week 52	6.85	6.15	6.85	↑6.23	6.85	↑6.31	↓6.15**	↓5.92
Week 78	6.54	6.38	↓6.28	↑6.77	↓6.15	↑6.46	↓5.69**	↓6.00
Week 98	6.08		↓6.00	—	↓6.00	—	↓5.85	—
Week 104	—	6.00	—	↑6.08	—	↑6.15	—	6.00

** $p < 0.01$

I. NECROPSY

Gross pathology

Treatment-related macroscopic findings were seen in males fed 20000 ppm and/or 6000 ppm in the kidneys, liver, prostate and testes. These findings consisted of a minor increase in incidence of enlarged kidneys, single masses in the liver, firmness of the prostate and a reduction in the incidence of reduced testes.

Additional findings were not considered to be treatment related.

Organ weights

Significant lower relative adrenal gland weight was noted at the interim kill in females fed 20000 ppm and 6000 ppm glyphosate acid. Furthermore, the liver weight was significantly lower at the interim kill in males fed 20000 ppm glyphosate acid. Significantly reduced the kidney weights were recorded at 6000ppm. The absolute ovary weight at the highest dose group was reduced although the ovary weight relative to body weight did not appear as significantly reduced.

There were no other significant and dose-related effects on organ weights.

Table 5.5-14 Glyphosate Acid: Two Year Dietary Toxicity and Oncogenicity Study in Rats ([redacted], 2001): Organ weight findings

WEEK 53		Males				Females			
ppm		0	2000	6000	20000	0	2000	6000	20000
Number of animals		11	11	11	12	12	12	12	11
Adrenal glands	Terminal body weight (g)	682.6	↓672.9	↑731.0	↓627.2	346.5	↑351.2	↓344.8	↓309.5
	Organ weight (g)	0.062	↓0.057	↑0.060	↓0.058	0.077	↓0.072	↓0.068*	↓0.063**
	Organ weights adjusted to b.w.	0.062	↓0.058	↓0.060	↓0.058	0.076	↓0.071	↓0.067*	↓0.065**
	Historical control data [range] 10 studies from 1990 to 1996 (116 control animals, female)	Not reported				0.050 – 0.16			
Brain	Terminal body weight (g)	682.6	↓672.9	↑731.0	↓627.2	346.5	↑351.2	↓344.8	↓309.5
	Organ weight (g)	2.26	↓2.20	↑2.27	↓2.23	2.04	↓2.02	↓2.01	↓1.98
	Organ weights adjusted to b.w.	2.26	↓2.21	↓2.24	↓2.25	2.03	↓2.01	↓2.00	↓2.02
Epididymidis	Terminal body weight (g)	682.6	↓672.9	↑731.0	↓627.2				
	Organ weight (g)	1.411	↑1.419	↑1.155	↓1.373	-	-	-	-
	Organ weights adjusted to b.w.	1.410	↑1.413	↑1.448	↓1.364	-	-	-	-
Heart	Terminal body weight (g)	682.6	↓672.9	↑731.0	↓627.2	346.5	↑351.2	↓344.8	↓309.5
	Organ weight (g)	1.758	↓1.679	↑1.863	↓1.686	1.152	↓1.132	↓1.152	↓1.068
	Organ weights adjusted to b.w.	1.758	↓1.684	↑1.761	↑1.782	1.136	↓1.108	↑1.140	↓1.132
Kidneys	Terminal body weight (g)	682.6	↓672.9	↑731.0	↓627.2	346.5	↑351.2	↓344.8	↓309.5
	Organ weight (g)	3.99	↑4.00	↑4.59**	↓3.75	2.39	↑2.45	↓2.34	↓2.25
	Organ weights adjusted to b.w.	3.95	↑4.01	↑4.38*	↓3.91	2.35	↑2.39	↓2.32	↑2.38
Liver	Terminal body weight (g)	682.6	↓672.9	↑731.0	↓627.2	346.5	↑351.2	↓344.8	↓309.5
	Organ weight (g)	24.4	↓23.5	↑25.9	↓21.3**	11.4	↑11.6	↓11.2	↓10.2*
	Organ weights adjusted to b.w.	24.2	↓23.5	↑24.4	↓22.4*	11.2	↑11.3	↓11.0	↓11.0
Ovaries	Terminal body weight (g)					346.5	↑351.2	↓344.8	↓309.5
	Organ weight (g)	-	-	-	-	0.099	↓0.092	↓0.094	↓0.078*
	Organ weights adjusted to b.w.	-	-	-	-	0.096	↓0.087	↓0.092	↓0.087
Spleen	Terminal body weight (g)	682.6	↓672.9	↑731.0	↓627.2	346.5	↑351.2	↓344.8	↓309.5
	Organ weight (g)	1.409	↓1.355	↑1.463	↓1.296	0.844	↓0.830	↓0.760	↓0.801
	Organ weights adjusted to b.w.	1.390	↓1.364	↑1.378	↓1.380	0.837	↓0.818	↓0.754	↓0.833
Testes	Terminal body weight	682.6	↓672.9	↑731.0	↓627.2				
	Organ weight	3.64	↓3.60	↑3.67	↓3.53	-	-	-	-
	Organ weights adjusted to b.w.	3.64	↓3.57	↓3.61	↓3.57	-	-	-	-

Table 5.5-14 Glyphosate Acid: Two Year Dietary Toxicity and Oncogenicity Study in Rats ([redacted], 2001): Organ weight findings

WEEK 53		Males				Females			
ppm		0	2000	6000	20000	0	2000	6000	20000
Number of animals		11	11	11	12	12	12	12	11
Uterus with cervix	Terminal body weight					346.5	↑351.2	↓344.8	↓309.5
	Organ weight	-	-	-	-	1.016	↓0.769	↓0.824	↓0.999
	Organ weights adjusted to b.w.	-	-	-	-	1.053	↓0.828	↓0.853	↓0.866

*/** Significantly different from the control at 5 % and 1 % level of probability, respectively.

Histopathology

A minor increase in the incidence but not severity of proliferative cholangitis in the liver was present in males fed 20000 ppm glyphosate acid at interim and terminal kill (see table below).

Moreover, in males fed 20000 ppm glyphosate acid an increased incidence of hepatitis and periodontal inflammation was observed. The incidence of prostatitis was higher than the control group in all treated males and there was a decrease in the incidence of tubular degeneration of the testis in males fed 20000 ppm glyphosate acid. The incidence of prostatitis was within historical background levels in all treated groups but, as the control value in this study was low, the relationship to treatment at the high dose level cannot be entirely dismissed.

The main changes in interim and terminal kill males and, to a lesser extent, females fed 20000 ppm glyphosate acid, were observed in the kidney. These changes consisted of slight increased incidence of papillary necrosis with varying degrees of mineralisation of the papilla and/or transitional cell hyperplasia. There was also a very small increased incidence of papillary mineralisation only (males and females fed 20000 ppm glyphosate acid) and transitional cell hyperplasia alone (20000 ppm males only).

All other observed differences in the incidence of findings either fall within the historical background level or are considered to be unrelated to the treatment with glyphosate acid.

Table 5.5-15 Glyphosate Acid: Two Year Dietary Toxicity and Oncogenicity Study in Rats ([redacted], 2001): Summary of histopathological findings

Finding	Dietary concentration of glyphosate (ppm)									
	Males (n=64)					Females (n=64)				
	Historical Control [%]	0	2000	6000	20000	Historical Control	0	2000	6000	20000
Liver Proliferative cholangitis	-	56	57	55	64	-	55	58	59	61
Hepatitis	4.7 [2 - 8]§	8	6	9	13	-	6	7	4	6
Hepatocellular adenoma	0-11.5#	0	2	0	5	-	0	0	1	0
Liposarcoma	-	0	0	1	0	-	0	0	0	0
Kidney Papillary necrosis	0.4 [0 – 2]	0	1	0	14	-	0	1	2	5
Transitional cell hyperplasia	-	2	3	0	5	-	3	1	0	1

Table 5.5-15 Glyphosate Acid: Two Year Dietary Toxicity and Oncogenicity Study in Rats ([redacted], 2001): Summary of histopathological findings

Finding	Dietary concentration of glyphosate (ppm)									
	Males (n=64)					Females (n=64)				
	Historical Control [%]	0	2000	6000	20000	Historical Control	0	2000	6000	20000
Prostate Prostatitis	23.4 [13 – 35]	13	22	23	37	-	-	-	-	-
Testis Unilateral tubular degeneration	-	18	13	18	5	-	-	-	-	-
Periodontal inflammation	-	25	27	23	42	-	18	24	32	28

n = number of animals per group
Historical control (mean and [range])
26 studies from the same laboratory between 1984-2003; §: 12 studies from the same laboratory between 1990 to 2001

There was no evidence of carcinogenicity and no differences between the groups in tumour incidence. An overview on the total number of tumour bearing animals is provided below.

Table 5.5-16 Glyphosate Acid: Two Year Dietary Toxicity and Oncogenicity Study in Rats ([redacted], 2001): Intergroup comparison of tumour bearing animals

	Males				Females			
ppm	**0**	**2000**	**6000**	**20000**	**0**	**2000**	**6000**	**20000**
Removal reason: Intercurrent								
Animals on Study	64	64	64	64	64	64	64	64
Animals completed	37	36	35	26	20	24	13	23
Number of tumour bearing animals	23	20	24	19	20	21	12	22
Animals with malignant tumours	9	6	10	3	12	15	6	13
Benign tumours	19	16	20	17	18	17	10	18
Multiple tumours	11	9	11	7	16	16	8	12
Single tumours	12	11	13	12	4	5	4	10
Multiple malignant tumours	2	1	0	0	0	3	2	0
Multiple benign tumours	10	7	6	7	8	6	3	3
Metastatic tumours	5	3	3	1	8	13	3	10
Removal reason: Interim								
Animals on study	64	64	64	64	64	64	64	64
Animals completed	11	11	11	12	12	12	12	11
Number of tumour bearing animals	2	2	0	1	7	4	5	4
Animals with malignant tumours	0	0	0	0	0	0	0	0
Benign tumours	2	2	0	1	7	4	5	4
Multiple tumours	0	0	0	0	0	1	0	0
Single tumours	2	2	0	1	7	3	5	4
Multiple malignant tumours	0	0	0	0	0	0	0	0
Multiple benign tumours	0	0	0	0	0	1	0	0
Metastatic tumours	0	0	0	0	0	0	0	0
Removal reason: Terminal								
Animals on study	64	64	64	64	64	64	64	64
Animals completed	16	17	18	26	32	28	39	30

Table 5.5-16 Glyphosate Acid: Two Year Dietary Toxicity and Oncogenicity Study in Rats ([redacted], 2001): Intergroup comparison of tumour bearing animals

	Males				Females			
ppm	**0**	**2000**	**6000**	**20000**	**0**	**2000**	**6000**	**20000**
Removal reason: Intercurrent								
Number of tumour bearing animals	14	14	16	23	29	26	39	29
Animals with malignant tumours	1	6	1	7	10	10	22	11
Benign tumours	14	13	16	21	26	25	36	28
Multiple tumours	7	8	6	13	19	17	29	13
Single tumours	7	6	10	10	10	9	10	16
Multiple malignant tumours	0	3	0	0	2	3	2	1
Multiple benign tumours	7	4	5	10	16	12	17	9
Metastatic tumours	1	4	0	4	5	7	15	6
Animals on study	64	64	64	64	64	64	64	64
Number of tumour bearing animals	39	36	40	43	56	51	56	55
Animals with malignant tumours	10	12	11	10	22	25	28	24

3. Assessment and conclusion

Assessment and conclusion by applicant:
In conclusion, glyphosate acid was not carcinogenic in the Wistar rats following continuous dietary exposure of up to 20000 ppm for 24 months (corresponding to 1214 mg/kg bw/day in males and 1498 mg/kg bw/day in females). The NOAEL for toxicity is 6000 ppm (corresponding to 361 mg/kg bw/day in males and 437 mg/kg bw/day in females). In addition, there was no evidence of neurotoxicity.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.5/003
Report author	[redacted]
Report year	1997
Report title	Combined Chronic Toxicity/Carcinogenicity Study of Glyphosate Technical in Sprague Dawley Rat
Report No	1231
Document No	Not reported
Guidelines followed in study	OECD 453 (1981)
Deviations from current test guideline (OECD 453, 2018)	Haematology was performed without determining haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, prothrombin time, activated partial thromboplastin time, platelet count; clinical chemistry was performed without determining inorganic phosphorous, calcium, chloride, sodium,

	potassium, cholesterol, creatinine, T3, T4, TSH; organ weights were not determined for all animals; weights of heart, spleen, (para)thyroids and uterus were not determined; histopathology was performed without determining Harderian gland, cervix, coagulating gland, gall bladder, lacrimal gland, upper respiratory tract, vagina.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Invalid: - Information on test substance identification not available and several deviations from the current OECD TG 453 evident.
Category study in AIR 5 dossier (L docs)	Category 3b

2. Full summary

The chronic toxicity and carcinogenic potential of Glyphosate technical was assessed in a 24-month feeding study in male and female Sprague Dawley rats. Groups of 50 rats per sex received daily dietary doses of 0, 3000, 15000, and 25000 ppm Glyphosate technical (equivalent to mean achieved dose levels of 0, 0.15, 0.78 and 1.29 g/kg bw/day (males) and 0, 0.21, 1.06 and 1.74 g/kg bw/day (females)). In addition 20 rats/sex/group were included for interim sacrifice at Week 52, to study non-neoplastic histopathological changes with a different high dose level of 30000 ppm. The dietary doses correspond to 0.18, 0.92 and 1.92 g/kg bw/day (males) and 0.24, 1.13 and 2.54 g/kg bw/day (females) for 3000, 15000 and 30000 ppm, respectively. Observations covered clinical signs, body weight, food consumption, haematology, clinical chemistry and urinalysis as well as organ weights, necropsy and histopathological examination.

There were no treatment-related deaths or clinical signs in any of the dose-groups. Moreover, there were no treatment-related effects on food consumption noted. Significantly reduced body weight gain that lasted throughout study until termination was observed in males receiving the highest dose. In all other groups body weight gain was comparable to the control at termination. Apart from increased alkaline phosphatase levels in the high dose of the carcinogenicity study at study termination, all other significant changes observed in haematological, biochemical and physio-pathological parameters of urine were within the range of the historical control data and hence appear to be of no biological significance.

Gross pathology and histopathological examination revealed no treatment-related and dose-dependent effects. Regarding organ weights, significant and dose-dependent effects after 52 weeks were found only in animals dosed at 30000 ppm. After 104 weeks the effects were seen as well in the mid dose group. Increased organ weights that were observed after 52 weeks but not after 104 weeks could be due to the different high dose level, e.g. 25000 ppm and 30000 ppm, respectively.

In conclusion, Glyphosate technical was not carcinogenic in the Sprague Dawley rats following continuous dietary exposure of up to 1.29 g/kg bw/day for males and 1.74 g/kg bw/day for females for 24 months. The NOAEL for toxicity is 1.29 g/kg bw/day for males and 1.74 g/kg bw/day for females.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate technical
Description:	No data given in the report
Lot/Batch #:	No data given in the report
Purity:	No data given in the report
Stability of test compound:	No data given in the report
2. Vehicle and/ or positive control:	Diet
3. Test animals:	
Species:	Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	Approx. 6 weeks
Sex:	Males and females
Weight at dosing:	Males: 70.0 – 93.2 g, females: 70.0 – 90.6 g
Acclimation period:	One week
Diet/Food:	Powdered rat feed (Lipton India Ltd, India), *ad libitum*
Water:	Filtered pure water, *ad libitum*
Housing:	Initially in groups of five in polypropylene cages, in groups of three from Week 24 to 52 and in groups of two from Week 53 to termination
Environmental conditions:	Temperature: 22 - 25 °C Humidity: 50 - 70 % Air changes: 10 - 15/hour 12 hours light/dark cycle

B: Study design and methods

In life dates: 1994-06-09 to 1996-06-12

Animal assignment and treatment:
In a combined chronic toxicity and carcinogenicity study groups of 50 Sprague Dawley rats per sex received daily dietary doses of 0, 3000, 15000 and 25000 ppm (equivalent to mean achieved dose levels of 0, 0.15, 0.78 and 1.29 g/kg bw/day (males) and 0, 0.21, 1.06 and 1.74 g/kg bw/day (females)) Glyphosate technical for two years. In addition, for the control and each dose group 20 rats per sex were included for interim sacrifice in Week 52 to study non-neoplastic histopathological changes (chronic toxicity study). Selected dose levels were the same except for the highest dose which was 30000 ppm. Here the dietary doses correspond to 0.18, 0.92 and 1.92 g/kg bw/day (males) and 0.24, 1.13 and 2.54 g/kg bw/day (females) for 3000, 15000 and 30000 ppm, respectively.

Test diets were prepared weekly by mixing appropriate amounts of the test substance with the basal diet. The stability and homogeneity of the test substance in food was determined in-house stability study at all dose levels before the start of dosing. Analyses for achieved concentrations were performed monthly during the study period.

Clinical observations
Rats were examined for toxic signs once and pre-terminal deaths twice a day. Ophthalmic examination was done at the start of the study, at interim sacrifice and at termination in the control and high dose group.

Body weight

Individual body weights were recorded on Day 0, at weekly intervals thereafter until the end of Week 13 and every 4 weeks thereafter until termination.

Food consumption and compound intake
Food consumption was recorded once weekly for each group from Week 1 to Week 13 and subsequently in Week 25, 38, 51, 65, 78, 92 and 104.

Haematology and clinical chemistry
Haematology
Individual blood samples were collected from 20 rats/sex/group of the main groups at 3, 6, 12, 18 and 24 months and from all surviving animals of the satellite group at 12 months. Before sampling animals were fasted overnight. The following parameters were measured: Haemoglobin, erythrocyte count, PCV, thrombocytes, total leukocyte count and differential leukocyte count.

Blood chemistry
Individual plasma samples were collected from 10 rats/sex/group of the main groups at 6, 12, 18 and 24 months and from all surviving animals of the satellite group at 12 months. Before sampling animals were fasted overnight. The following parameters were measured: Total serum proteins, albumin, ALT, AST, GGTP, SAP, blood urea nitrogen and blood glucose.

Urinalysis
Individual urine samples were collected from 20 rats/sex/group of the main groups at 3, 6, 12, 18 and 24 months and from all surviving animals of the satellite group at 12 months. The following measurements were made: Specific gravity, volume, appearance, pH, protein, glucose, occult blood, ketones, microscopy of sediments.

Sacrifice and pathology
Necropsy was performed on all animals at scheduled termination.

The following organ weights were determined from 10 rats per sex per main group and on all animals of the satellite groups: adrenals, brain, gonads, kidneys and liver.

Histopathological examination was carried out on all tissues collected at interim sacrifice, control and high dose groups; all pre-terminally dead and moribund sacrificed rats of the low and mid dose groups and on all lesions of the terminally sacrificed rats from the low and mid dose groups.

Tissue samples were taken from the following organs of all animals: adrenals, aorta, body cavities, brain, caecum, colon, duodenum, epididymides, eyes (both), femur, heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes (mesenteric and mandibular), mammary gland, oesophagus, ovaries, pancreas, pituitary, preputial gland, prostrate, rectum, salivary glands, sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord, spleen, sternum with bone marrow, stomach, testes, thymus, thyroid/parathyroid, trachea, urinary bladder and uterus.

Statistics
Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958). Animals withdrawn from study during the interval (those taken for moribund sacrifice) are taken into consideration by giving enough weightage.

The incidence of neoplasms was analysed by Life table analysis for fatal tumour incidence and Peto's incidental tumour analysis.

In addition to these tests the Fisher exact test for pairwise comparisons and the Cochran Armitage linear trend test for dose response trends were carried out. All reported P-values for the tumour incidence analysis

are one-sided.

The biochemical, haematological and organ weight data was analyzed for significance using Student 't' test or Cochran 't' test.

II. RESULTS

A. ANALYSIS OF DOSE FORMULATIONS

Analyses for concentrations showed that the diet preparations recovered 86.1 - 98.3 % of the target concentration. Thus, the concentrations of the test substance in the test diets were within acceptable limits. Analyses for homogeneity recovered 87.5 - 90.0 % for 3000 ppm, 91.7 - 93.9 % for 15000 ppm, 94.3 - 95.1 % for 25000 ppm and 91.8 - 92.6 % for 30000 ppm. Hence, the results indicated a good homogeneity. Moreover, stability analyses showed that recovery one month after diet preparation ranged between 87.5 and 95.0 %.

B. MORTALITY

No treatment-related clinical signs or deaths were observed in the satellite groups, e.g. the chronic toxicity study.

In the carcinogenicity study, e.g. after 104 weeks, male animals of the high dose group exhibited slight but statistically insignificant higher mortalities.

The numbers of pre-terminal deaths in the main group are displayed in the table below:

Table 5.5-17 Combined Chronic Toxicity/Carcinogenicity Study of Glyphosate Technical in Sprague Dawley Rat ([redacted], 1997): Cumulated mortalities after 104-week dietary exposure to Glyphosate technical*

Sex	Dose group (ppm)			
	0	3000	15000	25000
Male	16/50	17/50 (2)	18/50 (4)	23/50 (14)
Female	19/50	20/50 (2)	20/50 (2)	25/50 (12)

* Values in parentheses indicate increases in mortality compared to control in percent.

C. CLINICAL OBSERVATIONS

No significant toxic signs were observed in treated or control groups.

D. BODY WEIGHT

Significantly reduced body weight gain that lasted throughout study until Week 104 was observed in males receiving the highest dose. In all other groups body weight gain was comparable to the control at termination. At 104 weeks the reduction of body weight was 8 % in males of the satellite group and 10 % in males of the main group. The effect on body weight was not considered adverse as the decrease of body weight was only about 10 % and no general toxic signs or effects on organ function were observed. Furthermore, the effect was observed at doses above 1000 mg/kg bw/day.

Table 5.5-18 Combined Chronic Toxicity/Carcinogenicity Study of Glyphosate Technical in Sprague Dawley Rat ([redacted], 1997): Group mean body weights (satellite group)

Timepoint	Sex	Males				Females			
	Dose (ppm)	0	3000	15000	30000	0	3000	15000	30000
	No. of rats	20	20	20	20	20	20	20	20
Week 0		80.27 ± 5.97	↓78.45 ± 5.91	↓78.74 ± 5.20	↑81.09 ± 5.23	78.83 ± 4.88	↓77.56 ± 5.39	↓77.01 ± 5.91	↓77.83 ± 4.97
Week 1		106.20 ± 7.45	↓102.96 ± 6.89	↓101.15 ± 5.36	↓102.76 ± 7.08	97.22 ± 4.62	↑99.69 ± 4.03	↑98.75 ± 5.05	↑99.70 ± 4.31
Week 2		134.25 ± 10.67	↓132.56 ± 9.27	↓128.79 ± 11.39	↓132.95 ± 11.55	121.10 ± 6.93	↑125.20 ± 5.72	↑125.55 ± 7.17	↑125.91 ± 6.94
Week 3		163.89 ± 17.06	↓161.39 ± 11.78	↓160.42 ± 9.57	↓160.36 ± 14.26	143.97 ± 8.36	↑150.44 ± 7.39	↑150.43 ± 8.10	↑149.81 ± 7.62
Week 13		297.01 ± 30.02	↓295.50 ± 21.82	↓278.93 ± 21.44	↓266.65 ± 27.71	212.94 ± 17.66	↓212.78 ± 11.58	↑221.59 ± 12.82	↓203.96 ± 13.33
Week 52		408.89 ± 31.75	↑409.23 ± 32.55	↓389.42 ± 31.63	↓384.32 ± 38.06	268.24 ± 28.29	↓253.65 ± 16.80	↑268.85 ± 23.12	↓259.47 ± 24.17

Table 5.5-19: Combined Chronic Toxicity/Carcinogenicity Study of Glyphosate Technical in Sprague Dawley Rat ([redacted], 1997): Group mean body weights (main group)

Timepoint	Sex	Males				Females			
	Dose (ppm)	0	3000	15000	30000	0	3000	15000	30000
	No. of rats	20	20	20	20	20	20	20	20
Week 0		80.27 ± 6.22	↑80.68 ± 5.99	↓78.69 ± 5.65	↓80.01 ± 4.91	79.69 ± 5.77	↓79.34 ± 5.10	↓78.15 ± 4.87	↓77.98 ± 4.84
Week 1		103.52 ± 7.88	↑104.03 ± 7.71	↑104.33 ± 7.49	↑105.15 ± 7.18	101.02 ± 5.38	↑101.13 ± 5.60	↑101.56 ± 6.01	↑101.64 ± 6.31
Week 2		133.72 ± 12.36	↑134.29 ± 9.33	133.72 ± 9.12	↓132.34 ± 8.12	127.92 ± 7.44	↑129.04 ± 5.87	↓126.37 ± 7.40	↓126.71 ± 7.48
Week 3		163.95 ± 15.44	↑164.20 ± 11.55	↑164.26 ± 8.25	↓162.92 ± 8.70	155.85 ± 9.13	↑157.37 ± 6.64	↓154.25 ± 7.93	↓155.05 ± 8.73
Week 13		396.67 ± 31.82	↓285.08 ± 18.81	↓277.94 ± 24.92	↓270.84 ± 14.61	217.42 ± 17.46	↓213.09 ± 22.21	↓203.41 ± 15.10	↓206.27 ± 15.56
Week 53		403.19 ± 31.75	↓398.15 ± 32.55	↓384.34 ± 33.76	↓374.76 ± 29.85	280.45 ± 36.54	↓269.18 ± 29.30	↓257.82 ± 23.69	↓261.64 ± 26.85
Week 104		406.05 ± 61.32	↓385.51 ± 57.94	↓391.09 ± 55.86	↓366.58 ± 47.54	313.72 ± 58.93	↑315.60 ± 58.03	↓288.13 ± 42.77	↓284.52 ± 43.31

E. FOOD CONSUMPTION AND COMPOUND INTAKE

There were no treatment-related effects on food consumption for either sex or group noted during the study. The results show a higher test material intake for females when compared to males for each dose level. The mean intake in the chronic toxicity study for each dose group is 0.18, 0.92 and 1.92 g/kg bw/day (males) and 0.24, 1.13 and 2.54 g/kg bw/day (females) for 3000, 15000 and 30000 ppm, respectively.

The mean intake in the carcinogenicity study for each dose group is 0.15, 0.78 and 1.29 g/kg bw/day (males) and 0.21, 1.06 and 1.74 g/kg bw/day (females) for 3000, 15000 and 25000 ppm, respectively.

The group mean achieved doses are summarised below.

Table 5.5-20 Combined Chronic Toxicity/Carcinogenicity Study of Glyphosate Technical in Sprague Dawley Rat ([redacted], 1997): Group mean achieved dose levels

Dose group	Dietary concentration (ppm)	Mean achieved dose level (g/kg bw/day)*	
		Males	Females
		Chronic toxicity study (52 weeks)	
low	3000	0.18	0.24
mid	15000	0.92	1.13
high	30000	1.92	2.54
		Carcinogenicity study (104 weeks)	
low	3000	0.15	0.21
mid	15000	0.78	1.06
high	25000	1.29	1.74

* Calculations were done with values from Week 13 (chronic) and Week 25 (carcinogenicity)

F. OPHTHALMOLOGICAL EXAMINATION

Ophthalmological examinations revealed no abnormalities.

G. LABORATORY INVESTIGATION

Haematological examination did not reveal any abnormalities attributable to the treatment. Regarding the clinical chemical investigations, a significant increase in the alkaline phosphatase level was only seen in the high dose of the carcinogenicity study at study termination (see table below).

Other significant changes observed in haematological and biochemical parameters were within the range of the historical control data and hence appear to be of no biological significance.

Table 5.5-21 Combined Chronic Toxicity/Carcinogenicity Study of Glyphosate Technical in Sprague Dawley Rat ([redacted], 1997): Statistically significant changes in blood chemistry

	Dose group (ppm)							
	Males				Females			
	0	3000	15000	25000	0	3000	15000	25000
Alkaline phosphatase								
Month 6	25.58 ± 3.20	24.97 ± 3.61	24.85 ± 3.77	23.07 ± 2.75	24.96 ± 3.22	25.25 ± 3.70	25. 2 ± 3.26	25.11 ± 3.71
Month 12	25.64 ± 5.28	25.96 ± 3.84	27.64 ± 3.65	22.88 ± 3.12	19.04 ± 2.87	25.35* ± 3.62	28.3* ± 3.61	22.88* ± 3.72
Month 18	27.7 ± 4.55	25.94 ± 3.42	28.73 ± 2.89	26.68 ± 3.85	24.47 ± 5.56	28.42 ± 3.97	27.71 ± 3.84	25.28 ± 2.32
Month 24	26.04 ± 4.96	26.75 ± 4.22	28.42* ± 4.57	47.71* ± 5.70	24.87 ± 4.19	26.95* ± 3.00	25.75 ± 4.25	53.86* ± 5.49

*= $p \leq 0.05$

H. URINALYSIS

Urinalysis did not reveal any abnormalities attributable to the treatment.

I. NECROPSY

Gross pathology

There were no treatment-related macroscopic findings observed during the study period.

Organ weights

Significant and dose-dependent effects in the chronic toxicity study were found in both sexes of the high-dose group. In males, weights of kidneys, brain and testes were increased. In females, in addition to kidneys and brain, the liver weight was increased as well.

In the carcinogenicity study which lasted 52 weeks longer, significant and dose-dependent effects in males consisted of increased weight of brain and testes in the mid and high dose group. Effects on the kidneys were not observed, perhaps due to the lower dose level in the highest group compared to the chronic toxicity study, e.g. 25000 ppm to 30000 ppm, respectively.

In females, significant and dose-dependent effects after 24 months occurred only in kidneys. Like for male animals, this increase could be due to the different high dose levels.

Table 5.5-22 Combined Chronic Toxicity/Carcinogenicity Study of Glyphosate Technical in Sprague Dawley Rat ([redacted], 1997): Organ weights

mg/kg bw/d		Body Weight (g)	Liver (g)	Kidneys (g)	Brain (g)	Adrenals (g)	Testes (g)	Ovaries (g)
Satellite group (males) absolute values	0	393.70 ± 31.39	14.17 ± 1.73	3.16 ± 0.34	2.24 ± 0.11	0.067 ± 0.013	3.43 ± 0.30	-
	3000	393.07 ± 31.63	13.76 ± 1.75	3.01 ± 0.29	2.09* ± 0.26	0.059 ± 0.014	3.38 ± 0.35	-
	15000	372.79 ± 30.85	13.02 ± 1.92	2.98 ± 0.36	2.22 ± 0.17	0.058 ± 0.01	3.01* ± 0.53	-
	30000	369.52 ± 34.53	14.35 ± 2.80	3.20 ± 0.43	2.31 ± 0.12	0.059 ± 0.014	3.47 ± 0.41	-
Satellite group (males) relative values	0	393.69 ± 31.39	3.602 ± 0.363	0.805 ± 0.075	0.573 ± 0.048	0.017 ± 0.004	0.874 ± 0.087	-
	3000	393.08 ± 31.63	3.507 ± 0.416	0.768 ± 0.080	0.536 ± 0.081	0.015 ± 0.004	0.866 ± 0.119	-
	15000	372.76 ± 30.85	3.525 ± 0.668	0.806 ± 0.123	0.600 ± 0.068	0.058 ± 0.010	0.812 ± 0.151	-
	30000	369.52 ± 34.53	3.863 ± 0.450	0.866* ± 0.088	0.629* ± 0.064	0.016 ± 0.004	0.943* ± 0.098	-
Satellite group (females) absolute values	0	258.97 ± 26.83	8.63 ± 1.45	1.88 ± 0.24	1.94 ± 0.15	0.068 ± 0.014	-	0.115 ± 0.036
	3000	244.31 ± 14.87	7.89 ± 0.78	1.84 ± 0.09	2.00 ± 0.11	0.053* ± 0.012	-	0.121 ± 0.120
	15000	259.13 ± 21.71	8.15 ± 0.96	1.90 ± 0.14	2.06 ± 0.12	0.060* ± 0.009	-	0.133 ± 0.160
	30000	252.84 ± 23.56	9.25 ± 1.42	1.98 ± 0.16	2.08* ± 0.11	0.061 ± 0.01	-	0.123 ± 0.071
Satellite group (females) relative values	0	258.97 ± 26.93	3.324 ± 0.412	0.726 ± 0.070	0.756 ± 0.098	0.026 ± 0.006	-	0.045 ± 0.015
	3000	244.31 ± 14.87	3.229 ± 0.270	0.756 ± 0.047	0.819* ± 0.045	0.022* ± 0.006	-	0.049 ± 0.046
	15000	259.13 ± 21.70	3.163 ± 0.425	0.737 ± 0.057	0.799 ± 0.065	0.023 ± 0.004	-	0.037 ± 0.009
	30000	252.84 ± 23.56	3.662* ± 0.459	0.788* ± 0.073	0.828* ± 0.067	0.024 ± 0.006	-	0.049 ± 0.029
Main group (males) absolute values	0	445.17 ± 31.44	19.92 ± 2.46	3.91 ± 0.34	2.28 ± 0.13	0.080 ± 0.019	3.46 ± 0.55	-
	3000	422.03 ± 34.94	17.32 ± 3.88	3.41 ± 0.83	2.25 ± 0.14	0.089 ± 0.007	3.45 ± 1.16	-
	15000	397.69 ± 35.94	16.00* ± 2.50	3.48 ± 0.35	2.28 ± 0.14	0.077 ± 0.017	3.91 ± 0.83	-
	25000	379.14 ±	16.08* ±	3.36* ±	2.34 ± 0.16	0.069 ±	3.65 ± 0.45	-

Table 5.5-22 Combined Chronic Toxicity/Carcinogenicity Study of Glyphosate Technical in Sprague Dawley Rat ([redacted], 1997): Organ weights

mg/kg bw/d		Body Weight (g)	Liver (g)	Kidneys (g)	Brain (g)	Adrenals (g)	Testes (g)	Ovaries (g)
		19.21	2.52	0.34		0.006		
Main group (males) relative values	0	445.17 ± 31.44	4.45 ± 0.66	0.88 ± 0.07	0.51 ± 0.06	0.018 ± 0.004	0.78 ± 0.13	
	3000	422.03 ± 34.94	4.13 ± 1.00	0.81 ± 0.20	0.54 ± 0.05	0.021 ± 0.002	0.81 ± 0.25	-
	15000	397.69 ± 35.94	4.05 ± 0.69	0.88 ± 0.07	0.58* ± 0.058	0.019 ± 0.004	0.99* ± 0.24	-
	25000	379.40 ± 19.21	4.25 ± 0.68	0.89 ± 0.10	0.62* ± 0.06	0.018 ± 0.002	0.97* ± 0.12	-
Main group (females) absolute values	0	319.12 ± 32.62	14.06 ± 2.78	2.53 ± 0.36	2.18 ± 0.13	0.099 ± 0.009	-	0.174 ± 0.07
	3000	318.83 ± 41.15	12.00 ± 1.76	2.64 ± 0.10	2.28* ± 0.04	0.091 ± 0.009	-	0.180 ± 0.03
	15000	300.95 ± 13.72	11.23* ± 1.01	2.40 ± 0.12	2.19 ± 0.06	0.076* ± 0.01	-	0.125 ± 0.07
	25000	286.38 ± 38.69	12.65 ± 2.05	2.61 ± 0.39	2.10 ± 0.08	0.085 ± 0.023	-	0.187 ± 0.060
Main group (females) relative values	0	319.12 ± 32.62	4.42 ± 0.77	0.79 ± 0.10	0.69 ± 0.07	0.031 ± 0.004	-	0.056 ± 0.025
	3000	318.83 ± 41.15	3.79* ± 0.52	0.84 ± 0.07	0.73 ± 0.08	0.029 ± 0.003	-	0.057 ± 0.01
	15000	300.95 ± 13.72	3.74* ± 0.43	0.80 ± 0.07	0.73 ± 0.04	0.025* ± 0.003	-	0.042 ± 0.002
	25000	286.38 ± 38.69	4.49 ± 0.96	0.92* ± 0.14	0.76 ± 0.11	0.030 ± 0.008	-	0.066 ± 0.026

*= $p \leq 0.05$

Histopathology
Histopathological changes were found at all dose levels including control. Based on histopathology observation no treatment-related effects were observed.

Neoplastic changes
There were no treatment-related neoplasms observed.

3. Assessment and conclusion

Assessment and conclusion by applicant:
Based on the mild toxic effects on body weight gain and the increased organ weights without histopathological changes the NOAEL in rats after chronic exposure to Glyphosate technical for 24 month is 25000 ppm (corresponding to 1290 mg/kg bw/day for males and 1740 mg/kg bw/day for females). It is concluded that Glyphosate technical is not carcinogenic in rats.
Based on the deviations such as lack of information on test substance identification, storage conditions and several deviations from the current OECD TG 453 the study is not considered reliable for hazard and risk assessment. Drawing any reliable conclusion concerning a NOAEL from the study is not possible.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.5/004
Report author	[redacted]
Report year	1997
Report title	HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats
Report No	[redacted] 94-0150
Document No	NA
Guidelines followed in study	OECD 453 (1981), JMAFF 59 NohSan 3850 (1984), US-EPA (1989)
Deviations from current test guideline (OECD 453, 2018)	Yes, mortality was observed only once per week; prothrombin time and activated partial thromboplastin time were not investigated; organ weights of epididymides, heart, ovaries, spleen and uterus were not measured; gall bladder, lacrimal gland and upper respiratory tract were not investigated histopathologically. No historical control data available.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The chronic toxicity and carcinogenic potential of HR-001 (Glyphosate technical) was assessed in a 24-month feeding study in male and female Sprague-Dawley rats. Groups of 50 rats per sex received daily dietary doses of 0, 3000, 10000, and 30000 ppm HR-001 (equivalent to 0, 104, 354 and 1127 mg/kg bw/day for males and 0, 115, 393 and 1247 mg/kg bw/day for females). In addition, 30 rats/sex/group were included for interim sacrifice at 26, 52 and 78 weeks to study non-neoplastic histopathological changes. Observations covered clinical signs, body weight, food consumption, haematology, clinical chemistry and urinalysis as well as organ weights, necropsy and histopathological examination.

There were no treatment-related deaths in any of the dose-groups. Clinical observations consisted of loose faeces together with soiled fur in the perianal region in the high dose group as well as increased incidences of tail mass in the mid and high dose group. Moreover, decreases in body weight were observed in both sexes in the mid and high dose group along with a lower food consumption. Ophthalmological examinations, urinalysis and haematological and blood biochemical analyses did not demonstrate apparent toxicity of the test substance in either sex or group.

Necropsy supported the clinical signs of loose stool by increased incidences of distension of the caecum in the high dose group together with increased absolute and relative caecum weights in the mid and high dose group. Moreover, the increased incidences of thickened areas in the skin of the tail, corresponding to the increased incidences of tail mass, were histopathologically diagnosed as follicular hyperkeratosis and/or folliculitis/follicular abscess in the mid and high dose group.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate technical, Code: HR-001
Description:	White crystal
Lot/Batch #:	T-941209; T-950308
Purity:	97.56 %; 94.61 %
Stability of test compound:	No data given the report.

2. Vehicle and/or positive control: Diet

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley (Crj:CD)
Source:	[redacted]
Age:	5 weeks (males), 6 weeks (females)
Sex:	Males and females
Weight at dosing:	65 – 85 g
Acclimation period:	At least one week
Diet/Food:	MF Mash (Oriental Yeast Co., Ltd, Japan), *ad libitum*
Water:	Well water treated with sand and charcoal filter, HCl and UV rays, *ad libitum*
Housing:	In groups of ten animals of the same sex in wire-mesh stainless steel cages during the acclimatisation period. During the study males were housed in groups of 5 per cage until week 72, in groups of ≤3 until week 78 and individually thereafter. Females were housed in groups of five until week 78, and individually thereafter.
Environmental conditions:	Temperature: 24 ± 2 °C Humidity: 55 ± 15 % Air changes: 15/hour 12 hours light/dark cycle

B: Study design and methods

In life dates: 1994-12-19 to 1996-12-25

Animal assignment and treatment:
In a combined chronic toxicity and carcinogenicity study groups of 50 Sprague-Dawley rats/sex/group received daily dietary doses of 0, 3000, 10000 and 30000 ppm (equivalent to mean achieved dose levels of 0, 104.7, 354.0 and 1127.0 mg/kg bw/day in males and 0, 114.7, 393.0 and 1247.0 mg/kg bw/day in females) HR-001. In addition, 30 rats/sex/group were included for interim sacrifices at 26, 52 and 78 weeks.

Test diets were prepared weekly by mixing a known amount of the test substance with a small amount of basal diet. This pre-mix was then added to a larger amount of basal diet and blended by a blending machine. The stability of the test substance in food was previously determined in a 4-week dose-range finding study in mice. Homogeneity analyses were performed on samples of each dose level of the first diet preparation. Analyses for achieved concentrations were done for each dose level in monthly intervals.

Observations

Rats of all groups were examined for toxic signs and pre-terminal deaths once a day. In addition a detailed veterinary examination was made at least once per week. Ophthalmic examination was done at the start of the study and at termination.

Body weight
Individual body weights were recorded at weekly intervals until the end of Week 13 and every 4 weeks thereafter and before necropsy, except for dead or moribund satellite animals, which were discarded without body weight determination.

Food consumption and compound intake
Food consumption was measured for a period of three consecutive days weekly from Week 1 to 13 and every four weeks from Week 16 to 104. Mean individual food consumption, group mean food consumption and group compound intake were calculated.

Haematology and clinical chemistry
Blood samples were collected from 10 rats/sex/group of the satellite groups in Week 26, 52, from all surviving animals of the satellite group in Week 78 and from 10 rats/sex/group of the main group in week 104. Before sampling animals were fasted overnight. The following parameters were measured: hematocrit, haemoglobin, erythrocyte count, MCV, MCH, MCHC, platelet count, total leukocyte count, differential leukocyte count, alkaline phosphatase (ALP), glutamic-oxalacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), γ-glutamyl-transpeptidase, creatine phosphokinase, creatinine, blood urea nitrogen, total protein, glucose, albumin, globulin, albumin/globulin ratio, total bilirubin, total cholesterol, inorganic phosphorus, calcium, sodium, potassium, and chloride.

Urinalysis
Individual urine samples were collected from 10 rats/sex/group of the satellite groups in Week 26, 52, from all surviving animals of the satellite group in Week 78 and from 10 rats/sex/group of the main group in Week 104. The following measurements were made: density, volume, appearance, pH, protein, glucose, occult blood, ketones, urobilinogen, sediments.

Sacrifice and pathology
Necropsy and histopathological examinations were carried out on all tissues collected at interim and terminal sacrifice. The following organ weights were determined from all animals: adrenals, brain, caecum, kidneys, liver and testis.

Tissue samples were taken from the following organs: adrenals, aorta, bone & bone marrow (sternum and femur incl. joint), brain (cerebrum, cerebellum, pons and medulla oblongata), caecum, colon, duodenum, epididymides, eyes, gross lesions, Harderian glands, heart, ileum, jejunum, kidneys, liver, lungs, mammary gland, lymph nodes (cervical and mesenteric), oesophagus, ovaries, pancreas, pituitary, prostrate, rectum, salivary glands (submaxillary and sublingual), sciatic nerve, seminal vesicles and coagulating glands, skeletal muscle, skin (females only), spinal cord (cervical, thoracic and lumbar), spleen, stomach, testes, thymus, thyroid/parathyroid, trachea, urinary bladder, uterus (horns and cervix) and vagina.

Statistics
Statistical significance of the difference between the control group and the treated groups was estimated at 5 % and 1 % levels of probability.

The data of body weight (main group only), food consumption, urine specific gravity, urine volume, haematological parameters, blood biochemical parameters, and organ weights were evaluated by Bartlett's test for equality of variance. When group variances were homogeneous, a parametric analysis of variance of a one-way layout type was conducted to determine if any statistical differences existed among groups. When the analysis of variance was significant, Dunnett's (when sample size of each group was equal) or Scheffé's (when sample size of each group was different) multiple comparison test was applied to evaluate differences between the treated and the control groups. When the group variances were heterogeneous, the

data were analyzed by Kruskal-Wallis non-parametric analysis of variance. When significant, Dunnett type (when sample size of each group was equal) or Scheffé type (when sample size of each group was different) mean rank sum test was applied to determine if any significant differences existed between the treated and the control groups.

The data of urinalysis except for specific gravity and urine volume were assessed by Mann-Whitney's U test.

Mortality was analysed by Life table analysis.

The data of clinical sign (main group only), ophthalmology, necropsy, and histopathology were evaluated by Fisher's exact probability test.

II. RESULTS

A. ANALYSIS OF DOSE FORMULATIONS

The coefficient of variation for the homogeneity of the test substance for each dose level was 2.2 % and less. Hence, the results indicated a good homogeneity.

Analyses for concentrations showed that the diet preparations achieved 97 - 98 % of the target concentration. Thus, the concentrations of the test substance in the test diets were within acceptable limits.

B. MORTALITY AND CLINICAL SIGNS

In the high dose group neither sex showed an increase in mortality. Mortality in males was lower than the control during the last half of the treatment period with statistical significance in most of the weeks. In all other groups mortality was comparable to control. The final mortality is given in **Table 5.5-23**:

Table 5.5-23 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted], 1997): Final mortality at termination of treatment (%)

Sex	Dose group (ppm)*			
	0	3000	10000	30000
Male	32/50 (64)	30/50 (60)	32/50 (64)	21/50 (42)
Female	35/50 (70)	31/50 (62)	34/50 (68)	36/50 (72)

* number of mortalities / total number of rats/group (% mortality)

C. CLINICAL OBSERVATIONS

In the high dose group, significant increases in incidence of bradypnea, mass, and soiled fur were observed in males when compared to the control. Analysis of location of each mass showed that the ones in the tail were present in 27 males, which was apparently high in incidence compared to 11 of the control. The incidences of mass in other locations were comparable to the control. With respect to soiled fur, the sign was located at the external genital or perianal region. Males in this group also showed significant decreases in incidence of tactile hair loss, wound, and hair loss. In females, a significant increase in incidence of wetted fur was observed; the sign was mainly seen in the external genital region. Besides the signs mentioned above, loose stool was observed in all cages of this group from Week 24 in males and Week 23 in females until the end of the treatment. Animals showing loose stool could not be identified because of group housing, therefore the sign is only described here in the text but not included in the table below.

In the mid dose group, the incidence of tactile hair loss was significantly decreased in males and significantly increased in females when compared to the respective control.

In the low dose group, significant increases in incidence of decreased spontaneous motor activity,

bradypnea, and soiled fur and a significant decrease in incidence of tactile hair loss were observed in males. Analysis of location of the soiled fur demonstrated predominant occurrences of the sign in the external genital region and foreleg. Females in this group showed significant increases in incidence of ptosis and tactile hair loss.

Table 5.5-24 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted], 1997): Summary of changes in clinical signs

Conditions	Sex	Males				Females			
	Group #	G1	G2	G3	G4	G1	G2	G3	G4
	Dose (ppm)	0	3000	10000	30000	0	3000	10000	30000
	No. of rats	50	50	50	50	50	50	50	50
Appearance: Emaciation		12	16	14	14	23	28	18	26
Posture: Posterior paralysis		0	0	1	0	-	-	-	-
Behaviour: Decreased spontaneous motor activity		9	19*	9	13	23	22	20	26
Behaviour: Abnormal gait		1	0	0	0	0	0	1	2
Behaviour: Motor incoordination		-	-	-	-	0	0	1	0
Respiration: Brachypnea		3	10*	4	11*	7	14	6	12
Respiration: Abnormal respiratory sound		0	2	4	1	1	1	1	0
Respiration: Abnormal respiration		0	0	1	0	0	0	1	1
Eye: Opacity		1	5	4	3	2	1	4	0
Eye: Pale colour		7	5	14	10	8	6	10	8
Eye: Dark colour		-	-	-	-	0	2	1	1
Eye: Exophthalmos		0	0	1	0	1	0	1	1
Eye: Corsened corneal surface		0	1	1	1	-	-	-	-
Eye: Dried croneal surface		1	2	1	1	-	-	-	-
Eye: Lacrimation		2	0	1	1	-	-	-	-
Periocular region: Closed eye lid		-	-	-	-	1	0	0	0
Periocular region: Ptosis		7	6	4	6	4	12*	6	6
Nose: Distortion		0	0	2	0	1	0	0	0
Nose: Red discharge		-	-	-	-	1	0	0	0
Perinasal region: Tactile hair loss		5	0*	0*	0*	1	17**	9**	4
Oral cavity: Mass		1	0	0	1	-	-	-	-
Incisor: Elongation		0	3	3	3	3	2	1	0
Incisor: Malocclusion		3	2	1	0	4	1	1	1
Incisor: Partial loss		-	-	-	-	1	0	0	0
Incisor: Entire loss		-	-	-	-	0	0	0	0
Auricle: Partial loss		0	1	0	0	-	-	-	-
Neck: Torticollis		0	1	0	0	3	2	1	3
Abdominal region: Distention		0	1	0	1	2	0	3	0
Scrotum: Erosion/ulcer		1	0	0	0	-	-	-	-
Scrotum: Mass		0	1	0	0	-	-	-	-
Vagina: Red discharge		-	-	-	-	1	1	0	3
Foreleg: Entire loss		-	-	-	-	0	0	1	0
Finger: Partial loss		-	-	-	-	0	0	1	0

Table 5.5-24 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted], 1997): Summary of changes in clinical signs

Conditions	Sex	Males				Females			
	Group #	G1	G2	G3	G4	G1	G2	G3	G4
	Dose (ppm)	0	3000	10000	30000	0	3000	10000	30000
	No. of rats	50	50	50	50	50	50	50	50
Sole of hindpaw: Callus		26	21	18	19	9	14	17	16
Tail: Partial loss		3	0	0	0	0	0	0	1
Tail: Bending		1	0	0	2	1	0	0	0
Tail: Hardening		0	0	0	1	-	-	-	-
Integument: Red adhesive substance		7	10	10	11	15	19	16	19
Integument: Wound		7	4	6	0**	2	2	2	1
Integument: Erosion/ulcer		1	0	0	0	1	0	0	0
Integument: Scab		0	1	1	0	0	0	1	0
Integument: Thickened area		3	1	0	1	2	1	1	2
Integument: Swelling		10	5	8	10	4	2	3	2
Integument: Mass		22	26	21	37**	37	36	38	43
Integument: Pale-coloured skin		6	6	9	7	7	7	9	7
Integument: Yellow-coloured skin		0	1	0	1	0	1	0	1
Integument: Purple-coloured skin		-	-	-	-	0	0	1	0
Integument: Black-coloured skin		2	1	1	0	0	0	1	0
Integument: Ruffled fur		0	1	0	0	-	-	-	-
Integument: Hair loss		12	7	15	3*	16	13	21	25
Integument: Soiled fur		10	20*	12	21*	16	17	11	18
Integument: Wetted fur		9	7	7	16	5	5	5	15*
Integument: Piloerection		0	0	0	1	1	0	0	0

* Statistically significant ($p \leq 0.05$); ** Statistically significant ($p \leq 0.01$)

D. BODY WEIGHT

In the high dose group, body weights were lower than the control throughout the treatment period; significant decreases in their body weights were observed during Weeks 1 to 80 in males and at Week 7 and during Weeks 9 to 60 in females. The final group mean body weights of males and females at termination of the treatment period were both 93 % of the respective control.

In the mid dose group, males showed a decreased body weight gain during the first few weeks of treatment with a statistically significant difference from the control at Week 6. Their retarded growth persisted throughout the treatment period, and the final group mean body weight at termination of treatment was 95 % of the control. Body weight change in females was comparable to the control throughout the treatment period.

In the low dose group, body weights of both sexes were comparable to the control except for a significant increase in females at Week 16.

Table 5.5-25 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted], 1997): Summary of body weight (g)

Timepoint	Sex	Males				Females			
	Group #	G1	G2	G3	G4	G1	G2	G3	G4
	Dose (ppm)	0	3000	10000	30000	0	3000	10000	30000
	No. of rats	50	50	50	50	50	50	50	50
Week 0		151 ± 8	151 ± 8	151 ± 8	151 ± 8	116 ± 6	116 ± 6	116 ± 6	116 ± 6
Week 1		213 ± 12	213 ± 12	↓212 ± 13	↓205** ± 12	153 ± 10	153 ± 9	154 ± 8	150 ± 7
Week 2		275 ± 17	275 ± 17	↓271 ± 17	↓261** ± 16	179 ± 12	179 ± 12	178 ± 10	175 ± 10
Week 3		329 ± 20	↓326 ± 21	↓324 ± 20	↓310** ± 21	205 ± 15	↑206 ± 16	205 ± 13	↓201 ± 13
Week 4		371 ± 24	↓369 ± 24	↓362 ± 24	↓245** ± 24	226 ± 16	↑229 ± 18	↑228 ± 15	↓222 ± 15
Week 5		409 ± 28	↓407 ± 27	↓396 ± 30	↓378** ± 28	245 ± 18	↑250 ± 22	↑247 ± 19	↓238 ± 17
Week 6		439 ± 31	↓437 ± 31	↓422* ± 41	↓408** ± 32	260 ± 19	↑265 ± 23	↑261 ± 19	↓252 ± 18
Week 7		464 ± 34	↓462 ± 32	↓447 ± 44	↓431** ± 34	273 ± 20	↑280 ± 25	↑275 ± 20	↓262* ± 18
Week 8		484 ± 35	↓479 ± 34	↓467 ± 39	↓447** ± 37	280 ± 21	↑288 ± 26	↑283 ± 22	↓270 ± 19
Week 9		501 ± 37	↓496 ± 35	↓486 ± 41	↓459** ± 38	289 ± 21	↑298 ± 27	↑292 ± 22	↓277* ± 21
Week 10		520 ± 38	↓515 ± 38	↓502 ± 43	↓474** ± 40	296 ± 22	↑304 ± 28	↑297 ± 24	↓282* ± 21
Week 11		536 ± 39	↓532 ± 40	↓518 ± 46	↓487** ± 41	302 ± 22	↑312 ± 30	↑305 ± 24	↓288* ± 21
Week 12		549 ± 41	↓544 ± 43	↓530 ± 46	↓499** ± 44	310 ± 23	↑322 ± 32	↑312 ± 25	↓294** ± 23
Week 13		562 ± 43	↓557 ± 46	↓545 ± 48	↓511** ± 45	316 ± 24	↑328 ± 33	↑317 ± 27	↓297** ± 23
Week 80		840 ± 112	↑843 ± 116	↓828 ± 106	↓770* ± 91	525 ± 94	↑558 ± 93	↑544 ± 104	↓503 ± 70
Week 104		811 ± 114	↓800 ± 108	↓772 ± 172	↓758 ± 117	558 ± 119	↓521 ± 128	↓545 ± 118	↓517 ± 142

E. Food consumption and compound intake

In the high dose group, consistent with the decreasing body weight or decreasing body weight trends, food consumption showed a decreasing trend in males during the first few weeks.

In the other groups, food consumption in males and females was comparable to the respective control.

Table 5.5-26 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted], 1997): Summary of food consumption data (g)

Timepoint	Sex	Males				Females			
	Group #	G1	G2	G3	G4	G1	G2	G3	G4
	Dose (ppm)	0	3000	10000	30000	0	3000	10000	30000
	No. of rats	50	50	50	50	50	50	50	50
Week 1		16.7 ± 1.7	16.7 ± 0.8	↓16.6 ± 0.3	↓14.7** ± 1.0	13.0 ± 0.4	↑13.4 ± 0.5	↑13.5* ± 0.3	↓12.5 ± 0.5
Week 2		20.1 ± 0.9	↑20.4 ± 0.8	↑20.4 ± 0.6	↓19.9 ± 0.8	15.0 ± 0.8	↓14.6 ± 0.6	↓14.7 ± 0.6	↓14.7 ± 0.5
Week 3		23.0 ± 0.7	↓22.9 ± 0.8	↓22.4 ± 0.6	↓22.2 ± 0.9	15.3 ± 0.8	↑15.9 ± 0.7	↑15.8 ± 0.8	↑15.7 ± 0.4
Week 4		23.6 ± 0.7	↓22.9 ± 0.9	↓22.6* ± 0.4	↓22.4** ± 1.1	16.0 ± 0.8	↑16.2 ± 1.2	↑16.4 ± 0.9	↓15.8 ± 0.8
Week 5		22.9 ± 1.3	22.9 ± 1.0	↓22.5 ± 0.9	↓21.9 ± 1.2	17.1 ± 0.8	↑17.4 ± 0.9	↓16.6 ± 0.7	↓16.1* ± 1.0
Week 6		23.0 ± 1.3	↑23.3 ± 1.1	↓22.6 ± 1.7	↓22.7 ± 1.4	16.8 ± 0.8	↑17.5 ± 1.0	↑17.3 ± 0.8	↓16.2 ± 0.6
Week 13		24.2 ± 1.2	↓23.9 ± 1.4	↓23.6 ± 0.8	↓23.4 ± 1.0	15.7 ± 0.8	↑16.3 ± 0.8	↓15.5 ± 0.8	↓15.2 ± 0.9
Week 80		26.0 ± 2.1	↓25.6 ± 2.0	↓25.7 ± 1.7	↓25.4 ± 1.7	16.0 ± 1.4	↑16.6 ± 3.6	↑18.7* ± 2.1	↑16.5 ± 2.1
Week 104		26.0 ± 4.8	↓25.6 ± 7.3	↓25.4 ± 9.3	↓24.0 ± 2.6	16.7 ± 3.4	↑17.2 ± 4.3	↑17.6 ± 4.8	↑18.2 ± 5.7
Average		24.8	↓24.2	↓24.3	↓24.2	16.4	↑16.5	↑16.8	16.4

The group mean achieved doses are summarised below.

Table 5.5-27 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted], 1997): Group mean achieved dose levels in the main groups

Dose group	Dietary concentration (ppm)	Mean achieved dose level (mg/kg bw/day)	
		Males	Females
1 (control)	0		
2 (low)	3000	104	115
3 (mid)	10000	354	393
4 (high)	30000	1127	1247

The results show a higher test material intake for females when compared to males for each dose level.

F. OPHTHALMOLOGIC EXAMINATIONS

No abnormalities were observed.

Table 5.5-28 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted], 1997): Ophthalmological examinations

Site & Lesion	Sex	Males				Females			
	Group #	G1		G4		G1		G4	
	Dose (ppm)	0	0	30000	30000	0	0	30000	30000
	No. of rats	50	20	50	32	50	20	50	17
	Week	0	103	0	103	0	103	0	103
Eye ball: Pale colour		0	2	0	5	0	4	0	4
Cornea: Opacity		0	1	0	2	0	6	0	2
Cornea: Vascularisation		0	0	0	1	-	-	-	-
Cornea: Dried surface		0	0	0	2	0	1	0	0
Cornea: Keratoconus		0	0	0	1	-	-	-	-
Pupil: No reflex to mydriatic		0	1	0	1	0	1	0	0
Pupil: No reflex to light		0	0	0	3	0	1	0	0
Pupil: Infection		0	0	0	1	-	-	-	-
Lens/vitreous body: Opacity		0	1	0	7	0	3	0	3
Fundus: Pale colour		0	0	0	1	-	-	-	-

G. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematological and blood biochemical analyses did not demonstrate apparent toxicity of the test substance in either sex or group. The increase of haematocrit and decrease of platelet count is considered incidental and partly due to very low (RBC parameters) or high (platelets) control values when compared to other time points. These changes were only observed in one sex, at one time point and without any dose-response relationship. Compared to the control haematocrit values were 108 %, 111 % and 131 % for males and 99 %, 84 % and 96 % for females in the 3000, 10000 and 30000 ppm dose groups. No changes of these parameters have been observed at the other time points.

Statistically significant changes in haematology and blood chemistry are displayed in the tables below.

Table 5.5-29 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted], 1997): Statistically significant changes in haematology

Parameter	Sex	Males				Females			
	Group #	G1	G2	G3	G4	G1	G2	G3	G4
	Dose (ppm)	0	3000	10000	30000	0	3000	10000	30000
	No. of rats	10	10	10	10	10	10	10	10
Week 52 (satellite group)									
Haematocrit [%]		41.2 ± 0.9 (9)	↓38.9 ± 6.8	↓38.6 ± 7.3	↑41.6 ± 1.7	38.2 ± 2.9	↑39.2 ± 1.3	↑38.9 ± 2.0	↓37.5 ± 3.2
Haemoglobin [g/dL]		14.2 ± 0.4 (9)	↓13.4 ± 2.5	↓13.3 ± 2.7	↑14.3 ± 0.5	13.4 ± 0.9	↑13.7 ± 0.4	↑13.6 ± 0.7	↓13.1 ± 1.2
Red blood cell count [104/mm3]		7.99 ± 0.24 (9)	↓7.58 ± 1.40	↓7.50 ± 1.37	↑8.07 ± 0.41	7.02 ± 0.55	↑7.22 ± 0.28	↑7.07 ± 0.34	↓6.77 ± 0.87
Mean corpuscular volume [fL]		51.7 ± 2.1 (9)	↓51.6 ± 2.6	↓51.3 ± 2.6	51.7 ± 2.6	54.4 ± 1.7	↓54.3 ± 1.3	↑55.1 ± 1.4	↑55.7 ± 3.4
Mean corpuscular haemoglobin [pg]		17.8 ± 0.9 (9)	↓17.7 ± 0.8	↓17.7 ± 1.2	↓17.7 ± 0.9	19.1 ± 0.5	19.1 ± 0.4	↑19.2 ± 0.6	↑19.4 ± 1.2
Mean corpuscular		34.5 ±	↓34.3 ±	↓34.4 ±	↓34.3 ±	19.1 ±	19.1 ±	↑19.2 ±	↑19.4 ±

Table 5.5-29 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted], 1997): Statistically significant changes in haematology

Parameter	Sex	Males				Females			
	Group #	G1	G2	G3	G4	G1	G2	G3	G4
	Dose (ppm)	0	3000	10000	30000	0	3000	10000	30000
	No. of rats	10	10	10	10	10	10	10	10
haemoglobin concentration [g/dL]		0.7 (9)	0.8	0.9	0.8	0.5	0.4	0.6	1.2
Platelet count [103/mm3]		1208 ± 142 (9)	↓1198 ± 214	↓1176 ± 218	↑1252 ± 118	922 ± 120	↑952 ± 67	↑928 ± 130	↑1087 ± 177
Total leucocyte count [103/mm3]		13.3 ± 3.5 (9)	13.3 ± 3.9	↓12.6 ± 2.6	↓11.4 ± 2.4	7.1 ± 1.8	7.1 ± 1.3	↓6.3 ± 1.3	↓6.5 ± 2.1
Differential leukocyte count: Lymphocytes [103/mm3]		9.2 ± 2.1 (9)	↑9.7 ± 2.6	↓8.6 ± 1.8	↓8.6 ± 1.8	4.5 ± 1.4	↑4.7 ± 1.2	↓4.3 ± 0.8	↓4.4 ± 2.0
Differential leukocyte count: Neutrophils (stab form) [103/mm3]		0.0 ± 0.1 (9)	↑0.1 ± 0.1	↑0.1 ± 0.2	↑0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Differential leukocyte count: Neutrophiles (segmented form) [103/mm3]		3.9 ± 2.0 (9)	↓53.1 ± 1.3	↓3.6 ± 1.3	↓2.5 ± 0.8	2.5 ± 1.2	↓2.2 ± 0.6	↓1.9 ± 0.8	↓1.9 ± 0.7
Differential leukocyte count: Monocytes [103/mm3]		0.1 ± 0.1 (9)	↑0.3 ± 0.4	↑0.2 ± 0.3	0.1 ± 0.1	0.0 ± 0.1	↑0.1 ± 0.1	0.0 ± 0.1	0.0 ± 0.0
Differential leukocyte count: Eosinophils [103/mm3]		0.1 ± 0.1 (9)	0.1 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
Differential leukocyte count: Basophils [103/mm3]		0.0 ± 0.0 (9)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Differential leukocyte count: Unclassified cells [103/mm3]		0.0 ± 0.0 (9)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Week 104 (main group)									
Haematocrit [%]		26.6 ± 7.6	↑28.7 ± 5.7	↑29.6 ± 7.4	↑34.9* ± 4.9	31.4 ± 5.0	↓31.0 ± 6.3	↓26.5 ± 6.5	↓30.2 ± 6.9
Haemoglobin [g/dL]		9.5 ± 3.1	↑10.6 ± 2.4	↑10.7 ± 3.3	↑12.9 ± 1.8	11.5 ± 2.0	↓11.4 ± 2.6	↓9.4 ± 2.7	↓10.7 ± 2.9
Red blood cell count [104/mm3]		5.82 ± 1.51	↓5.68 ± 0.75	↑5.95 ± 1.21	↑6.77 ± 0.97	5.46 ± 1.17	↓5.42 ± 1.19	↓4.69 ± 1.12	↓5.36 ± 1.18
Mean corpuscular volume [fL]		46.2 ± 72.	↑50.4 ± 6.0	↑49.5 ± 6.5	↑51.6 ± 1.8	58.2 ± 4.7	↓57.5 ± 3.9	↓56.4 ± 2.9	↓56.4 ± 6.0
Mean corpuscular haemoglobin [pg]		16.2 ± 2.6	↑18.5 ± 2.9	↑17.8 ± 3.5	↑19.0 ± 1.1	21.3 ± 1.9	↓21.0 ± 1.6	↓19.8 ± 1.6	↓19.8 ± 2.5
Mean corpuscular haemoglobin concentration [g/dL]		35.2 ± 2.7	↑36.6 ± 2.0	↑35.6 ± 3.0	↑36.9 ± 1.2	36.6 ± 1.3	36.6 ± 1.6	↓35.0 ± 2.1	↓35.0 ± 2.4
Platelet count [103/mm3]		1770 ± 409	↓1617 ± 313	↓1564 ± 475	↓1172** ± 208	1099 ± 419	↑1158 ± 292	↑1261 ± 254	↑1148 ± 197
Total leucocyte count [103/mm3]		16.2 ± 4.7	↓12.5 ± 4.0	↓16.0 ± 6.1	↓12.3 ± 5.2	9.2 ± 3.4	↑9.3 ± 2.3	↑9.4 ± 2.1	↓8.5 ± 2.5
Differential leukocyte count: Lymphocytes		8.0 ± 3.6	↓6.0 ± 2.0	↑8.3 ± 3.9	↓6.4 ± 1.7	4.0 ± 1.7	↑4.7 ± 2.3	↑4.1 ± 0.9	↑4.3 ± 1.7

Table 5.5-29 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted], 1997): Statistically significant changes in haematology

Parameter	Sex	Males				Females			
	Group #	G1	G2	G3	G4	G1	G2	G3	G4
	Dose (ppm)	0	3000	10000	30000	0	3000	10000	30000
	No. of rats	10	10	10	10	10	10	10	10
[103/mm3]									
Differential leukocyte count: Neutrophils (stab form) [103/mm3]		0.1 ± 0.2	0.1 ± 0.1	↑0.2 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	↑0.2 ± 0.1	0.1 ± 0.1
Differential leukocyte count: Neutrophiles (segmented form) [103/mm3]		7.6 ± 2.4	↓6.0 ± 2.6	↓7.2 ± 4.0	↓5.5 ± 3.7	5.0 ± 2.2	↓4.1 ± 1.2	5.0 ± 1.5	↓3.9 ± 1.9
Differential leukocyte count: Monocytes [103/mm3]		0.3 ± 0.3	0.3 ± 0.3	0.3 ± 0.2	↓0.2 ± 0.2	0.1 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
Differential leukocyte count: Eosinophils [103/mm3]		0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	↑0.2 ± 0.6	↓0.0 ± 0.0	0.1 ± 0.1
Differential leukocyte count: Basophils [103/mm3]		0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Differential leukocyte count: Unclassified cells [103/mm3]		0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.1	0.0 ± 0.0

* Statistically significant ($p \leq 0.05$) ; * Statistically significant ($p \leq 0.01$); numbers in braces represent the animal numbers of the respective endpoint different from 10 animals per group

Table 5.5-30 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Statistically significant changes in blood chemistry

Parameter	Sex	Males				Females			
	Group #	G1	G2	G3	G4	G1	G2	G3	G4
	Dose (ppm)	0	3000	10000	30000	0	3000	10000	30000
	No. of rats	10	10	10	10	10	10	10	10
Week 26 (satellite group)									
Alkaline phosphatase (ALP) [U/L]		69 ± 15	↓66 ± 15	↑81 ± 21	↑86 ± 15	30 ± 7	↑36 ± 11	↑35 ± 15	↑37 ± 20
Glutamic oxaloacetic transaminase (GOT) [U/L]		97 ± 21	↑99 ± 28	↓85 ± 13	↓79 ± 10	91 ± 54	↓77 ± 40	↓73 ± 16	↓73 ± 9
Glutamic pyruvic transaminase (GPT) [U/L]		42 ± 8	↓41 ± 10	↓40 ± 10	↓36 ± 7	41 ± 26	↓36 ± 25	↓32 ± 9	↓31 ± 4
y-Glutamyl transpeptidase (GGTP) [U/L]		1 ± 1	1 ± 1	1 ± 0	1 ± 1	1 ± 1	1 ± 0	1 ± 0	↓0 ± 0
Creatine phosphokinase (CPK) [U/L]		108 ± 12	↑122 ± 69	↓107 ± 19	↓106 ± 27	71 ± 11	↑79 ± 10	↑74 ± 12	↑75 ± 10
Creatinine (Creat) [mg/dL]		0.91 ± 0.04	↑0.93 ± 0.08	↓0.90 ± 0.08	↓0.88 ± 0.05	1.04 ± 0.08	↓0.99 ± 0.08	↓0.95* ± 0.06	↓0.93** ± 0.06
Blood urea nitrogen (BUN) [mg/dL]		12.9 ± 1.4	↓12.7 ± 1.0	↑13.1 ± 1.9	↑13.0 ± 1.3	15.5 ± 1.5	↓15.3 ± 1.7	↓14.8 ± 1.4	↓15.4 ± 2.0
Total protein (TP) [g/dL]		6.69 ±	↓6.61 ±	↓6.50 ±	↓6.60 ±	7.29 ±	↓7.00 ±	↓7.10 ±	↓6.90 ±

Table 5.5-30 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Statistically significant changes in blood chemistry

Parameter	Sex	Males				Females			
	Group #	G1	G2	G3	G4	G1	G2	G3	G4
	Dose (ppm)	0	3000	10000	30000	0	3000	10000	30000
	No. of rats	10	10	10	10	10	10	10	10
		0.18	0.25	0.26	0.25	0.35	0.37	0.27	0.30
Albumin (Alb) [g/dL]		3.05 ± 0.25	↓3.04 ± 0.06	↓3.04 ± 0.13	↑3.14 ± 0.16	4.06 ± 0.28	↓3.73* ± 0.32	↓4.02 ± 0.21	↓3.84 ± 0.24
Globulin (Glob) [g/dL]		3.65 ± 0.25	↓3.57 ± 0.25	↓3.46 ± 0.19	↓3.46 ± 0.16	3.23 ± 0.17	↑3.28 ± 0.18	↓3.08 ± 0.13	↓3.06* ± 0.12
Albumin/globulin ratio (A/G ratio)		0.84 ± 0.10	↑0.86 ± 0.07	↑0.88 ± 0.05	↑0.91 ± 0.05	1.26 ± 0.10	↓1.14* ± 0.11	↑1.30 ± 0.08	↓1.25 ± 0.08
Glucose (Glue) [mg/dL]		136 ± 16	↑137 ± 7	↑145 ± 15	↓132 ± 9	137 ± 8	↑143 ± 22	↓136 ± 22	↓119** ± 8
Total cholesterol (T.Chol) [mg/dL]		79 ± 10	↓74 ± 23	↓74 ± 8	↓73 ± 16	84 ± 8	↑87 ± 14	↑85 ± 18	84 ± 14
Triglyceride (TG) [mg/dL]		151 ± 39	↓146 ± 36	↑186 ± 94	↓138 ± 32	192 ± 142	↓133 ± 58	↑277 ± 331	↓129 ± 60
Total bilirubin (T.Bil) [mg/dL]		0.16 ± 0.02	0.16 ± 0.03	↓0.15 ± 0.01	↑0.17 ± 0.02	0.25 ± 0.05	↓0.20** ± 0.02	↓0.24 ± 0.02	↓0.22 ± 0.02
Calcium (Ca) [mg/dL]		9.8 ± 0.3	↓9.7 ± 0.3	↓9.7 ± 0.1	9.8 ± 0.4	10.4 ± 0.3	↓10.1 ± 0.4	↓10.3 ± 0.2	↓10.2 ± 0.3
Inorganic phosphorus (P) [mg/dL]		4.9 ± 0.4	4.9 ± 0.4	4.9 ± 0.4	↓4.7 ± 0.4	4.0 ± 0.6	↓3.7 ± 0.7	↓3.7 ± 0.6	↓3.8 ± 0.6
Sodium (Na) [mEq/dL]		146.0 ± 0.9	↓145.6 ± 1.0	↓146.1 ± 0.9	↓145.8 ± 1.0	144.8 ± 0.8	↓144.7 ± 1.1	↑145.1 ± 1.4	↓144.6 ± 1.2
Potassium (K) [mEq/dL]		3.47 ± 0.23	↓3.46 ± 0.27	↓3.45 ± 0.11	↓3.35 ± 0.21	3.25 ± 0.20	↑3.59 ± 0.67	↓3.13 ± 0.27	↑3.34 ± 0.15
Chloride (Cl) [mEq/dL]		105.4 ± 1.3	↓105.1 ± 1.4	↑106.3 ± 1.4	↓104.6 ± 1.8	106.3 ± 1.2	↓105.8 ± 1.4	↓105.6 ± 1.3	↓105.7 ± 2.1
Week 52 (satellite group)									
Alkaline phosphatase (ALP) [U/L]		58 ± 11 (9)	↑75 ± 41	↑84 ± 54	↑79 ± 15	22 ± 8	↑28 ± 9	↑26 ± 10	↑47** ± 25
Glutamic oxaloacetic transaminase (GOT) [U/L]		96 ± 18 (9)	↑127 ± 138	↑152 ± 155	↓72 ± 14	113 ± 74	↓108 ± 31	↓86 ± 12	↓73 ± 14
Glutamic pyruvic transaminase (GPT) [U/L]		48 ± 17 (9)	↓45 ± 15	↑71 ± 43	↓32* ± 6	53 ± 40	↓48 ± 16	↓40 ± 11	↓35 ± 8
y-Glutamyl transpeptidase (GGTP) [U/L]		1 ± 1 (9)	↑2 ± 1	↑2 ± 4	1 ± 1	1 ± 1	1 ± 0	1 ± 0	1 ± 0
Creatine phosphokinase (CPK) [U/L]		118 ± 36 (9)	↓110 ± 36	↓114 ± 61	↓114 ± 75	142 ± 34	↓140 ± 62	↓105 ± 22	↓110 ± 46
Creatinine (Creat) [mg/dL]		1.01 ± 0.10 (9)	↑1.09 ± 0.11	↑1.08 ± 0.17	↑1.03 ± 0.09	0.98 ± 0.08	↑0.99 ± 0.09	↓0.92 ± 0.07	↓0.91 ± 0.07
Blood urea nitrogen (BUN) [mg/dL]		12.7 ± 1.7 (9)	↑15.3 ± 7.0	↑15.0 ± 10.1	↑14.3 ± 1.6	14.6 ± 2.5	↓13.9 ± 0.09	↓12.5 ± 1.6	↓13.6 ± 1.9
Total protein (TP) [g/dL]		6.79 ± 0.35 (9)	↓6.76 ± 0.49	↓6.76 ± 0.31	↓6.75 ± 0.25	7.50 ± 0.30	↑7.55 ± 0.22	↓7.19* ± 0.32	↓7.20 ± 0.27
Albumin (Alb) [g/dL]		2.64 ± 0.10 (9)	↓2.50 ± 0.33	↓2.56 ± 0.45	↓2.61 ± 0.17	3.70 ± 0.25	↑3.73 ± 0.23	↓3.65 ± 0.21	↓3.45 ± 0.35
Globulin (Glob) [g/dL]		4.15 ± 0.31 (9)	↑4.26 ± 0.79	↑4.20 ± 0.66	4.15 ± 0.24	3.80 ± 0.22	↑3.82 ± 0.11	↓3.54* ± 0.19	↓3.75 ± 0.52
Albumin/globulin ratio (A/G ratio)		0.64 ± 0.05 (9)	↓0.61 ± 0.14	↓0.63 ± 0.15	0.63 ± 0.07	0.98 ± 0.09	0.98 ± 1.03	↑1.03 ± 0.07	↓0.94 ± 0.17
Glucose (Glue) [mg/dL]		143 ±	143 ±	↓138 ±	143 ±	143 ±	↓136 ±	↓136 ±	↓130 ±

Table 5.5-30 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Statistically significant changes in blood chemistry

Parameter	Sex	Males				Females			
	Group #	G1	G2	G3	G4	G1	G2	G3	G4
	Dose (ppm)	0	3000	10000	30000	0	3000	10000	30000
	No. of rats	10	10	10	10	10	10	10	10
		11 (9)	17	20	32	21	15	16	15
Total cholesterol (T.Chol) [mg/dL]		104 ± 19 (9)	↓92 ± 24	↓90 ± 26	↓90 ± 20	95 ± 30	↑97 ± 21	↑97 ± 22	↑109 ± 54
Triglyceride (TG) [mg/dL]		217 ± 47 (9)	↓171 ± 43	↓205 ± 85	↓176 ± 48	379 ± 199	↑511 ± 321	↑509 ± 364	↓337 ± 199
Total bilirubin (T.Bil) [mg/dL]		0.20 ± 0.02 (9)	↑0.24 ± 0.09	0.24 ± 0.05	↓0.21 ± 0.02	0.29 ± 0.07	↑0.35 ± 0.11	↑0.33 ± 0.13	↓0.25 ± 0.06
Calcium (Ca) [mg/dL]		10.2 ± 0.2 (9)	10.2 ± 0.1	↑10.1 ± 0.2	10.2 ± 0.2	10.7 ± 0.3	10.7 ± 0.2	↓10.5 ± 0.3	↓10.5 ± 0.2
Inorganic phosphorus (P) [mg/dL]		4.2 ± 0.3 (9)	↑4.5 ± 0.5	↑4.6 ± 0.8	↑4.4 ± 0.3	3.4 ± 0.7	3.4 ± 0.6	↓3.3 ± 0.5	↓3.2 ± 0.7
Sodium (Na) [mEq/dL]		145.9 ± 0.7 (9)	↑146.3 ± 1.0	145.9 ± 1.1	↓145.7 ± 1.0	144.3 ± 0.7	↑144.4 ± 1.3	↓144.1 ± 0.9	144.3 ± 1.1
Potassium (K) [mEq/dL]		3.51 ± 0.15 (9)	↓3.49 ± 0.28	↓3.33 ± 0.28	↓3.46 ± 0.16	3.16 ± 0.43	↓2.83 ± 0.45	↓3.06 ± 0.28	↑3.24 ± 0.63
Chloride (Cl) [mEq/dL]		106.0 ± 1.4 (9)	↑106.6 ± 0.9	106.0 ± 1.0	↓104.9 ± 1.9	106.0 ± 1.8	↓103.9 ± 3.6	↓104.9 ± 2.1	↓105.1 ± 2.1
Week 78 (satellite group)									
Alkaline phosphatase (ALP) [U/L]		48 ± 16	↑89* ± 19	↑74 ± 19	↑82 ± 29	32 ± 12 (8)	↑97 ± 147 (9)	↑34 ± 14 (8)	↑37 ± 21 (8)
Glutamic oxaloacetic transaminase (GOT) [U/L]		97 ± 38	↓84 ± 22	↓85 ± 20	↓76 ± 9	147 ± 84(8)	↓107 ± 36 (9)	↓101 ± 61 (8)	↓122 ± 48 (8)
Glutamic pyruvic transaminase (GPT) [U/L]		41 ± 15	↓32 ± 9	↓37 ± 13	↓36 ± 8	89 ± 66 (8)	↓47 ± 24 (9)	↓48 ± 20 (8)	↓45 ± 17 (8)
y-Glutamyl transpeptidase (GGTP) [U/L]		1 ± 2	↑2 ± 2	1 ± 1	1 ± 1	2 ± 1 (8)	2 ± 1 (9)	↓1 ± 0 (8)	↓1 ± 0 (8)
Creatine phosphokinase (CPK) [U/L]		136 ± 36	↑185 ± 115	↓122 ± 43	↓125 ± 22	87 ± 22 (8)	↓77 ± 18 (9)	↓79 ± 11 (8)	↑118 ± 90 (8)
Creatinine (Creat) [mg/dL]		1.03± 0.09	↑1.16 ± 0.22	↓1.01 ± 0.11	↑1.04 ± 0.21	1.05 ± 0.08 (8)	↓0.99 ± 0.06 (9)	↓1.01 ± 0.08 (8)	↑1.08 ± 0.22 (8)
Blood urea nitrogen (BUN) [mg/dL]		16.4 ± 4.7	↑19.7 ± 9.0	↓15.5 ± 4.1	↑18.1 ± 12.6	13.6 ± 2.0 (8)	↓13.2 ± 1.9 (9)	↑14.2 ± 3.3 (8)	↑16.7 ± 9.4 (8)
Total protein (TP) [g/dL]		6.50 ± 0.32	↓6.14 ± 0.50	↓6.27 ± 0.37	↑6.53 ± 0.18	6.83 ± 0.38 (8)	↓6.69 ± 0.49 (9)	↑7.09 ± 0.44 (8)	↑7.08 ± 0.41 (8)
Albumin (Alb) [g/dL]		2.47 ± 0.49	↓1.95 ± 0.56	↓2.42 ± 0.31	↑2.68 ± 0.10	3.13 ± 0.42 (8)	↑3.72 ± 0.67 (9)	↑3.35 ± 0.32 (8)	↓2.84 ± 0.63 (8)
Globulin (Glob) [g/dL]		4.03 ± 0.33	↑4.19 ± 0.18	↓3.85 ± 0.43	↓3.85 ± 0.18	3.70 ± 0.35 (8)	↑3.97 ± 0.52 (9)	↑3.73 ± 0.30 (8)	↑4.25 ± 0.70 (8)
Albumin/globulin ratio (A/G ratio)		0.62 ± 0.17	↓0.47 ± 0.14	↑0.64 ± 0.14	↑0.70 ± 0.05	0.86 ± 0.17 (8)	↓0.71 ± 0.22 (9)	↑0.90 ± 0.10 (8)	↓0.70 ± 0.22 (8)
Glucose (Glue) [mg/dL]		131 ± 21	↓123 ± 18	↑140 ± 14	↑132 ± 13	126 ± 8 (8)	↓116 ± 21 (9)	↓116 ± 24 (8)	↓102 ± 24 (8)
Total cholesterol (T.Chol) [mg/dL]		140 ± 79	↓101 ± 47	↓124 ± 34	↓119 ± 38	107 ± 18 (8)	↓94 ± 25 (9)	↓100 ± 25 (8)	↓93 ± 34 (8)
Triglyceride (TG) [mg/dL]		353 ± 335	↓155 ± 56	↓221 ± 88	↓200 ± 67	395 ± 230 (8)	↓275 ± 162 (9)	↓235 ± 87 (8)	↓241 ± 167 (8)
Total bilirubin (T.Bil) [mg/dL]		0.22 ± 0.07	↓0.17 ± 0.05	↓0.20 ± 0.02	↓0.20 ± 0.03	0.25 ± 0.05 (8)	↓0.21 ± 0.05 (9)	↓0.23 ± 0.04 (8)	↓0.23 ± 0.04 (8)
Calcium (Ca) [mg/dL]		10.0 ±	↓9.8 ±	↑10.1 ±	10.0 ±	10.2 ±	10.2 ±	↑10.5 ±	↑10.3 ±

Table 5.5-30 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Statistically significant changes in blood chemistry

Parameter	Sex	Males				Females			
	Group #	G1	G2	G3	G4	G1	G2	G3	G4
	Dose (ppm)	0	3000	10000	30000	0	3000	10000	30000
	No. of rats	10	10	10	10	10	10	10	10
		0.5	0.4	0.4	0.5	0.2 (8)	0.2 (9)	0.4 (8)	0.5 (8)
Inorganic phosphorus (P) [mg/dL]		4.5 ± 1.0	↑4.7 ± 0.2	↓4.3 ± 0.6	↑4.8 ± 1.8	3.6 ± 0.6 (8)	↑3.9 ± 0.7 (9)	↑3.9 ± 1.1 (8)	↑4.1 ± 0.8 (8)
Sodium (Na) [mEq/dL]		145.0 ± 1.3	145.0 ± 0.8	↑145.3 ± 0.9	↓144.2 ± 1.5	142.2 ± 1.9 (8)	↑142.3 ± 1.4 (9)	↑142.6 ± 1.6 (8)	↑145.5 ± 10.8 (8)
Potassium (K) [mEq/dL]		3.32 ± 0.18	↑3.87 ± 0.76	↑3.49 ± 0.26	↑3.40 ± 0.32	2.90 ± 0.37 (8)	↑3.15 ± 0.39 (9)	↑3.25 ± 1.12 (8)	↓2.88 ± 0.40 (8)
Chloride (Cl) [mEq/dL]		106.0 ± 2.4	↓105.9 ± 2.2	↓105.8 ± 1.7	↓104.1 ± 1.2	104.8 ± 4.0 (8)	↓103.8 ± 2.9 (9)	↓104.6 ± 2.1 (8)	↓104.6 ± 6.8 (8)
Week 104 (main group)									
Alkaline phosphatase (ALP) [U/L]		64 ± 32	↓53 ± 25	↑84 ± 33	↑95 ± 85	39 ± 27	↑41 ± 30	↑61 ± 22	↑61 ± 26
Glutamic oxaloacetic transaminase (GOT) [U/L]		107 ± 43	↓87 ± 26	↓95 ± 20	↓86 ± 20	240 ± 280	↓110 ± 71	↓98 ± 39	↓99 ± 73
Glutamic pyruvic transaminase (GPT) [U/L]		32 ± 11	↓31 ± 8	32 ± 14	↑33 ± 10	74 ± 82	↓37 ± 16	↓45 ± 32	↓45 ± 30
y-Glutamyl transpeptidase (GGTP) [U/L]		3 ± 2	↓2 ± 1	3 ± 1	↓2 ± 1	1 ± 1	↑2 ± 1	1 ± 1	1 ± 1
Creatine phosphokinase (CPK) [U/L]		206 ± 216	↓125 ± 74	↓148 ± 129	↓92 ± 20	118 ± 54	↓100 ± 48	↓101 ± 45	↓92 ± 25
Creatinine (Creat) [mg/dL]		1.11 ± 0.19	↑1.33 ± 0.33	↑1.19 ± 0.24	1.11 ± 0.38	1.14 ± 0.33	↓1.04 ± 0.09	↓1.03 ± 0.07	↓1.01 ± 0.07
Blood urea nitrogen (BUN) [mg/dL]		16.0 ± 7.6	↑21.8 ± 11.7	↑17.2 ± 4.9	↑20.3 ± 22.9	15.8 ± 5.6	↓14.9 ± 4.0	↓13.9 ± 2.4	↓12.9 ± 2.4
Total protein (TP) [g/dL]		6.32 ± 0.38	↑6.45 ± 0.61	↓6.27 ± 0.68	↑6.49 ± 0.29	7.15 ± 0.28	↓6.90 ± 0.61	↓6.78 ± 0.35	↓7.02 ± 0.44
Albumin (Alb) [g/dL]		1.84 ± 0.37	↑1.88 ± 0.43	↓1.72 ± 0.30	↑2.24 ± 0.40	2.84 ± 0.40	↓2.61 ± 0.49	↓2.42 ± 0.41	↓2.71 ± 0.52
Globulin (Glob) [g/dL]		4.48 ± 0.37	↑4.57 ± 0.51	↑4.55 ± 0.55	↓4.26 ± 0.35	4.31 ± 0.49	↓4.29 ± 0.51	↑4.37 ± 0.32	4.31 ± 0.56
Albumin/globulin ratio (A/G ratio)		0.42 ± 0.10	0.42 ± 0.11	↓0.38 ± 0.08	↑0.53* ± 0.13	0.67 ± 0.16	↓0.62 ± 0.14	↓0.56 ± 0.12	↓0.65 ± 0.18
Glucose (Glue) [mg/dL]		108 ± 20	↓92 ± 20	↓99 ± 16	↑115 ± 8	106 ± 12	↑112 ± 17	↑107 ± 17	↑115 ± 17
Total cholesterol (T.Chol) [mg/dL]		101 ± 35	↑156 ± 77	↑121 ± 57	↑108 ± 36	122 ± 52	↓113 ± 35	↓83 ± 24	↓109 ± 35
Triglyceride (TG) [mg/dL]		156 ± 65	↑286 ± 343	↑197 ± 232	↓126 ± 52	331 ± 305	↓259 ± 248	↓163 ± 59	↓253 ± 358
Total bilirubin (T.Bil) [mg/dL]		0.17 ± 0.02	↑0.23 ± 0.19	0.17 ± 0.04	↑0.18 ± 0.02	0.20 ± 0.05	0.20 ± 0.06	↓0.18 ± 0.08	↓0.19 ± 0.10
Calcium (Ca) [mg/dL]		10.2 ± 0.5	↑10.4 ± 0.6	↑10.3 ± 0.5	↑10.3 ± 0.3	10.4 ± 0.7	↓10.1 ± 0.3	↓10.0 ± 0.2	↓10.1 ± 0.4
Inorganic phosphorus (P) [mg/dL]		4.8 ± 1.8	↑5.2 ± 1.7	↑5.1± 1.0	↓4.4 ± 1.0	4.3 ± 1.5	↓4.1 ± 0.7	↓4.0 ± 0.6	↓3.6 ± 0.8
Sodium (Na) [mEq/dL]		148.7 ± 1.8	↓147.8 ± 1.3	↓148.1 ± 1.2	↑148.9 ± 1.2	143.9 ± 2.2	↑144.4 ± 1.2	↑144.0 ± 1.9	↑144.5 ± 1.0
Potassium (K) [mEq/dL]		3.26 ±	↓3.06 ±	↓3.06 ±	↑3.29 ±	3.06 ±	↓3.04 ±	↓2.96 ±	↑3.12 ±

Table 5.5-30 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Statistically significant changes in blood chemistry

Parameter	Sex	Males				Females			
	Group #	G1	G2	G3	G4	G1	G2	G3	G4
	Dose (ppm)	0	3000	10000	30000	0	3000	10000	30000
	No. of rats	10	10	10	10	10	10	10	10
		0.44	0.36	0.33	0.22	0.25	0.49	0.39	0.28
Chloride (Cl) [mEq/dL]		108.7 ± 2.0	↓105.4* ± 2.1	↓105.3* ± 3.9	↓106.7 ± 1.9	103.5 ± 2.8	↑104.0 ± 3.7	103.5 ± 2.9	↑104.6 ± 2.3

* Statistically significant ($p \leq 0.05$) ; * Statistically significant ($p \leq 0.01$); numbers in brackets represent the animal numbers of the respective endpoint different from 10 animals per group

G. URINALYSIS

Urinalysis did not demonstrate apparent toxicity of the test substance in either sex or group. Statistically significant changes in urinalysis parameters are displayed in **Table 5.5-31**. Data on time points other than 104 weeks were only included in the table below in case of any differences to the 104 week time point.

Table 5.5-31 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Summary of urinalysis (satellite group)

Time point	Sex	Males				Females			
	Group #	G1	G2	G3	G4	G1	G2	G3	G4
	Dose (ppm)	0	3000	10000	30000	0	3000	10000	30000
	No. of rats	10	10	10	10	10	10	10	10
Specific gravity									
Week 26 (satellite group)		1.065 ± 0.012	↑1.070 ± 0.015	1.065 ± 0.014	↑1.079 ± 0.015	1.064 ± 0.016	↓1.044 ± 0.027	↓1.059 ± 0.028	↓1.050 ± 0.020
Week 52 (satellite group)		1.066 ± 0.14	↑1.068 ± 0.014	↑1.072 ± 0.018	↑1.075 ± 0.011	1.046 ± 0.014	↓1.040 ± 0.012	↑1.058 ± 0.019	↑1.057 ± 0.014
Week 78 (satellite group)		1.075 ± 0.15	↓1.055 ± 0.028	↓1.059 ± 0.014	↓1.064 ± 0.021	1.036 ± 0.007 (8)	↓1.034 ± 0.008 (9)	↓1.031 ± 0.016 (8)	↑1.062 ± 0.020 (8)
Week 104 (main group)		1.057 ± 0.016	↓1.040 ± 0.019	↓1.052 ± 0.021	↑1.061 ± 0.019	1.038 ± 0.007	↑1.040 ± 0.026	↑1.054 ± 0.025	↑1.053 ± 0.014
Glucose									
Week 104 (main group)	Negative	10	10	10	10	10	10	10	10
	Slight	-	-	-	-	-	-	-	-
	Moderate	-	-	-	-	-	-	-	-
	Severe	-	-	-	-	-	-	-	-
	Extreme	-	-	-	-	-	-	-	-
Ketones									
Week 104 (main group)	Negative	10	10	10	10	10	10	10	10
	Slight	-	-	-	-	-	-	-	-
	Moderate	-	-	-	-	-	-	-	-
	Severe	-	-	-	-	-	-	-	-
	Extreme	-	-	-	-	-	-	-	-
Occult blood									

Table 5.5-31 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Summary of urinalysis (satellite group)

Time point	Sex	Males				Females			
	Group #	G1	G2	G3	G4	G1	G2	G3	G4
	Dose (ppm)	0	3000	10000	30000	0	3000	10000	30000
	No. of rats	10	10	10	10	10	10	10	10
Week 26 (satellite group)	Negative	10	10	9	10	10	10	10	10
	Slight	-	-	-	-	-	-	-	-
	Moderate	-	-	1	-	-	-	-	-
	Severe	-	-	-	-	-	-	-	-
Week 52 (satellite group)	Negative	10	10	8	9	10	10	9	10
	Slight	-	-	-	-	-	-	-	-
	Moderate	-	-	1	-	-	-	-	-
	Severe	-	-	1	1	-	-	1	-
Week 78 (satellite group)	Negative	7	4	10	7	7 (8)	8 (9)	7 (8)	7 (8)
	Slight	-	1	-	-	-	-	-	-
	Moderate	-	-	-	1	1 (8)	-	1 (8)	1 (8)
	Severe	-	-	-	-	-	1 (8)	-	-
Week 104 (main group)	Negative	8	8	8	9	10	9	9	10
	Slight	-	1	-	-	-	-	-	-
	Moderate	1	-	1	-	-	-	-	-
	Severe	1	1	1	1	-	1	1	-
pH									
Week 26 (satellite group)	5.0	-	-	-	-	-	-	-	-
	6.0	-	1	4	10**	5	3	5	8
	6.5	5	3	5	-	3	2	2	2
	7.0	1	1	1*	-	1	1	2	-
	7.5	1	2	-	-	-	3	1	-
	8.0	3	2	-	-	1	1	-	-
	8.5	-	1	-	-	-	-	-	-
Week 52 (satellite group)	5.0	-	-	-	4	-	-	-	5
	6.0	1	-	3	6**	4	6	7	5**
	6.5	2	5	4	-	1	3	2	-
	7.0	1	1	3*	-	3	1	1	-
	7.5	4	4	-	-	2	-	-	-
	8.0	2	-	-	-	-	-	-	-
	8.5	-	-	-	-	-	-	-	-
Week 78 (satellite group)	5.0	-	-	-	-	-	-	-	2 (8)
	6.0	2	-	4	8*	6 (8)	9 (9)	6 (8)	6 (8)
	6.5	1	3	5	-	1 (8)	-	2 (8)	-
	7.0	3	-	1	-	1 (8)	-	-	-
	7.5	1	2	-	-	-	-	-	-
	8.0	-	-	-	-	-	-	-	-
	8.5	-	-	-	-	-	-	-	-
Week 104 (main group)	5.0	-	-	-	1	-	-	-	3
	6.0	1	1	5	9**	7	4	6	7
	6.5	4	6	1	-	1	4	4	-
	7.0	4	3	4	-	1	1	-	-
	7.5	1	-	-	-	1	1	-	-
	8.0	-	-	-	-	-	-	-	-
	8.5	-	-	-	-	-	-	-	-
Protein									
Week 26	Negative	-	-	-	-	1	5	2	6

Table 5.5-31 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Summary of urinalysis (satellite group)

Time point	Sex	Males				Females			
	Group #	G1	G2	G3	G4	G1	G2	G3	G4
	Dose (ppm)	0	3000	10000	30000	0	3000	10000	30000
	No. of rats	10	10	10	10	10	10	10	10
(satellite group)	Slight	1	3	3	4	7	2	4	3
	Moderate	5	5	7	6*	1	2	3	1
	Severe	4	2	-	-	1	1	1	-
	Extreme	-	-	-	-	-	-	-	-
Week 52 (satellite group)	Negative	-	-	-	-	-	-	2	1
	Slight	-	-	-	1	4	5	2	5
	Moderate	5	5	7	7	3	1	5	3
	Severe	5	5	3	2	3	4	1	1
	Extreme	-	-	-	-	-	-	-	-
Week 78 (satellite group)	Negative	-	-	-	-	-	1 (9)	4 (8)	-
	Slight	-	-	-	-	5 (8)	4 (9)	1 (8)	1 (8)
	Moderate	-	-	1	-	1 (8)	3 (9)	3 (8)	2 (8)
	Severe	3	2	4	5	2 (8)	1 (8)	-	3 (8)
	Extreme	4	3	5	3	-	-	-	2 (8)
Week 104 (main group)	Negative	-	-	-	-	-	-	-	-
	Slight	-	-	-	-	1	-	3	5
	Moderate	-	-	-	-	4	6	2	2
	Severe	7	8	7	10	4	2	2	2
	Extreme	3	2	3	-	1	2	3	1
Urobilirubin [Ehrlich unit/dL]									
Week 104 (main group)	Negative	10	10	10	10	10	10	10	10
	Slight	-	-	-	-	-	-	-	-
	Moderate	-	-	-	-	-	-	-	-
	Severe	-	-	-	-	-	-	-	-
Appearance									
Week 26 (satellite group)	Colourless	-	-	-	-	-	-	-	-
	Pale yellow	-	1	-	-	1	-	-	-
	Yellow	10	8	10	10	9	10	10	9
	Yellow brown	-	2	-	-	-	-	-	1
	Brown	-	-	-	-	-	-	-	-
Week 52 (satellite group)	Colourless	-	-	-	-	-	-	-	-
	Pale yellow	1	-	-	-	-	-	-	-
	Yellow	9	9	9	10	10	10	10	10
	Yellow brown	-	1	1	-	-	-	-	-
	Brown	-	-	-	-	-	-	-	-
Week 78 (satellite group)	Colourless	-	-	-	-	-	-	-	-
	Pale yellow	2	1	-	-	1 (8)	4 (9)	3 (8)	-
	Yellow	5	3	10	8	7 (8)	5 (9)	5 (8)	8 (8)
	Yellow brown	-	-	-	-	-	-	-	-
	Brown	-	1	-	-	-	-	-	-
Week 104 (main group)	Colourless	-	-	-	-	1	1	-	-
	Pale	-	3	-	1	7	1	3	2

Table 5.5-31 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Summary of urinalysis (satellite group)

Time point	Sex	Males				Females			
	Group #	G1	G2	G3	G4	G1	G2	G3	G4
	Dose (ppm)	0	3000	10000	30000	0	3000	10000	30000
	No. of rats	10	10	10	10	10	10	10	10
	yellow								
	Yellow	6	6	6	7	2	7	6	8*
	Yellow brown	3	1	2	1	-	-	-	-
	Brown	1	-	2	1	-	1*	1*	-
Urine volume [mL]									
Week 26 (satellite group)		10.2 ± 2.4	↑12.5 ± 4.9	↑13.3 ± 5.1	↑12.3 ± 6.2	13.5 ± 5.9	↑15.8 ± 7.9	↓12.4 ± 5.1	↓11.7 ± 6.3
Week 52 (satellite group)		7.0 ± 3.5	↑10.8 ± 5.3	↑11.6 ± 12.2	↑9.2 ± 3.2	15.8 ± 5.0	↑25.2 ± 13.2	↓13.9 ± 9.1	↓11.3 ± 7.0
Week 78 (satellite group)		9.0 ± 12.5	↑20.9 ± 30.2	↓7.2 ± 4.1	↑11.6 ± 13.3	12.1 ± 6.9 (8)	↑19.4 ± 9.7 (9)	↑16.7 ± 11.8 (8)	↓7.8 ± 3.0 (8)
Week 104 (main group)		11.4 ± 7.0	↑29.0 ± 16.6*	↑17.2 ± 13.4	↓10.4 ± 4.8	18.8 ± 9.3	↑27.6 ± 31.7	↑19.4 ± 10.0	↓18.5 ± 8.3
Urinary sediments: Red blood cells									
Week 26 (satellite group)	Nil	9	10	10	10	10	10	10	10
	A few in some fields	1	-	-	-	-	-	-	-
	A few in all fields	-	-	-	-	-	-	-	-
	many in all fields	-	-	-	-	-	-	-	-
Week 52 (satellite group)	Nil	10	9	9	10	10	10	10	10
	A few in some fields	-	-	-	-	-	-	-	-
	A few in all fields	-	-	-	-	-	-	-	-
	many in all fields	-	1	1	-	-	-	-	-
Week 78 (satellite group)	Nil	7	4	10	8	7 (8)	8 (9)	8 (8)	7 (8)
	A few in some fields	-	-	-	-	1 (8)	1 (8)	-	-
	A few in all fields	-	-	-	-	-	-	-	1 (8)
	many in all fields	-	-	-	-	-	-	-	-
Week 104 (main group)	Nil	9	9	9	9	9 (9)	5 (6)	2 (3)	- (0)
	A few in some fields	-	-	1	-	-	1 (6)	1 (3)	- (0)
	A few in all fields	-	-	-	-	-	-	-	- (0)
	many in all fields	1	1	-	1	-	-	-	- (0)
Urinary sediments: White blood cells									
Week 104 (main group)	Nil	10	10	10	10	9 (9)	6 (6)	3 (3)	- (0)
	A few in some fields	-	-	-	-	-	-	-	- (0)
	A few in	-	-	-	-	-	-	-	- (0)

Table 5.5-31 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats (1997): Summary of urinalysis (satellite group)

Time point	Sex	Males				Females			
	Group #	G1	G2	G3	G4	G1	G2	G3	G4
	Dose (ppm)	0	3000	10000	30000	0	3000	10000	30000
	No. of rats	10	10	10	10	10	10	10	10
	all fields								
	many in all fields	-	-	-	-	-	-	-	- (0)
Urinary sediments: Epithelial cells									
Week 104 (main group)	Nil	10	10	10	10	9 (9)	6 (6)	3 (3)	- (0)
	A few in some fields	-	-	-	-	-	-	-	- (0)
	A few in all fields	-	-	-	-	-	-	-	- (0)
	many in all fields	-	-	-	-	-	-	-	- (0)
Urinary sediments: Casts									
Week 104 (main group)	Nil	10	10	10	10	9 (9)	6 (6)	3 (3)	- (0)
	A few in some fields	-	-	-	-	-	-	-	- (0)
	A few in all fields	-	-	-	-	-	-	-	- (0)
	many in all fields	-	-	-	-	-	-	-	- (0)
Urinary sediments: Abnormal crystals (Crystals of cholesterol, tyrosine, leucine, cystein or test substance, etc)									
Week 104 (main group)	Nil	10	10	10	10	9 (9)	6 (6)	3 (3)	- (0)
	A few in some fields	-	-	-	-	-	-	-	- (0)
	A few in all fields	-	-	-	-	-	-	-	- (0)
	many in all fields	-	-	-	-	-	-	-	- (0)
Urinary smear: Red blood cells									
Week 105 (main group)	Nil	-	-	-	-	15 (15)	19 (19)	16 (16)	14 (14)
	A few in some fields	-	-	-	-	-	-	-	-
	A few in all fields	-	-	-	-	-	-	-	-
	many in all fields	-	-	-	-	-	-	-	-
Urinary smear: White blood cells									
Week 105 (main group)	Nil	-	-	-	-	15 (15)	19 (19)	16 (16)	14 (14)
	A few in some fields	-	-	-	-	-	-	-	-
	A few in all fields	-	-	-	-	-	-	-	-
	many in all fields	-	-	-	-	-	-	-	-
Urinary smear: Epithelial cells									
Week 105 (main group)	Nil	-	-	-	-	15 (15)	19 (19)	16 (16)	14 (14)
	A few in some fields	-	-	-	-	-	-	-	-
	A few in	-	-	-	-	-	-	-	-

Table 5.5-31 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Summary of urinalysis (satellite group)

Time point	Sex	Males				Females			
	Group #	G1	G2	G3	G4	G1	G2	G3	G4
	Dose (ppm)	0	3000	10000	30000	0	3000	10000	30000
	No. of rats	10	10	10	10	10	10	10	10
	all fields								
	many in all fields	-	-	-	-	-	-		-
Urinary smear: Casts									
Week 105 (main group)	Nil	-	-	-	-	15 (15)	19 (19)	16 (16)	14 (14)
	A few in some fields	-	-	-	-	-		-	-
	A few in all fields	-	-	-	-	-	-	-	-
	many in all fields	-	-	-	-	-	-	-	-
Urinary smear: Abnormal crystals									
Week 105 (main group)	Nil	-	-	-	-	15 (15)	19 (19)	16 (16)	14 (14)
	A few in some fields	-	-	-	-	-	-	-	-
	A few in all fields	-	-	-	-	-	-	-	-
	many in all fields	-	-	-	-	-	-	-	-

* Statistically significant ($p \leq 0.05$) ; * Statistically significant ($p \leq 0.01$); numbers in braces represent the animal numbers of the respective endpoint different from 10 animals per group

Metabolism of glyphosate after absorption from the intestine is minimal. Thus, most of the glyphosate is excreted *via* urine as the unchanged parent compound. In the urine glyphosate dissociates into the free acid, which can lead to a reduction of the urinary pH. Therefore, the reduced urinary pH might be of no toxicological significance.

H. NECROPSY

In the high dose group significant increases in incidence of distension of the caecum were observed in both sexes, accompanied by soiled fur in the perianal region in males. Moreover, significant increases in absolute and relative weights of the caecum in both sexes in the high and mid dose group were seen, but not associated with histopathological abnormalities (Tables below).

Table 5.5-32 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Absolut organ weights at interim/terminal kills - Group mean values (males)

mg/kg bw/d		Group mean values in male rats (Satellite)							
		No. of animals	Body Weight (g)	Brain (mg)	Liver (g)	Kidneys (mg)	Adrenals (mg)	Testes (mg)	Cecum (mg)
Week 26 (satellite group)	0	10	660 ± 45	2224 ± 91	15.32 ± 2.08	3335 ± 362	60.8 ± 8.4	3593 ± 222	3310 ± 653
	3000	10	↓647 ± 54	↓2223 ± 47	↓14.88 ± 1.91	↑ 3453 ± 252	↑65.1 ± 9.0	↓3525 ± 200	↑3405 ± 956
	10000	10	↓652 ± 71	↑2268 ± 121	↑15.46 ± 2.01	↑3351 ± 347	↑61.2 ± 4.4	↑3648 ± 219	↑4500* ± 1227

Table 5.5-32 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Absolut organ weights at interim/terminal kills - Group mean values (males)

		Group mean values in male rats (Satellite)							
mg/kg bw/d		No. of animals	Body Weight (g)	Brain (mg)	Liver (g)	Kidneys (mg)	Adrenals (mg)	Testes (mg)	Cecum (mg)
	30000	10	↓594 ± 51	↑2257 ± 41	↑15.49 ± 2.04	↓3305 ± 356	↓57.0 ± 7.5	↑3684 ± 191	↑6234** ± 1159
Week 52 (satellite group)	0	10	782 ± 58	2375 ± 103	18.62 ± 2.38	3936 ± 588	60.4 ± 9.6	3697 ± 189	3691 ± 529
	3000	10	↑798 ± 63	↓2339 ± 58	↑23.05 ± 15.28	↑4461 ± 1824	↑63.6 ± 9.4	↓3671 ± 309	↓3272 ± 1125
	10000	10	↑791 ± 130	↓2359 ± 107	↑20.62 ± 8.37	↑4528 ± 2891	↑65.1 ± 12.0	↓3627 ± 389	↑4493 ± 1386
	30000	10	↓701 ± 74	↓2296 ± 90	↓18.16 ± 2.64	↑3936 ± 477	↓59.1 ± 9.2	↓3595 ± 490	↑5896** ± 823
Week 78 (satellite group)	0	6	851 ± 94	2376 ± 63	20.42 ± 4.38	4722 ± 1273	79.6 ± 21.9	3317 ± 885	3719 ± 666
	3000	5	↓784 ± 119	↑2475 ± 151	↓17.83 ± 1.75	↓4491 ± 584	↑120.0 ± 95.7	↑3343 ± 784	↑4427 ± 978
	10000	10	↓849 ± 75	↑2417 ± 56	↑21.35 ± 3.71	↓4542 ± 731	↑92.7 ± 40.9	↓3313 ± 499	↑4807 ± 975
	30000	8	↓738 ± 90	↓2341 ± 55	↑21.36 ± 5.00	↓4516 ± 1135	↓73.3 ± 26.3	↑3686 ± 385	↑6873** ± 1826
Week 104 (main group)	0	10	773 ± 121	2400 ± 67	18.44 ± 2.40	4588 ± 686	107.0 ± 44.0	3720 ± 1059	3413 ± 909
	3000	10	↓746 ± 67	↓2371 ± 134	↑19.23 ± 3.11	↑5656 ± 1513	↑110.5 ± 31.5	↓3040 ± 809	↑3799 ± 1990
	10000	10	↓768 ± 196	↓2337 ± 126	↑18.67 ± 3.15	↑4808 ± 1275	↓92.4 ± 18.7	↓3362 ± 446	↑4539 ± 961
	30000	10	↓739 ± 115	↑2432 ± 117	↓17.70 ± 2.24	↓4267 ± 1039	↓85.3 ± 19.9	↓3564 ± 465	↑7272** ± 2841

*/** Significantly different from the control at 5 % and 1 % level of probability, respectively.

Table 5.5-33 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Relative organ weights at interim/terminal kills - Group mean values (males)

		Group mean values in male rats (Satellite)						
mg/kg bw/d		No. of animals	Brain (mg)	Liver (g)	Kidneys (mg)	Adrenals (mg)	Testes (mg)	Cecum (mg)
Week 26 (satellite group)	0	10	0.34 ± 0.02	2.32 ± 0.18	0.51 ± 0.04	0.009 ± 0.001	0.55 ± 0.06	0.50 ± 0.08
	3000	10	↑0.35 ± 0.03	↓2.30 ± 0.17	↑0.54 ± 0.05	↑0.010 ± 0.001	0.55 ± 0.07	↑0.53 ± 0.16
	10000	10	↑0.35 ± 0.04	↑2.37 ± 0.11	↑0.52 ± 0.05	0.009 ± 0.001	↑0.57 ± 0.08	↑0.70 ± 0.23
	30000	10	↑0.38** ± 0.04	↑2.60** ± 0.18	↑0.56 ± 0.04	↑0.010 ± 0.001	↑0.63* ± 0.07	↑1.06** ± 0.25
Week 52 (satellite group)	0	10	0.31 ± 0.02	2.38 ± 0.25	0.50 ± 0.06	0.008 ± 0.001	0.47 ± 0.04	0.47 ± 0.07
	3000	10	↓0.30 ± 0.03	↑3.02 ± 2.46	↑0.58 ± 0.31	0.008 ± 0.002	↓0.46 ± 0.05	↓0.42 ± 0.16
	10000	10	0.31 ± 0.04	↑2.69 ± 1.41	↑0.61 ± 0.48	0.008 ± 0.002	0.47 ± 0.08	↑0.58 ± 0.20
	30000	10	↑0.33 ± 0.04	↑2.59 ± 0.25	↑0.57 ± 0.08	0.008 ± 0.001	↑0.52 ± 0.09	↑0.85** ± 0.13

Table 5.5-33 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Relative organ weights at interim/terminal kills - Group mean values (males)

		Group mean values in male rats (Satellite)						
mg/kg bw/d		No. of animals	Brain (mg)	Liver (g)	Kidneys (mg)	Adrenals (mg)	Testes (mg)	Cecum (mg)
Week 78 (satellite group)	0	6	0.28 ± 0.03	2.39 ± 0.33	0.55 ± 0.13	0.009 ± 0.002	0.39 ± 0.11	0.45 ± 0.11
	3000	5	↑0.32 ± 0.07	↓2.32 ± 0.45	↑0.59 ± 0.14	↑0.017 ± 0.017	↑0.42 ± 0.05	↑0.58 ± 0.17
	10000	10	↑0.29 ± 0.03	↑2.52 ± 0.44	↓0.54 ± 0.08	↑0.011 ± 0.005	0.39 ± 0.07	↑0.57 ± 0.15
	30000	8	↑0.32 ± 0.05	↑2.99 ± 1.16	↑0.64 ± 0.28	↑0.011 ± 0.006	↑0.51 ± 0.11	↑0.97** ± 0.40
Week 104 (main group)	0	10	0.32 ± 0.05	2.42 ± 0.36	0.61 ± 0.15	0.014 ± 0.008	0.48 ± 0.11	0.45 ± 0.15
	3000	10	0.32 ± 0.03	↑2.59 ± 0.42	↑0.77 ± 0.22	↑0.015 ± 0.006	↓0.41 ± 0.10	↑0.51 ± 0.24
	10000	10	0.32 ± 0.07	↑2.52 ± 0.25	↑0.67 ± 0.25	↑0.013 ± 0.004	↓0.45 ± 0.09	↑0.62 ± 0.17
	30000	10	↑0.34 ± 0.05	↑2.43 ± 0.38	↓0.59 ± 0.17	↑0.012 ± 0.003	↑0.49 ± 0.10	↑0.97** ± 0.29

*/** Significantly different from the control at 5 % and 1 % level of probability, respectively.

Table 5.5-34 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Absolut organ weights at interim/terminal kills - Group mean values (females)

		Group mean values in female rats (Satellite)						
mg/kg bw/d		No. of animals	Body Weight (g)	Brain (mg)	Liver (g)	Kidneys (mg)	Adrenals (mg)	Cecum (mg)
Week 26 (satellite group)	0	10	361 ± 39	2058 ± 74	8.28 ± 0.87	1912 ± 155	72.3 ± 10.6	2747 ± 596
	3000	10	↓340 ± 49	↓2044 ± 54	↓8.04 ± 1.20	↑1936 ± 215	↓67.7 ± 8.9	↑2773 ± 716
	10000	10	↓356 ± 356	↓2022 ± 38	↓8.36 ± 0.92	↑1947 ± 158	↓69.6 ± 10.2	↑3055 ± 1071
	30000	10	↓321 ± 37	↓2040 ± 91	↓8.05 ± 1.24	↑1929 ± 131	↓66.2 ± 8.6	↑5359** ± 1572
Week 52 (satellite group)	0	10	419 ± 86	2061 ± 67	10.05 ± 1.90	2110 ± 193	72.0 ± 15.5	2113 ± 683
	3000	10	↑484 ± 88	↑2077 ± 109	↑10.75 ± 1.74	↑2307 ± 312	↑89.0 ± 22.6	↑2580 ± 449
	10000	10	↑479 ± 479	↑2076 ± 101	↑10.50 ± 1.46	↑2303 ± 140	↑77.6 ± 12.5	↑2995* ± 595
	30000	10	↓402 ± 39	↑2088 ± 69	↑10.45 ± 2.34	↑2602 ± 1138	↑73.1 ± 11.8	↑4806** ± 772
Week 78 (satellite group)	0	8	555 ± 101	2131 ± 77	12.78 ± 2.05	2466 ± 278	91.2 ± 32.1	2911 ± 726
	3000	9	↓505 ± 88	↑2133 ± 144	↓12.23 ± 2.34	↑2732 ± 406	↑134.2 ± 88.4	↑3101 ± 3101
	10000	8	↓481 ± 65	↑2147 ± 78	↓10.98 ± 1.25	↑2512 ± 281	↓88.1 ± 32.9	↑3947 ± 3947
	30000	8	↓433 ± 146	↑2141 ± 87	↓11.01 ± 2.95	↑2612 ± 328	↑101.8 ± 34.9	↑4857 ± 4857
Week 104	0	10	528 ± 92	2143 ± 119	14.18 ± 3.20	3224 ± 1112	117.3 ± 50.4	2670 ± 1021

Table 5.5-34 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Absolut organ weights at interim/terminal kills - Group mean values (females)

		Group mean values in female rats (Satellite)						
mg/kg bw/d		No. of animals	Body Weight (g)	Brain (mg)	Liver (g)	Kidneys (mg)	Adrenals (mg)	Cecum (mg)
(main group)	3000	10	↑544 ± 93	↑2147 ± 97	↑14.47 ± 4.19	↓2978 ± 351	↓101.4 ± 35.2	↑4193* ± 1287
	10000	10	↑567 ± 71	↑2160 ± 80	↑14.98 ± 2.60	↓3001 ± 236	↑154.1 ± 85.3	↑3741 ± 1536
	30000	10	↑540 ± 80	↓2128 ± 64	↑14.62 ± 1.99	↓2991 ± 348	↓114.0 ± 37.5	↑4909** ± 1318

*/** Significantly different from the control at 5 % and 1 % level of probability, respectively.

Table 5.5-35 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Relative organ weights at interim/terminal kills - Group mean values (females)

		Group mean values in female rats (Satellite)					
mg/kg bw/d		No. of animals	Brain (mg)	Liver (g)	Kidneys (mg)	Adrenals (mg)	Cecum (mg)
Week 26 (satellite group)	0	10	0.58 ± 0.06	2.30 ± 0.16	0.53 ± 0.05	0.020 ± 0.003	0.78 ± 0.23
	3000	10	↑0.61 ± 0.08	↑2.37 ± 0.24	↑0.58 ± 0.07	0.020 ± 0.004	↑0.83 ± 0.23
	10000	10	↓0.57 ± 0.06	↑2.35 ± 0.16	↑0.55 ± 0.04	0.020 ± 0.004	↑0.88 ± 0.34
	30000	10	↑0.64 ± 0.07	↑2.50 ± 0.19	↑0.61* ± 0.06	↑0.021 ± 0.003	↑1.71** ± 0.59
Week 52 (satellite group)	0	10	0.51 ± 0.09	2.42 ± 0.27	0.52 ± 0.08	0.018 ± 0.005	0.53 ± 0.20
	3000	10	↓0.44 ± 0.08	↓2.33 ± 0.14	↓0.48 ± 0.08	↑0.019 ± 0.004	↑0.56 ± 0.17
	10000	10	↓0.44 ± 0.07	↓2.21 ± 0.22	↓0.50 ± 0.09	↓0.017 ± 0.004	↑0.65 ± 0.21
	30000	10	↑0.52 ± 0.04	↑2.62 ± 0.69	↑0.66 ± 0.32	0.018 ± 0.004	↑1.20** ± 0.15
Week 78 (satellite group)	0	8	0.40 ± 0.08	2.32 ± 0.24	0.46 ± 0.08	0.017 ± 0.006	0.54 ± 0.17
	3000	9	↑0.43 ± 0.08	↑2.42 ± 0.16	↑0.55 ± 0.05	↑0.026 ± 0.017	↑0.63 ± 0.18
	10000	8	↑0.45 ± 0.06	↓2.30 ± 0.21	↑0.53 ± 0.08	↑0.018 ± 0.006	↑0.84 ± 0.24
	30000	8	↑0.56 ± 0.22	↑2.61 ± 0.28	↑0.66 ± 0.21	↑0.027 ± 0.017	↑1.22* ± 0.74
Week 104 (main group)	0	10	0.42 ± 0.06	2.75 ± 0.70	0.65 ± 0.35	0.024 ± 0.014	0.52 ± 0.20
	3000	10	↓0.41 ± 0.07	↓2.68 ± 0.64	↓0.56 ± 0.09	↓0.019 ± 0.007	↑0.80* ± 0.30
	10000	10	↓0.39 ± 0.07	↓2.67 ± 0.50	↓0.54 ± 0.09	↑0.028 ± 0.015	↑0.66 ± 0.27
	30000	10	↓0.40 ± 0.06	↓2.72 ± 0.27	↓0.56 ± 0.09	↓0.022 ± 0.009	↑0.91** ± 0.24

*/** Significantly different from the control at 5 % and 1 % level of probability, respectively.

The incidences of thickened areas in the skin of the tail, corresponding to the tail mass in the clinical observations, were significantly increased in the mid and high dose group. The lesion was diagnosed as follicular hyperkeratosis and/or folliculitis/follicular abscess. An increased incidence of hair loss was also observed in high-dosed females, but it lacked corresponding histopathological changes (Table 5.5-36).

Table 5.5-36 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Gross pathology

		Males				Females			
ppm		0	3000	10000	30000	0	3000	10000	30000
Findings/Number of animals (Satellite)									
Digestive system	Intestine								
	Distention of cecum								
	Week 26	0/10	0/10	0/10	9/10**	0/10	0/10	2/10	10/10**
External appearance	Fur								
	Soiled in perianal region								
	Week 52	0/10	0/10	0/10	5/10*	0/10	0/10	0/10	2/10
Digestive system	Intestine								
	Distention of cecum								
	Week 78	0/6	0/5	0/10	6/8**	0/8	0/9	0/8	4/8*
Findings/Number of animals (Main group) Week 104 – Terminal Kill									
Digestive system	Intestine								
	Distention of cecum	0/18	1/20	1/18	16/29**	-	-	-	-
Integumentary system	Skin								
	Callosity in paw	12/18	11/20	11/18	8/29**	6/15	8/19	13/16*	7/14
External Appearance	Fur								
	Loss of tactile hair	-	-	-	-	0/35	5/31*	1/34	1/36
	Soiled in fore-limb	3/32	11/30*	2/32	4/21	-	-	-	-
Urinary system	Kidney								
	Coarse surface	3/32	5/30	6/32	7/21*	-	-	-	-
Genital system	Uterus								
	Mass(es)	-	-	-	-	4/15	0/19*	1/16	2/14
Endocrine system	Pituitary								
	Mass(es)	19/32	16/30	10/32*	13/21	-	-	-	-
	Adrenals								
	Enlargement	-	-	-	-	8/35	3/31	1/34	2/36
Integumentary system	Skin								
	Hair loss	-	-	-	-	3/35	5/31	5/34	11/36*
	Callosity in paw	-	-	-	-	3/35	6/31	7/34	10/36*
	Thickened area	4/32	3/30	1/32	9/21*	0/35	0/31	4/34	5/36*
Body cavities	Thoracic cavity								
	Hydrothorax	2/31	0/30	8/32*	2/21	-	-	-	-

*/** Significantly different from the control at 5 % and 1 % level of probability, respectively.

Table 5.5-37 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Histopathology of main and satellite groups – Incidence of microscopic non-neoplastic lesions

	Males				Female			
ppm	0	3000	10000	30000	0	3000	10000	30000
Total animals investigated	76	75	80	78	78	79	78	78
Cardiovascular System								
Heart								

Table 5.5-37 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Histopathology of main and satellite groups – Incidence of microscopic non-neoplastic lesions

	Males				Female			
ppm	**0**	**3000**	**10000**	**30000**	**0**	**3000**	**10000**	**30000**
NAD	24	18	22	27	53	52	53	65
Myocardial atrophy/fibrosis	47	49	45	45	22	19	20	8**
Endocardial hyperplasia	0	1	1	0	0	0	0	1
Myocarditis	6	6	10	6	3	6	4	2
Endocardial mineralisation	0	0	1	4	1	0	0	1
Epicarditis	0	0	1	0	0	0	0	0
Auricular thrombus	0	1	2	1	0	0	2	0
Ventricular thrombus	0	0	1	0	0	0	0	0
Haemorrhage	0	0	1	0	0	0	0	0
Arteritis	0	2	0	1	0	0	0	0
Aorta								
NAD	76	75	79	74	76	78	77	76
Mineralisation	0	0	1	4	2	0	1	2
Endothelial degeneration	0	0	0	0	0	1	0	0
Haematopoietic & Lymphatic System								
Bone marrow (femur)								
NAD	50	44	51	60	56	53	50	53
Necrosis	1	0	0	0	0	0	0	0
Increased haematopoiesis	23	31	27	18	21	25	27	24
Fibrosis	0	0	1	1	0	0	1	0
Haemorrhage	1	0	0	1	0	0	0	0
Bone marrow (sternum)								
NAD	49	44	53	61	57	53	50	53
Increased haematopoiesis	24	31	26	17	21	25	27	24
Fibrosis	0	1	0	0	0	0	1	0
Bone marrow (vertebra)								
NAD	50	44	53	61	57	53	50	53
Increased haematopoiesis	23	31	26	17	21	25	27	24
Thymus								
NAD	75	73	77	77	77	78	76	77
Atrophy	0	0	2	0	0	0	0	0
Lymphoid cell hyperplasia	0	0	0	1	0	1	3	1
Epithelial reticulum cell hyperplasia	0	1	0	0	0	0	0	0
Lymph nodes (cervical)								
NAD	68	64	69	75	72	75	72	75
Lymphoid cell hyperplasia	2	3	1	0	0	1	3	1
Plasma cell hyperplasia	3	5	5	3	0	0	0	0
Sinal dilatation	1	1	1	0	1	0	0	0
Sinal foam cell accumulation	0	0	1	0	0	0	0	0
Lymph nodes (mesenteric)								
NAD	73	72	77	75	74	75	78	73
Lymphoid cell hyperplasia	2	1	0	1	1	1	0	1
Plasma cell hyperplasia	0	0	0	1	0	0	0	0
Sinal dilatation	0	0	1	0	1	0	0	0
Granuloma	0	0	0	1	1	2	0	2
Lymph nodes (others)								
Lymph adenitis	0 (7)	1 (8)	0 (10)	0 (5)	0	0	0	0
Plasma cell hyperplasia	4 (7)	5 (8)	6 (10)	5 (5)	2	4	6	3

Table 5.5-37 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Histopathology of main and satellite groups – Incidence of microscopic non-neoplastic lesions

	Males				Female			
ppm	**0**	**3000**	**10000**	**30000**	**0**	**3000**	**10000**	**30000**
Sinal dilatation	1 (7)	3 (8)	3 (10)	0 (5)	0	1	1	1
Spleen								
NAD	50	43	47	51	36	27	30	26
Necrosis	0	1	1	0	0	1	1	2
Lymphoid cell hyperplasia	1	0	1	1	0	0	3	0
Plasma cell hyperplasia	1	0	1	0	0	0	0	2
Capsular cyst	2	0	1	0	0	1	0	0
Fibrosis	3	3	1	1	0	1	3	1
Increased brown pigment deposition	10	9	7	8	20	25	22	23
Capsulitis/capsular fibrosis	0	1	0	1	0	0	0	0
Increased extramedullary haematopoiesis	11	18	23*	18	22	28	23	26
Haemorrhage	0	0	1	0	0	0	0	0
Arteritis	0	2	0	0	0	0	0	0
Digestive System								
Pancreas								
NAD	55	51	61	53	59	62	61	62
Acinar cell hyperplasia	1	2	1	1				
Basophilic acinar cell focus	5	4	5	5	2	3	3	2
Acinar cell atrophy	10	17	10	17	11	12	12	10
Pancreatitis	2	0	0	0	0	1	0	1
Ductal proliferation	1	0	1	0	0	0	0	1
Fibrosis	0	0	0	0	1	0	0	0
Islet cell hyperplasia	1	1	0	0	0	0	0	1
Granuloma	0	0	0	1	1	1	0	0
Arteritis	1	6	4	3	2	1	1	1
Integumentary System								
Skin								
NAD	35	32	44	35	62	56	45	52
Epithelial hyperplasia	2	0	2	0	0	0	0	0
Necrosis	0	1	0	0	0	0	1	0
Dermatitis	3	4	4	3	2	1	3	2
Epidermal cyst	3	0	1	4	0	0	2	1
Erosion/ulcer	1	0	0	0	0	1	0	0
Fibrosis	1	1	0	2	0	0	0	0
Follicular epithelial hyperplasia	0	0	0	1	0	0	0	0
Folliculitis/follicular abscess	5	7	1	9	0	1	6*	4
Follicular dilatation	3	5	4	3	0	0	0	0
Follicular hyperkeratosis	7	5	2	23**	0	2	2	6*
Plantar granuloma	27	25	21	19	10	17	22*	17
Palmar granuloma	1	0	0	0	0	0	0	0
Subcutaneous cyst	0	0	1	0	0	0	0	0
Subcutaneous oedema	0	0	2	0	0	0	0	1
Subcutaneous inflammation	3	2	0	1	0	0	0	1
Subcutaneous abscess	2	1	1	2	0	2	1	2

Numbers in brackets represent the animal number of the respective finding
* Significantly different from control ($p \leq 0.05$ %)
** Significantly different from control ($p \leq 0.01$ %)
NAD= No abnormalities detected

All changes regarding neoplastic lesions were not statistically significant.

Table 5.5-38 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Histopathology of main and satellite groups – Incidence of microscopic neoplastic lesions

	Males				Female			
ppm	**0**	**3000**	**10000**	**30000**	**0**	**3000**	**10000**	**30000**
Total animals investigated	76	75	80	78	78	79	78	78
General organs								
Systemic histiocytic sarcoma	0	0	1	0	1	0	0	0
Systemic malignant fibrous histiocytoma	2	0	0	0	-	-	-	-
Cardiovascular System								
Heart schwannoma	0	1	1	1	0	0	0	1
Hematopoietic & Lymphatic System								
Myelogenic leukemia	1	0	0	0	-	-	-	-
Malignant lymphoma	0	2	1	0	1	1	1	2
Mononuclear cell leukemia	0	1	0	0	-	-	-	-
Spleen Histiocystic sarcoma								
Respiratory System								
Lung adenoma	2	3	1	3	-	-	-	-
Squamous cell carcinoma	0	0	1	0	1 (2)	-	0 (3)	0 (1)
Adenocarcinoma	0	0	0	1	-	-	-	-
Digestive System								
Leiomysarcoma	0	0	1	0	-	-	-	-
Leiomyoma	0	0	0	1	0	0	1	0
Adenocarcinoma	0	0	1	0	-	-	-	-
Malignant schwannoma	0	1	0	0	-	-	-	-
Malignant histiocytoma	-	-	-	-	1	0	0	0
Liver								
Hepatocellular adenoma	1	0	2	1	1	1	0	0
Hepatocellular carcinoma	0	1	2	1	-	-	-	-
Pancreas								
Acinar cell adenoma	0	1	1	2	-	-	-	-
Islet cell adenoma	4	1	1	1	3	2	1	1
Islet cell carcinoma	0	0	1	0	0	1	0	0
Urinary system								
Kidney								
Adenoma	0	0	0	4	-	-	-	-
Transitional cell carcinoma	-	-	-	-	1	0	0	0
Lipoma	0	0	0	1	0	1	0	0
Urinary bladder								
Papilloma	-	-	-	-	0	1	0	0
Genital System								
Testis interstitial cell tumour	3	2	0	2	-	-	-	-
Coagulating gland adenoma	0	0	0	1	-	-	-	-
Ovary								
Granulosa cell tumour	-	-	-	-	1	0	0	0
Luteoma	-	-	-	-	0	0	1	0
Uterus								
Plyp/endometrial stromal polyp	-	-	-	-	5	8	2	5
Granular cell tumour	-	-	-	-	1	0	0	0

Table 5.5-38 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Histopathology of main and satellite groups – Incidence of microscopic neoplastic lesions

	Males				Female			
ppm	**0**	**3000**	**10000**	**30000**	**0**	**3000**	**10000**	**30000**
Adenocarcinoma	-	-	-	-	1	1	0	0
Malignant schwannoma	-	-	-	-	0	0	0	1
Vagina								
Polyp	-	-	-	-	1	0	1	1
Leiomyosarcoma	-	-	-	-	0	0	0	1
Clitoral gland								
Squamous cell carcinoma	-	-	-	-	1 (1)	-	-	0 (1)
Endocrine System								
Pituitary								
Anterior adenoma	38	40	33	42	54	54	47 (77)	52
Anterior adenocarcinoma	-	-	-	-	1	0	0 (77)	0
Intermediate adenoma	0	1	0	0	-	-	-	-
Thyroid								
Follicular adenoma	3	2	1	0	0	2	1 (76)	0
C-cell adenoma	6	10	5	6	4	7	8 (76)	4
Follicular adenocarcinoma	1	0	0	0	-	-	-	-
C-cell carcinoma	2	0	1	1	-	-	-	-
Adrenal								
Cortical adenoma	1	2	1	0	0	1	1	0
Pheochromocytoma	14	9	5*	10	2	0	2	1
Cortical adenocarcinoma	0	0	1	0	-	-	-	-
Ganglioneuroma	-	-	-	-	0	1	0	0
Nervous System								
Meningioma	-	-	-	-	0	1	0 (77)	0
Glioma	1	0	1	1	-	-	-	-
Malignant reticulosis	1	0	0	0	1	1	0 (77)	0
Granula cell tumour (cerebellum)	0	0	1	0	0	0	1 (77)	0
Musculo-skeletal System								
Osteochondroma (femur)	0	0	1	0	0	0	0	1
Chondroma (vertebra)	-	-	-	-	1	0	0	0
Osteosarcoma (other bone)	0	0	1	0	-	-	-	-
Osteochondroma (other bone)	-	-	-	-	2	0	0	3
Sense Organs								
Eye								
Schwannoma	1	0	0	0	-	-	-	-
Ear								
Zymbal's gland carcinoma	-	0	1	-	-	-	-	-
Auricle								
Papilloma	1 (3)	0 (1)	-	0 (2)	0 (3)	0 (1)	1 (2)	0 (3)
Fibroma	-	-	-	-	1 (3)	0 (1)	0 (2)	0 (3)
Malignant schwannoma	0 (3)	0 (1)	-	1 (2)	-	-	-	-
Integumentary System								
Skin								
Papilloma	2	5	3	0	0	1	1	0
Keratoacanthoma	4	3	0	7	0	0	0	1
Trichoepithelioma	0	1	0	0	-	-	-	-
Sebaceous gland adenoma	0	1	0	0	-	-	-	-
Basal cell adenoma	0	0	0	3	-	-	-	-
Fibroma	4	4	4	5	4	3	2	4
Lipoma	4	2	2	1	4	1	3	0

Table 5.5-38 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Histopathology of main and satellite groups – Incidence of microscopic neoplastic lesions

	Males				Female			
ppm	**0**	**3000**	**10000**	**30000**	**0**	**3000**	**10000**	**30000**
Sqamous cell carcinoma	0	0	1	1	-	-	-	-
Basal cell carcinoma	0	0	0	1	-	-	-	-
Fibrosarcoma	0	1	1	0	-	-	-	-
Liposarcoma	1	0	0	0	-	-	-	-
Hemangiosarcoma	0	0	1	0	-	-	-	-
Malignant haemangiopericytoma	0	0	0	1	-	-	-	-
Osterosarcoma	0	1	0	1	-	-	-	-
Malignant schwannoma	0	0	0	1	-	-	-	-
Histiocytic sarcoma	0	0	1	0	-	-	-	-
Mammary gland								
Adenoma	1 (4)	0 (5)	0 (2)	0 (2)	1	2	1 (77)	0
Fibroadenoma	2 (4)	3 (5)	1 (2)	0 (2)	25	30	27 (77)	30
Adenocarcinoma	0 (4)	0 (5)	1 (2)	2 (2)	11	8	11 (77)	8
Body cavities								
Thoracic cavity								
Malignant mesothelioma	-	-	1 (1)	-	-	-	-	-
Abdominal cavity								
Lipoma	-	-	-	-	0 (1)	0 (2)	1 (1)	-
Malignant schwannoma	0 (1)	0 (5)	1 (4)	0 (2)	1 (1)	0 (2)	0 (1)	-
Malignant mesothelioma	0 (1)	1 (5)	0 (4)	0 (2)	-	-	-	-
No. of benign neoplasms	92	91	64	92	110	116	102	104
No. of malignant neoplasms	8	9	19	11	21	12	12	12
No. of benign and malignant neoplasms	100	100	83	103	131	128	114	116
No. of animals with benign neoplasms	51	54	43	51	60	55	54	59
No. of animals with malignant neoplasms	8	9	19	9	17	12	12	12
No. of animals with neoplasms	55	54	52	51	60	58	54	60

Numbers in brackets represent the animal number of the respective finding
* Significantly different from control ($p \leq 5$ %)

From this, it is concluded that the test compound at the doses tested does not cause treatment or dose related gross and histopathological changes and it is not carcinogenic under the testing conditions.

3. Assessment and conclusion

Assessment and conclusion by applicant:
In conclusion, HR-001 was not carcinogenic in the Sprague-Dawley rats following continuous dietary exposure of up to 30000 ppm for 24 months. The NOAEL for toxicity is 3000 ppm, corresponding to 104.0 mg/kg bw/day for males and 114.7 mg/kg bw/day for females.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.5/005
Report author	[redacted]
Report year	1996
Report title	Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats
Report No	886.C.C-R
Document No	Not reported
Guidelines followed in study	OECD 453 (1981)
Deviations from current test guideline (OECD 453, 2018)	Yes, individual animals exceed the 20 % range in body weight; mortality was observed only once per day (instead of twice per day); haematological parameters: prothrombin time, activated partial thromboplastin time, reticulocyte count were not observed; clinical chemistry: P, Cl, Na, K, cholesterol, bilirubin, creatinine, T3, T4 and TSH were not observed; urinalysis: osmolality/spec gravity and occult blood was not determined; organ weights of epididymis, heart, spleen, thyroid/parathyroid and uterus were not determined. Histopathological examination of the Harderian gland, cervix, coagulating glands, gall bladder, lacrimal gland, rectum and vagina was not performed. An evaluation with regards to historical control data was provided in the study report by the study director.
Previous evaluation	Yes, accepted in the RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The chronic toxicity and carcinogenic potential of glyphosate technical was assessed in a 24-month feeding study in male and female Wistar rats. Groups of 50 rats per sex received daily dietary doses of 0, 100, 1000, and 10000 ppm glyphosate technical (equivalent to mean achieved dose levels of 0, 7.4, 73.9 and 764.8/740.6 mg/kg bw/day for 12/24 months respectively). In addition, one vehicle control with ten rats per sex and one high dose group with 20 rats per sex were included for interim sacrifice at the 12th month to study non-neoplastic histopathological changes. Observations covered clinical signs, body weight, food consumption, haematology, clinical chemistry and urinalysis, as well as organ weights, necropsy and histopathological examination.

There were no treatment-related deaths or clinical signs in any of the dose-groups. Moreover, there were no treatment-related effects on body weight gain or food consumption. All dose- or treatment-related significant changes observed in haematological, biochemical parameters as well as the urinalysis were within the range of the historical control data and hence appear to be of no biological significance.

Gross pathology, organ weight data and histopathological examination demonstrated no treatment-related and dose-dependent effects.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate technical
Description:	White odourless crystals
Lot/Batch #:	60; 046
Purity:	96.8 %; 96 %
Stability of test compound:	More than two years at ambient temperature
2. Vehicle and/ or positive control:	Diet
3. Test animals:	
Species:	Rat
Strain:	Wistar
Source:	[redacted]
Age:	6 weeks
Sex:	Males and females
Weight at dosing:	Males: 90 – 179 g, females: 80 – 151 g
Acclimation period:	At least one week
Diet/Food:	Standard "Gold Mohur" (M/S Lipton India Ltd, India), *ad libitum*
Water:	Deep bore well water treated with charcoal filter and UV rays, *ad libitum*
Housing:	Initially in groups of five per sex in polypropylene cages and in groups of three from Week 12 onwards.
Environmental conditions:	Temperature: 19 - 25 °C Humidity: 40 - 70 % Air changes: not reported 12 hours light/dark cycle

B: Study design and methods

In life dates: 1992-03-04 to 1994-03-04

Animal assignment and treatment:
In a combined chronic toxicity and carcinogenicity study groups of 50 Wistar rats per sex received daily dietary doses of 0, 100, 1000 and 10000 ppm (equivalent to mean achieved dose levels of 0, 7.4, 73.9 and 740.6 mg/kg bw/day for 24 months respectively) glyphosate technical. In additional one vehicle control with ten rats per sex and one high dose group with 20 rats per sex were included for interim sacrifice at the 12th month to study non-neoplastic histopathological changes.

Test diets were prepared fortnightly by mixing a known amount of the test substance with a small amount of basal diet. This pre-mix was then added to larger amount of basal diet and blended for further 20 minutes. The stability of the test substance in food was determined at day 1, day 15 and day 30 in an in-house stability study at 2000 and 20000 ppm. It was stated that homogeneity of samples was also determined but no results were provided.

Observations
Veterinary examination was made before and after grouping and at the end of each month of experimental schedule. Rats were examined for toxic signs and pre-terminal deaths once a day. Ophthalmic examination

was done at the start of the study and at termination.

Body weight
Individual body weights were recorded before dosing, at weekly intervals until the end of week 13 and every 4 weeks thereafter until termination.

Food consumption and compound intake
Food consumption was recorded once weekly for each cage group from Week 1 to Week 13 and subsequently over one week in every 4 weeks until termination.

Haematology and clinical chemistry
Haematology
Individual blood samples were collected from 20 rats/sex/group at 3, 6, 12, 18 and 24 months. The following parameters were measured: Haemoglobin, haematocrit, erythrocyte count, clotting time and total leukocyte count and differential leukocyte count.

Blood chemistry
At the scheduled intervals of 6, 12, 18 and 24 months, blood collected from 10 rats/sex/group was subjected to clinical chemistry analysis. The following parameters were measured: Total proteins, albumin, ALT, AST, GGT, ALP, blood urea nitrogen and blood glucose.

Urinalysis
Individual urine samples were collected from 10 rats/sex/group at 3, 6, 12, 18 and 24 months. The following measurements were made: Volume, appearance, pH, nitrite, urobilinogen, bilirubin, erythrocytes, protein, glucose, ketones, microscopy of sediments.

Sacrifice and pathology
Histopathological examination was carried out on all tissues collected at interim sacrifice, control and high dose groups; all pre-terminally dead and moribund sacrificed rats of the low and mid dose groups and on all lesions of the terminally sacrificed rats from the low and mid dose groups.

The following organ weights were determined from 10 rats per sex per group: adrenals, brain, gonads, kidneys and liver.

Tissue samples were taken from the following organs: adrenals, aorta (main group animals), bone & bone marrow (sternum and femur incl. joint), brain, caecum, colon, duodenum, epididymides (main group animals), eyes (with optic nerve), heart, ileum, jejunum, kidneys, liver, lungs, mammary gland, lymph nodes (mesenteric, mandibular and mediastinal), muscle (femoral), oesophagus, ovaries, pancreas, pituitary, prostrate, rectum, salivary glands, sciatic nerve, seminal vesicles and coagulating glands, skin, spinal cord (cervical, thoracic and lumbar), spleen, stomach, testes, thymus, thyroid/parathyroid, trachea, tumour/mass, urinary bladder and uterus.

A detailed histopathological examination was performed on all sampled tissues of the control and high-dose animals, and on animals that died or were killed in extremis. In addition, gross lesions and masses from low and intermediate dose groups at termination were examined microscopically.

Statistics
Using specific computer programs, body weight, net body weight gain, food consumption, haematology, clinical chemistry and organ weight data of different groups were compared by Bartlett's test for homogeneity of intra group variances. When the variances proved to be heterogeneous, the data were transformed using appropriate transformation. The data with homogeneous intra group variances were subjected to one-way analysis of variance (ANOVA - Snedecor and Cochran, 1980). When 'F' value was significant, Dunnett's pair wise comparison (Scheffe, 1953) of means of treated groups with control mean was done individually.

Net food intake (g/kg bw/day) and test compound intake (mg/kg bw/day) was calculated for the whole study period using calculated means and food intake was statistically analysed by the procedure given above. Incidence of gross, histopathological changes of mass(es) and incidence of benign and malignant neoplasia in the treatment groups were statistically compared with control group by Z-test wherever it was applicable/necessary. The incidence of neoplasms was analysed by Cochran-Armitage linear trend test, Life table analysis for fatal tumour incidence and Peto's incidental tumour analysis.
When a significant difference to the control was observed in any of the treatment groups, the dose correlation co-efficient was estimated and subjected to t-test.

II. RESULTS

A. ANALYSIS OF DOSE FORMULATIONS

Analyses for achieved concentrations showed that the diet preparations of the control, low, mid- and high dose group were within an acceptable range. The mean achieved concentrations of the test substance of eight batches of the prepared test substance diets were 0.0, 99.1 ± 4.7, 995.3 ± 36.8 and 9993.1 ± 277.5 ppm, for the control, low, mid and high dose group, respectively.

B. MORTALITY

There were no treatment-related deaths observed during the study.
The numbers of pre-terminal deaths in the carcinogenicity study groups are displayed in the table below.

C. CLINICAL OBSERVATIONS

There were no treatment-related clinical signs of toxicity observed during the study.

Table 5.5-39 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Summary of toxic signs, physical examination and pre-terminal deaths

Conditions	Sex	Males						Females					
	Period	1 to 12 month		1 to 24 month				1 to 12 month		1 to 24 month			
	Group #	G1IS	G4IS	G1	G2	G3	G4	G1IS	G4IS	G1	G2	G3	G4
	Dose (ppm)	0	10000	0	100	1000	10000	0	10000	0	100	1000	10000
	No. of rats	10	20	50	50	50	50	10	20	50	50	50	50
Dull/Lethargy		0	0	0	0	0	0	0	0	0	0	0	0
Weak/Loss of body weight/Emaciation		0	0	13	20	21	17	0	2	10	16	7	14
Nasal discharge		1	5	42	46	41	40	2	3	33	33	34	36
Snuffling/Respiratory problems		3	7	40	42	42	40	1	1	28	32	37	37
Epistaxis		0	0	0	0	0	0	0	0	0	0	0	0
Conjunctivitis/Lacrimation		0	0	1	4	0	0	0	0	0	1	0	1
Microphthalmia		0	0	2	1	0	5	0	1	1	2	2	2
Exophthalmia/Swelling of Eye		0	0	2	0	0	2	0	0	0	0	0	1
Blindness/Opacity		1	0	9	1	2	6	0	0	2	1	4	1
Cataract		0	0	6	5	1	7	0	0	2	1	3	2
Ear infection		0	0	0	0	0	0	0	0	0	0	0	0
Circling symptom		0	0	4	5	6	4	1	0	9	9	8	2
Soft stool/Diarrhoea		3	3	16	7	15	3	0	0	1	0	0	0

Table 5.5-39 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Summary of toxic signs, physical examination and pre-terminal deaths

Conditions	Sex	Males						Females					
	Period	1 to 12 month		1 to 24 month				1 to 12 month		1 to 24 month			
	Group #	G1IS	G4IS	G1	G2	G3	G4	G1IS	G4IS	G1	G2	G3	G4
	Dose (ppm)	0	10000	0	100	1000	10000	0	10000	0	100	1000	10000
	No. of rats	10	20	50	50	50	50	10	20	50	50	50	50
Obesity/Distended abdomen		0	0	3	1	2	1	2	3	5	7	5	3
Urine incontinence		0	0	5	3	2	1	0	0	1	12	3	1
External Genitalia affection		0	0	3	2	2	2	0	0	0	0	0	0
Piloerection/Rough hair coat		0	0	0	0	0	1	0	0	0	0	0	0
Alopecia		0	0	18	19	18	17	0	0	7	5	5	4
Hyperesthesia		0	0	0	0	0	0	0	0	0	0	0	0
Paralysis		0	0	0	0	0	0	0	0	0	0	0	0
Mass/Growth		0	0	6	8	6	2	0	0	10	9	3	7
Injury/Wound		0	0	0	3	1	1	0	0	3	2	0	3
Overgrown tooth		0	0	0	0	0	0	0	0	0	1	0	0
Moribund Sacrifice		0	0	3	8	9	5	0	0	10	10	7	11
Moribund Sacrifice after 730 days		0	0	0	0	0	0	0	0	0	2	0	0
Mortality		0	0	23	21	23	16	0	0	16	11	10	18
Mortality after 730 days		0	0	4	1	0	0	0	0	0	1	0	0
Survival		0	0	20	20	18	29	0	0	24	26	33	21

D. BODY WEIGHT

There were no treatment-related effects on male and female overall body weight gain during the conduct of study. During the first weeks of exposure statistically significant decreases of body weights were observed in males only. As the slight reduction of body weight (<10 %) was only observed in males at the beginning of the study following no dose-response relationship it was considered no adverse effect.

Table 5.5-40 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Body weights

Sex	Males						Females					
Period	1 to 12 month		1 to 24 month				1 to 12 month		1 to 24 month			
Group #	G1IS	G4IS	G1	G2	G3	G4	G1IS	G4IS	G1	G2	G3	G4
Dose (ppm)	0	10000	0	100	1000	10000	0	10000	0	100	1000	10000
Initial	142 ± 19.7	↑148 ± 16.1	139 ± 14.0	↓133 ± 17.3	139 ± 17.4	↓128* ± 16.1	123 ± 11.9	↓114 ± 14.4	116 ± 12.2	↓113 ± 13.3	↓112 ± 11.7	↓111 ± 14.2
Week 1	183 ± 23.8	↓176 ± 19.6	171 ± 19.0	↓158* ± 22.3	171 ± 22.9	↓161*± 20.6	141 ± 12.0	↓135 ± 16.7	132 ± 12.8	↑134 ± 13.9	↓130 ± 12.5	↓129 ± 13.5
Week 2	215 ± 21.1	↓203 ± 20.3	198 ± 19.2	↓191 ± 23.1	↓192 ± 26.2	↓186* ± 24.5	151 ± 11.7	↓149 ± 16.5	144 ± 14.8	↑146 ± 14.1	↑146 ± 12.2	↓140 ± 16.4

Table 5.5-40 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Body weights

Sex	Males						Females					
Period	1 to 12 month		1 to 24 month				1 to 12 month		1 to 24 month			
Group #	G1IS	G4IS	G1	G2	G3	G4	G1IS	G4IS	G1	G2	G3	G4
Dose (ppm)	0	10000	0	100	1000	10000	0	10000	0	100	1000	10000
Week 3	244 ± 27.2	↓234 ± 22.1	228 ± 21.0	↓216* ± 26.0	↓215* ± 25.2	↓215* ± 28.4	171 ± 15.3	↓161 ± 16.8	159 ± 13.9	↑160 ± 15.5	↓158 ± 14.1	↓154 ± 17.0
Week 4	267 ± 22.2	↓257 ± 22.1	248 ± 23.6	↓239 ± 26.8	↓236* ± 27.6	↓236* ± 26.7	185 ± 15.9	↓176 ± 16.3	172 ± 15.1	↑175 ± 16.9	↑173 ± 15.4	↓167 ± 17.3
Week 5	279 ± 17.1	↓273 ± 23.4	261 ± 21.2	↓255 ± 24.8	↓258 ± 30.4	↓253 ± 29.2	190 ± 13.8	↓182 ± 14.4	178 ± 15.7	↑184* ± 17.0	↑181 ± 16.7	↓175 ± 17.2
Week 6	283 ± 13.7	283 ± 24.1	274 ± 21.7	↓266 ± 24.8	↓270 ± 29.9	↓266 ± 29.1	194 ± 12.2	↓180 ± 11.8	186 ± 15.4	↑191 ± 16.4	↓184 ± 18.3	↓181 ± 19.5
Month 12	408 ± 21.3	↑410 ± 35.2	398 ± 30.2	↓384* ± 37.8	↑401 ± 41.7	↓390 ± 37.5	261 ± 62.7	↓253 ± 38.0	241 ± 26.1	↑254* ± 28.1	↑246 ± 32.3	↑248 ± 32.4
Month 24	-	-	401 ± 48.3	↓386 ± 45.7	↑407 ± 72.1	↓396 ± 41.6	-	-	278 ± 25.9	↑289 ± 33.5	↑282 ± 26.2	↑279 ± 39.7

* Statistically significant ($p \leq 0.05$)

Table 5.5-41 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Body weight gain

Sex	Males						Females					
Period	1 to 12 month		1 to 24 month				1 to 12 month		1 to 24 month			
Group #	G1IS	G4IS	G1	G2	G3	G4	G1IS	G4IS	G1	G2	G3	G4
Dose (ppm)	0	10000	0	100	1000	10000	0	10000	0	100	1000	10000
Week 13	190 ± 19.4	↓186 ± 23.8	187 ± 22.9	↓185 ± 22.4	↑189 ± 26.3	↑194 ± 23.6	93 ± 16.4	↑96 ± 20.1	94 ± 16.0	↑101 ± 14.7	↑100 ± 22.5	↑96 ± 18.0
Month 6	239 ± 22.6	↓237 ± 30.0	234 ± 26.6	↓233 ± 32.6	↑236 ± 33.8	↑240 ± 32.5	108 ± 25.7	↑115 ± 27.4	108 ± 20.3	↑117 ± 17.4	↑116 ± 24.0	↑115 ± 21.7
Month 12	266 ± 22.1	↓262 ± 31.6	259 ± 27.9	↓251 ± 37.3	↑262 ± 36.8	↑262 ± 32.5	138 ± 58.4	↑139 ± 42.5	126 ± 27.0	↑141* ± 23.7	↑134 ± 30.7	↑137 ± 29.4
Month 18	-	-	270 ± 31.7	↓261 ± 49.0	↓260 ± 39.4	↑273 ± 40.1	-	-	154 ± 31.0	↑169 ± 37.8	↑162 ± 32.1	↑162 ± 36.2
Month 24	-	-	260 ± 48.4	↓253 ± 43.5	↑269 ± 67.9	↑265 ± 43.8	-	-	162 ± 25.4	↑176 ± 33.1	↑171 ± 29.5	162 ± 36.2

* Statistically significant ($p \leq 0.05$)

E. FOOD CONSUMPTION AND COMPOUND INTAKE

There were no treatment-related effects on food consumption for either sex noted during the study.

Table 5.5-42 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Food consumption

Sex	Males						Females					
Period	1 to 12 month		1 to 24 month				1 to 12 month		1 to 24 month			
Group #	G1IS	G4IS	G1	G2	G3	G4	G1IS	G4IS	G1	G2	G3	G4
Dose (ppm)	0	10000	0	100	1000	10000	0	10000	0	100	1000	10000
Week 1	12.8 ± 0.35	↓12.0 ± 0.41	13.2 ± 0.75	↓13.1 ± 0.52	↓12.7 ± 0.47	↓12.5 * ± 0.58	12.3 ± 0.35	↑12.5 ± 0.91	13.1 ± 0.46	↓12.9 ± 0.75	↓12.7 ± 0.63	↓12.5 ± 0.75
Week 2	20.0 ± 0.00	↓15.8 ± 2.63	18.5 ± 1.72	↓18.2 ± 2.10	↓17.8 ± 2.30	↓17.6 ± 1.84	21.5 ± 0.71	↓16.5 ± 1.91	15.7 ± 1.70	↑16.5 ± 1.58	↑16.5 ± 1.35	↑15.8 ± 1.38
Week 3	18.5 ± 1.41	↓16.1 ± 1.44	19.0 ± 2.95	↓16.3 * ± 1.32	↓17.9 ± 2.04	↓16.9 ± 1.02	17.8 ± 1.77	↓16.6 ± 1.25	16.3 ± 1.51	↑16.7 ± 2.37	↑17.4 ± 1.70	↑15.8 ± 1.23
Week 4	19.3 ± 1.06	↓16.8 ± 0.65	19.0 ± 1.08	↓17.4 * ± 1.02	↓17.6 * ± 1.63	↓17.2 * ± 0.47	18.8 ± 1.77	↓17.0 ± 0.71	17.0 ± 0.80	17.0 ± 0.67	17.0 ± 0.50	↓16.5 ± 0.69
Week 5	18.5 ± 0.71	↑18.8 ± 2.49	18.0 ± 0.94	↓17.4 ± 0.61	↑18.9 ± 2.14	↑19.1 * ± 0.80	17.3 ± 0.35	↓17.0 ± 2.56	17.0 ± 1.50	↑17.5 ± 0.72	↑18.5 ± 1.73	↑19.0 ± 2.75
Week 6	18.8 ± 3.18	↑19.0 ± 1.58	19.1 ± 0.86	↓18.0 ± 1.58	↑18.5 ± 1.62	↓18.9 ± 1.31	17.8 ± 1.77	↑18.1 ± 1.49	17.2 ± 1.55	↑18.3 ± 1.06	↓17.0 ± 1.73	↑18.5 * ± 1.01
Week 13	18.4 ± 4.20	↑19.0 ± 1.75	20.1 ± 2.43	↑20.7 ± 2.41	↓19.8 ± 2.58	20.1 ± 2.34	20.4 ± 3.97	↓19.2 ± 2.67	18.7 ± 2.68	↑19.9 ± 3.47	↑18.9 ± 2.17	↑19.1 ± 2.12
Month 8	21.1 ± 0.95	↑21.6 ± 1.79	22.6 ± 1.52	↓21.8 ± 1.32	↓21.8 ± 1.51	↓21.1 ± 1.33	21.2 ± 3.42	↓19.8 ± 1.03	21.9 ± 1.66	↓20.7 * ± 1.31	↓20.5 * ± 1.02	↓20.8 * ± 1.49
Month 12	22.1 ± 2.59	↑22.9 ± 0.69	23.2 ± 1.76	↓23.1 ± 1.24	↓22.5 ± 1.25	↓22.5 ± 1.21	21.4 ± 3.02	↑22.0 ± 1.60	23.4 ± 2.28	↓22.6 ± 1.08	↓22.0 * ± 1.02	↓22.2 ± 1.14
Month 15	-	-	23.9 ± 3.50	↑25.6 ± 4.90	↓23.7 ± 4.20	↑24.0 ± 5.10	-	-	25.8 ± 4.40	↓23.5 ± 4.70	↓24.7 ± 4.60	↓24.0 ± 3.70
Month 20	-	-	23.9 ± 1.70	↑24.2 ± 2.70	↑24.4 ± 1.90	↓22.8 ± 2.40	-	-	25.0 ± 1.80	↓22.6 * ± 2.20	↓23.3 * ± 2.20	↓22.4 * ± 2.50
Month 24	-	-	28.1 ± 6.20	↓24.1 * ± 3.10	↓24.9 ± 4.40	↓25.4 ± 4.90	-	-	23.9 ± 6.10	↓20.3 ± 5.20	↓22.2 ± 3.40	↓23.8 ± 4.20

Table 5.5-42 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Food consumption

Sex	Males						Females					
Period	1 to 12 month		1 to 24 month				1 to 12 month		1 to 24 month			
Group #	G1IS	G4IS	G1	G2	G3	G4	G1IS	G4IS	G1	G2	G3	G4
Dose (ppm)	0	10000	0	100	1000	10000	0	10000	0	100	1000	10000

* Statistically significant ($p \leq 0.05$)

The group mean achieved doses are summarised below.

Table 5.5-43 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Group mean achieved dose levels in the main groups

Dose group	Dietary concentration (ppm)	Mean achieved dose level (mg/kg bw/day)		
		Males	Females	Overall mean
1 (control)	0			
2 (low)	100	6.3	8.6	7.4
3 (mid)	1000	59.4	88.5	73.9
4 (high)	10000	595.2	886.0	740.6

The results show a higher test material intake for females when compared to males for each dose level. The mean intake for each dose group is 7.4, 73.9 and 740.6 mg/kg bw/day for 100, 1000, and 10000 ppm, respectively.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

The following significant dose related changes of the blood chemistry parameters were seen at the high dose:

decrease in GGT activity at 12 months in male rats
decrease in Albumin level at 6 months in female rats
increase in AP (alkaline phosphatase) activity at 6, 12 and 18 months in female rats. In the absence of further liver findings this is not considered to be an adverse effect.

Table 5.5-44 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Haematological data

Sex	Males				Females			
Group #	G1	G2	G3	G4	G1	G2	G3	G4
Dose (ppm)	0	100	1000	10000	0	100	1000	10000
13th Week								
WBC [103/Cu mm]	15.1 ± 4.36	↓13.3 ± 1.88	↓11.9* ± 1.93	↓13.3 ± 1.81	12.6 ± 4.37	12.6 ± 4.16	↑13.1 ± 4.67	↑13.2 ± 2.67
RBC [106/Cu.mm]	8.14 ± 0.93	↑8.54 ± 0.91	↑9.10* ± 0.51	↑8.88* ± 0.61	8.31 ± 0.58	↓8.28 ± 0.80	↓8.12 ± 1.12	↓8.04 ± 0.78
HB [g/dL]	14.9 ± 0.95	↑15.4 ± 0.80	↑16.3* ± 0.61	↑16.4* ± 0.55	14.5 ± 1.93	↑15.5 ± 1.63	↑15.3 ± 1.47	↑16.6* ± 1.08
Hct [%]	37.4 ± 3.96	↑39.8* ± 3.33	↑42.0* ± 3.13	↑40.5* ± 2.87	39.5 ± 3.63	↑39.9 ± 4.17	↓38.7 ± 5.09	↑40.0 ± 3.87
Clot [s]	68 ±	↑71 ±	↓65 ±	↑70 ±	69 ±	↑74 ±	↑83* ±	↑73 ±

Table 5.5-44 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Haematological data

Sex	Males				Females			
Group #	G1	G2	G3	G4	G1	G2	G3	G4
Dose (ppm)	0	100	1000	10000	0	100	1000	10000
	14.1	16.4	12.6	19.0	16.6	16.1	14.1	19.1
Neut. [%]	17 ± 3.79	ND	ND	↓14* ± 2.87	18 ± 4.23	ND	ND	↑19 ± 2.01
Lymp. [%]	82 ± 3.81	ND	ND	↑84 ± 3.53	79 ± 4.83	ND	ND	↓77 ± 2.59
Eosi. [%]	1 ± 1.08	ND	ND	↑2* ± 1.21	3 ± 1.49	ND	ND	↑4* ± 2.43
6th Month								
WBC [103/Cu mm]	14.8 ± 3.68	↓13.6 ± 2.78	↓11.8* ± 2.06	↓13.5 ± 2.95	10.2 ± 2.07	↑10.4 ± 3.43	↑11.2 ± 6.70	↑11.2 ± 3.61
RBC [106/Cu.mm]	9.26 ± 1.80	↑9.31 ± 1.47	↓8.41 ± 0.96	↓8.66 ± 1.68	9.30 ± 0.44	↓9.08 ± 1.20	↑9.54 ± 0.57	↑9.68* ± 0.45
HB [g/dL]	15.2 ± 0.68	15.2 ± 0.66	15.2 ± 1.11	↓15.1 ± 0.50	15.2 ± 0.77	↓15.0 ± 0.57	↓14.7* ± 0.31	15.2 ± 0.66
Hct [%]	45.6 ± 3.92	↓44.3 ± 4.44	↓43.7 ± 4.43	↓43.1 ± 4.33	48.8 ± 2.38	↓48.2 ± 6.44	↑49.8 ± 2.98	↑52.0* ± 3.47
Clot [s]	74 ± 9.79	↑85 ± 14.7	↑91* ± 17.3	↑81 ± 15.8	84 ± 16.8	↓80 ± 14.4	↓81 ± 13.1	84 ± 13.2
Neut. [%]	20 ± 4.43	ND	ND	↑18 ± 3.72	13 ± 3.24	ND	ND	↑16* ± 4.41
Lymp. [%]	77 ± 4.68	ND	ND	↓79 ± 4.10	85 ± 4.07	ND	ND	↓80* ± 5.37
Eosi. [%]	3 ± 1.16	ND	ND	3 ± 1.65	2 ± 1.68	ND	ND	↑4* ± 2.13
12th Month								
WBC [103/Cu mm]	13.4 ± 3.45	↓12.8 ± 2.98	↓13.3 ± 3.14	↑13.6 ± 2.37	9.21 ± 2.63	↑10.2 ± 2.97	↓8.72 ± 2.69	↑10.8 ± 3.80
RBC [106/Cu.mm]	9.28 ± 1.25	↑9.48 ± 0.97	↑10.1* ± 0.88	↑9.46 ± 1.19	9.10 ± 0.98	↑9.13 ± 0.60	↓8.87 ± 0.68	↓8.51 ± 0.87
HB [g/dL]	15.0 ± 1.11	↑15.7 ± 1.19	↑16.0* ± 1.58	↑15.7 ± 1.36	16.1 ± 1.41	↑16.7 ± 1.02	↑16.9 ± 1.27	↑16.3 ± 1.27
Hct [%]	38.7 ± 5.86	↑40.5 ± 6.08	↑43.2* ± 5.48	↑40.7 ± 5.38	40.5 ± 5.92	↓39.8 ± 3.45	↓39.6 ± 4.80	↓38.4 ± 3.06
Clot [s]	78 ± 20.9	↑97* ± 24.1	↑92 ± 23.2	↑90 ± 18.8	96 ± 17.5	↑101 ± 26.7	↓95 ± 23.8	↓95 ± 12.8
Neut. [%]	22 ± 3.93	ND	ND	↑25 ± 5.50	22 ± 3.66	ND	ND	↑26* ± 5.00
Lymp. [%]	75 ± 4.87	ND	ND	↓71 ± 5.30	75 ± 4.32	ND	ND	↓69* ± 5.51
Eosi. [%]	3 ± 3.45	ND	ND	↑4 ± 1.76	3 ± 1.32	ND	ND	↑5* ± 1.33
18th Month								
WBC [103/Cu mm]	8.0 ± 2.07	↑9.0 ± 1.71	↑9.7* ± 2.02	↑8.1 ± 2.01	6.3 ± 1.78	↑7.3 ± 2.10	↓5.9 ± 1.27	↓6.3 ± 1.81
RBC [106/Cu.mm]	8.8 ± 0.88	↓8.47 ± 0.74	↑9.30 ± 0.93	↑9.16 ± 0.57	8.33 ± 0.70	↑8.71 ± 0.89	↑8.72 ± 0.77	↓8.07 ± 0.65
HB [g/dL]	14.8 ± 1.20	↓14.5 ± 0.83	↑15.4 ± 0.90	↑15.2 ± 0.72	14.5 ± 0.69	↑14.8 ± 0.91	↓14.3 ± 0.77	↑14.6 ± 0.64
Hct [%]	43.5 ± 6.65	↓38.6* ± 4.30	↓43.1 ± 6.64	↓40.6 ± 3.29	40.0 ± 3.08	↑41.9 ± 4.91	↓38.5 ± 3.82	↓38.8 ± 3.33
Clot [s]	131 ± 26.1	↑146 ± 28.0	↑145 ± 29.9	↑140 ± 16.8	112 ± 21.2	↑126* ± 18.8	↑137* ± 24.4	↑144* ± 26.4

Table 5.5-44 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Haematological data

Sex	Males				Females			
Group #	G1	G2	G3	G4	G1	G2	G3	G4
Dose (ppm)	0	100	1000	10000	0	100	1000	10000
Neut. [%]	22 ± 8.76	ND	ND	↑28 ± 8.96	29 ± 7.78	ND	ND	↑33 ± 10.5
Lymp. [%]	75 ± 9.20	ND	ND	↓68* ± 8.85	69 ± 7.94	ND	ND	↓62* ± 8.995
Eosi. [%]	2 ± 1.90	ND	ND	↑4* ± 2.48	2 ± 1.63	ND	ND	↑5* ± 5.73
24th Month								
WBC [103/Cu mm]	9.9 ± 4.47	↑11.4 ± 2.53	↑10.0 ± 2.89	↓8.2 ± 1.64	7.5 ± 2.01	↑7.8 ± 2.29	↓7.1 ± 1.76	↑8.2 ± 3.45
RBC [106/Cu.mm]	6.88 ± 0.63	↑7.42* ± 0.95	↑8.12* ± 0.93	↑7.40* ± 0.67	6.99 ± 0.56	↑7.35 ± 1.19	↑7.10 ± 0.83	↑7.2 ± 0.67
HB [g/dL]	14.4 ± 0.82	↑15.1* ± 1.01	↑15.1 ± 1.39	↑15.3* ± 1.19	14.6 ± 1.10	↑15.4 ± 1.65	↑15.0 ± 1.06	↑15.4 ± 1.44
Hct [%]	32.2 ± 2.86	↑34.4 ± 4.34	↑36.9* ± 3.95	↑33.8 ± 3.13	35.5 ± 3.08	↑37.1 ± 5.69	↑35.9 ± 4.05	↑36.8 ± 3.28
Clot [s]	115 ± 29.7	↓110 ± 28.2	↑123 ± 29.9	↓110 ± 27.5	98 ± 29.7	↑109 ± 17.3	↑108 ± 25.1	98 ± 18.0
Neut. [%]	30 ± 7.15	ND	ND	↓28 ± 6.76	26 ± 6.82	ND	ND	↑32* ± 9.88
Lymp. [%]	68 ± 7.42	ND	ND	↑70 ± 7.19	72 ± 7.04	ND	ND	↓66* ± 10.1
Eosi. [%]	2 ± 1.53	ND	ND	2 ± 1.90	2 ± 1.15	ND	ND	2 ± 1.67
Differential leukocyte count on moribund sacrificed rats								
Neut. [%]	39 ± 11.5	39 ± 23.7	42 ± 27.7	47 ± 20.1	49 ± 26.2	48 ± 26.2	48 ± 21.4	51 ± 29.1
Lymp. [%]	59 ± 12.1	60 ± 32.0	57 ± 33.6	52 ± 20.3	50 ± 26.2	52 ± 28.4	51 ± 22.2	49 ± 27.7
Eosi. [%]	2 ± 1.5	1 ± 0.5	1 ± 0.5	1 ± 0.9	1 ± 1.4	0 ± 1.1	1 ± 1.4	0 ± 0.3

ND not determined
* Statistically significant ($p \leq 0.05$)

No other dose or treatment related significant changes were observed in haematological, and biochemical parameters. These changes observed were only temporal and were not consistently seen at all sampling periods throughout the study. The dose related changes were also within the range of the historical control data and hence appear to be of no biological significance.

Table 5.5-45 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Clinical chemistry data

Sex	Males				Females			
Group #	G1	G2	G3	G4	G1	G2	G3	G4
Dose (ppm)	0	100	1000	10000	0	100	1000	10000
6th Month								
ALT [IU/L]	33 ± 4.87	↑37 ± 6.96	↑37 ± 4.21	33 ± 8.78	35 ± 5.12	↓32 ± 3.92	35 ± 7.32	↑38 ± 5.34
AST [IU/L]	70 ± 5.16	↑78* ± 11.1	↑84* ± 14.9	↑89* ± 20.6	85 ± 17.8	↓84 ± 14.6	↓80 ± 11.8	↓82 ± 8.58
Alp [IU/L]	213 ± 99.2	↑251 ± 61.8	↑227 ± 77.6	↓185 ± 30.6	133 ± 42.6	↑146 ± 39.2	↑152 ± 58.2	↑235* ± 117.3
GGT [IU/L]	5.8 ±	↑6.9 ±	↑7.2 ±	↑8.5 ±	7.1 ±	↑7.4 ±	↓6.0 ±	↑9.5 ±

Table 5.5-45 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Clinical chemistry data

Sex	Males				Females			
Group #	G1	G2	G3	G4	G1	G2	G3	G4
Dose (ppm)	0	100	1000	10000	0	100	1000	10000
	2.68	3.42	2.68	3.35	1.72	3.13	3.25	3.20
Total Protein [g/dL]	8.1 ± 0.43	↑8.2 ± 0.30	8.1 ± 0.26	↓8.0 ± 0.60	8.2 ± 0.52	↓7.8 ± 0.65	↓7.7* ± 0.22	↓7.7 ± 0.63
Alb [g/dL]	4.0 ± 0.17	↓3.9 ± 0.26	4.0 ± 0.33	↓3.9 ± 0.19	3.7 ± 0.18	3.7 ± 0.55	3.7 ± 0.16	↓3.5* ± 0.19
BUN [mg/dL]	17 ± 1.2	↓16 ± 2.0	↓14* ± 1.2	↓15* ± 1.8	19 ± 1.5	↓16* ± 2.0	↓18 ± 1.4	↓18 ± 2.0
Glu [mg/dL]	117 ± 8.78	↓114 ± 12.6	↑118 ± 15.9	↑124 ± 16.0	113 ± 6.75	↓108 ± 9.01	↑116 ± 8.55	↑117 ± 10.4
12th Month								
ALT [IU/L]	49 ± 15.3	↓47 ± 14.2	↓42 ± 11.5	↑50 ± 11.4	29 ± 9.68	↑31 ± 7.44	↑38 ± 15.7	↑34 ± 11.4
AST [IU/L]	77 ± 12.6	77 ± 12.0	↓72 ± 11.4	↓73 ± 10.4	75 ± 10.6	↑84 ± 7.55	↑79 ± 20.7	↓74 ± 10.4
Alp [IU/L]	244 ± 82.3	↓228 ± 101.5	↑298 ± 69.4	↓128 ± 76.9	141 ± 34.2	↑158 ± 34.1	↑203* ± 70.4	↑231* ± 74.6
GGT [IU/L]	8.3 ± 2.06	8.3 ± 4.09	↑8.4 ± 3.50	↓5.1* ± 2.46	5.8 ± 2.20	↑7.7 ± 3.24	↑6.3 ± 2.68	↓5.3 ± 2.18
Total Protein [g/dL]	9.2 ± 0.46	↓9.1 ± 0.32	↑9.3 ± 0.58	↓8.7* ± 0.34	8.3 ± 0.98	↓8.0 ± 0.59	↑8.4 ± 0.78	↓8.5 ± 0.94
Alb [g/dL]	3.9 ± 0.23	3.9 ± 0.27	↑4.3* ± 0.34	↑4.2 ± 0.40	3.7 ± 0.21	↓3.5* ± 0.09	↓3.5 ± 0.07	↓3.5 ± 0.10
BUN [mg/dL]	18 ± 1.1	18 ± 1.8	↑19 ± 2.5	18 ± 1.5	14 ± 2.4	↑15 ± 1.9	14 ± 1.6	↓13 ± 2.1
Glu [mg/dL]	130 ± 14.8	↓115* ± 12.3	↓111* ± 14.2	↓114* ± 8.72	117 ± 11.3	↓113 ± 5.14	117 ± 6.80	↓120 ± 13.9
18th Month								
ALT [IU/L]	73 ± 15.0	↑85 ± 24.1	↓67 ± 22.2	↑75 ± 8.06	59 ± 13.5	↑68 ± 18.2	↑77 ± 24.2	↑79* ± 14.7
AST [IU/L]	117 ± 17.2	↑142 ± 42.8	↑118 ± 24.3	↓115 ± 27.0	105 ± 15.9	↑131 ± 43.7	↑122 ± 55.5	↑125* ± 25.1
Alp [IU/L]	211 ± 90.5	↑289 ± 159	↓188 ± 77.5	↓174 ± 49.5	101 ± 36.7	↑139 ± 60.6	↑197* ± 105	↑194* ± 86.3
GGT [IU/L]	8.4 ± 4.44	↑10.4 ± 2.78	↑8.98 ± 3.43	↓6.9 ± 3.15	5.3 ± 2.29	↑7.1 ± 1.40	↑8.4 ± 4.28	↑7.6 ± 3.59
Total Protein [g/dL]	7.6 ± 0.28	↓7.5 ± 0.63	7.6 ± 0.50	↓7.4 ± 0.50	8.0 ± 0.46	8.0 ± 0.48	8.0 ± 0.57	↓7.8 ± 0.49
Alb [g/dL]	3.1 ± 0.18	↓2.7* ± 0.24	↑3.2 ± 0.32	3.1 ± 0.21	3.8 ± 0.13	↓3.5* ± 0.16	↓3.6* ± 0.27	↓3.6 ± 0.29
BUN [mg/dL]	19 ± 1.67	↑20 ± 2.38	↓17 ± 1.40	19 ± 2.01	18 ± 2.02	18 ± 2.86	↑21 ± 3.38	↑20 ± 2.23
Glu [mg/dL]	114 ± 8.13	↓104* ± 12.9	↓103 ± 15.5	↓105* ± 8.46	124 ± 11.1	↓122 ± 20.4	↓111* ± 13.4	↓117 ± 5.62
24th Month								
ALT [IU/L]	97 ± 39.7	↓85 ± 20.3	↑270 ± 455	↑103 ± 29.6	65 ± 23.5	↑72 ± 22.2	↑69 ± 45.2	↑73 ± 20.5
AST [IU/L]	128 ± 57.7	↑130 ± 28.8	↑226 ± 269	↓111 ± 11.1	153 ± 126	↓138 ± 70.7	↑175 ± 220	↑155 ± 146
Alp [IU/L]	261 ± 68.4	↑351* ± 80.7	↑281 ± 177	↑292 ± 104	254 ± 117	↑274 ± 109	↓220 ± 70.1	↓249 ± 88.3
GGT [IU/L]	6.8 ± 2.59	↓5.3 ± 2.95	↑19.1 ± 44.2	↓6.5 ± 11.8	15.1 ± 33.9	↓5.1 ± 3.18	↓4.1 ± 6.77	↓2.5 ± 1.18

Table 5.5-45 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Clinical chemistry data

Sex	Males				Females			
Group #	G1	G2	G3	G4	G1	G2	G3	G4
Dose (ppm)	0	100	1000	10000	0	100	1000	10000
Total Protein [g/dL]	8.0 ± 0.53	↑8.2 ± 0.50	↓7.6 ± 0.34	8.0 ± 0.27	8.2 ± 0.52	↑8.6 ± 0.34	8.2 ± 0.86	↓7.4* ± 0.36
Alb [g/dL]	2.8 ± 0.27	↓2.7 ± 0.13	↓2.7 ± 0.16	↓2.7 ± 0.28	3.3 ± 0.3	↑3.4 ± 0.35	↓3.1 ± 0.30	↓3.1* ± 0.16
BUN [mg/dL]	18 ± 2.95	18 ± 1.65	↓17 ± 2.78	↑19 ± 2.27	17 ± 1.60	↓16 ± 1.91	↑18 ± 3.16	↓16 ± 1.96
Glu [mg/dL]	129 ± 13.0	↓122 ± 12.4	↓116 ± 30.0	↓105* ± 13.1	130 ± 16.5	↓117 ± 16.7	↓110* ± 9.24	↓129 ± 7.71

* Statistically significant ($p \leq 0.05$)

G. URINALYSIS

There were no treatment-related findings.

Table 5.5-46 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Clinical chemistry data

Sex	Males				Females			
Group #	G1	G2	G3	G4	G1	G2	G3	G4
Dose (ppm)	0	100	1000	10000	0	100	1000	10000
13th Week								
Leukocytes	8	10	10	10	0	6	2	3
Nitrite	2	3	2	2	0	2	0	0
pH (mean values)	7.2	7.1	6.5	7.0	8.1	7.8	7.6	7.2
Protein	2	0	0	0	0	0	0	0
Glucose	0	0	0	0	0	0	0	0
Ketone bodies	0	0	0	0	0	0	0	0
Urobilinogen	0	0	0	0	0	0	0	0
Bilirubin	0	0	0	0	0	0	0	0
Erythrocytes	5	5	7	5	0	4	2	4
6th Month								
Leukocytes	7	5	9	6	3	3	2	7
Nitrite	0	0	0	0	0	1	0	0
pH (mean values)	6.3	6.7	7.0	6.5	6.9	7.3	6.9	6.4
Protein	2	2	5	4	0	1	1	5
Glucose	0	0	0	0	0	0	0	0
Ketone bodies	0	0	2	0	0	0	0	0
Urobilinogen	4	1	0	0	0	0	0	0
Bilirubin	0	0	2	3	3	7	4	6
Erythrocytes	4	7	6	8	9	8	5	7
12th Month								
Leukocytes	10	10	10	10	10	10	10	10
Nitrite	0	0	0	1	3	0	0	0
pH (mean values)	6.7	7.0	6.3	6.3	7.6	7.7	7.7	7.8
Protein	10	10	10	10	5	2	8	6
Glucose	0	0	0	0	0	0	0	0
Ketone bodies	2	2	1	1	0	0	0	0
Urobilinogen	0	0	0	0	0	0	0	0
Bilirubin	2	5	5	5	3	2	2	0
Erythrocytes	7	9	9	8	8	8	6	9
18th Month								

Table 5.5-46 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Clinical chemistry data

Sex	Males				Females			
Group #	G1	G2	G3	G4	G1	G2	G3	G4
Dose (ppm)	0	100	1000	10000	0	100	1000	10000
Leukocytes	10	10	10	10	10	10	10	10
Nitrite	6	2	7	1	0	1	2	2
pH (mean values)	7.8	7.5	7.8	7.4	7.3	7.2	7.5	7.9
Protein	1	4	5	5	0	2	1	6
Glucose	0	0	0	0	0	0	0	0
Ketone bodies	0	0	0	0	0	0	0	0
Urobilinogen	0	0	0	0	0	0	0	0
Bilirubin	1	0	1	0	0	0	0	0
Erythrocytes	9	10	10	10	10	10	10	10
24th Month								
Leukocytes	10	10	10	10	6	10	10	10
Nitrite	1	1	0	0	0	0	0	1
pH (mean values)	7.1	7.1	6.8	6.7	6.6	7.2	6.9	6.7
Protein	10	9	9	10	5	9	8	9
Glucose	0	0	0	0	0	0	0	0
Ketone bodies	0	1	2	0	1	0	1	2
Urobilinogen	0	0	0	0	0	0	0	0
Bilirubin	1	0	1	0	1	1	1	0
Erythrocytes	10	10	10	10	10	10	10	10

H. NECROPSY

Gross pathology

The incidence of liver lesions was increased in the high dose group males; three small mesenteric masses were seen in the males and females of the high dose group. The incidence of other changes was low and they appeared to be not related to the compound. Number of animals showing gross pathology changes was higher in high dose group compared to control group, this was especially due to findings in liver and lungs.

Organ weights

There were no treatment-related findings observed in organ weights or relative organ weights.

Table 5.5-47 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Organ weights

Dose group	Nominal dose (ppm)	Organ weight	Organ weight ratios [%]
Adrenal glands (males)			
G1IS (control)	0	0.044 ± 0.009	0.012 ± 0.002
G4IS (high)	10000	0.044 ± 0.005	0.011 ± 0.001
G1 (control)	0	0.056 ± 0.020	0.015 ± 0.005
G2 (low)	100	↑0.063 ± 0.022	0.019 ± 0.006
G3 (mid)	1000	↑0.177 ± 0.340	0.043 ± 0.083
G4 (high)	10000	↑0.065 ± 0.029	0.017 ± 0.008
Adrenal glands (females)			
G1IS (control)	0	0.054 ± 0.007	0.023 ± 0.005
G4IS (high)	10000	↓0.051 ± 0.014	0.020 ± 0.005
G1 (control)	0	0.058 ± 0.010	0.021 ± 0.004
G2 (low)	100	↑0.064 ± 0.010	0.026 ± 0.012

Table 5.5-47 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Organ weights

Dose group	Nominal dose (ppm)	Organ weight	Organ weight ratios [%]
G3 (mid)	1000	↑0.106 ± 0.095	0.045 ± 0.046
G4 (high)	10000	↑0.063 ± 0.018	0.024 ± 0.009
Testes (males)			
G1IS (control)	0	3.28 ± 0.22	0.848 ± 0.060
G4IS (high)	10000	↑3.38 ± 0.22	0.883 ± 0.111
G1 (control)	0	2.94 ± 0.67	0.784 ± 0.205
G2 (low)	100	↓2.34 ± 0.83	0.688 ± 0.240
G3 (mid)	1000	↑3.02 ± 0.69	0.724 ± 0.136
G4 (high)	10000	↓2.53 ± 0.78	0.643 ± 0.208
Ovaries (females)			
G1IS (control)	0	0.055 ± 0.023	0.022 ± 0.009
G4IS (high)	10000	↑0.062 ± 0.016	0.024 ± 0.004
G1 (control)	0	0.068 ± 0.028	0.025 ± 0.010
G2 (low)	100	↑0.087 ± 0.089	0.032 ± 0.029
G3 (mid)	1000	↑0.346 ± 0.873	0.151 ± 0.389
G4 (high)	10000	↑0.091 ± 0.073	0.031 ± 0.020
Kidneys (males)			
G1IS (control)	0	2.57 ± 0.29	0.664 ± 0.060
G4IS (high)	10000	↑2.66 ± 0.31	0.689 ± 0.041
G1 (control)	0	2.73 ± 0.31	0.721 ± 0.091
G2 (low)	100	2.73 ± 0.27	0.810 ± 0.150
G3 (mid)	1000	↑3.06 ± 0.39	0.743 ± 0.111
G4 (high)	10000	↑2.84 ± 0.38	0.719 ± 0.074
Kidneys (females)			
G1IS (control)	0	1.65 ± 0.22	0.68 ± 0.12
G4IS (high)	10000	↑1.66 ± 0.18	0.66 ± 0.10
G1 (control)	0	2.04 ± 0.24	0.74 ± 0.10
G2 (low)	100	↓1.97 ± 0.27	0.76 ± 0.12
G3 (mid)	1000	↓2.03 ± 0.20	0.83 ± 0.07
G4 (high)	10000	↑2.12 ± 0.54	0.78 ± 0.21
Liver (males)			
G1IS (control)	0	11.4 ± 1.31	2.94 ± 0.21
G4IS (high)	10000	↑11.5 ± 1.30	2.99 ± 0.20
G1 (control)	0	21.5 ± 11.8	5.78 ± 3.49
G2 (low)	100	↑23.5 ± 9.18	7.12 ± 3.73
G3 (mid)	1000	↑22.6 ± 10.7	5.89 ± 4.01
G4 (high)	10000	↓19.9 ± 9.62	5.05 ± 2.49
Liver (females)			
G1IS (control)	0	8.07 ± 1.62	3.28 ± 0.25
G4IS (high)	10000	↑8.27 ± 1.20	3.23 ± 0.32
G1 (control)	0	12.3 ± 3.09	4.44 ± 1.20
G2 (low)	100	↓11.3 ± 3.64	4.36 ± 1.60
G3 (mid)	1000	↑14.6 ± 7.96	5.91 ± 3.04
G4 (high)	10000	↑12.5 ± 4.42	4.62 ± 1.93
Brain (males)			
G1IS (control)	0	2.08 ± 0.08	0.539 ± 0.036
G4IS (high)	10000	↑2.09 ± 0.14	0.544 ± 0.048
G1 (control)	0	2.08 ± 0.33	0.549 ± 0.091
G2 (low)	100	↓1.99 ± 0.13	0.589 ± 0.077
G3 (mid)	1000	↓2.07 ± 0.09	0.507 ± 0.083

Table 5.5-47 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Organ weights

Dose group	Nominal dose (ppm)	Organ weight	Organ weight ratios [%]
G4 (high)	10000	↓1.97 ± 0.11	0.505 ± 0.072
Brain (females)			
G1IS (control)	0	1.88 ± 0.11	0.79 ± 0.14
G4IS (high)	10000	↑1.92 ± 0.09	0.77 ± 0.15
G1 (control)	0	1.90 ± 0.13	0.69 ± 0.09
G2 (low)	100	↓1.86 ± 0.09	0.72 ± 0.08
G3 (mid)	1000	↓1.83 ± 0.09	0.75 ± 0.06
G4 (high)	10000	↓1.86 ± 0.12	0.69 ± 0.11

Pathology and Histopathology

None of the significant microscopic changes, both increased and decreased incidences (in liver, spleen, lymph nodes, adrenals, thymus, gonads, uterus, mammary gland) observed have shown dose relationship, hence appeared to be incidental and not related to the treatment with the test compound.

Cataract frequency was also comparable between control and dosing groups. Single incidences in the mid and high dose group were higher than in the control group, but were lacking a dose response relationship. Furthermore, when taking into account the physical examination of the eye (see table below) six control animals showed cataracts, meaning that 3 animals with cataract have not been evaluated at necropsy (possibly due to autolysis). Taking into account the physical examination no difference between control and dose group was observed. In addition, when taking into account damaged eye, cataract and corneal opacity no difference between control and dose groups was observed.

Table 5.5-48 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Summary of findings in the eyes – terminally sacrificed animals

	Dose group (ppm)							
	Males				Females			
	G1 0	G2 100	G3 1000	G4 10000	G1 0	G2 100	G3 1000	G4 10000
Necropsy findings in terminally sacrificed rats								
No. of rats examined	20	20	18	29	24	26	33	21
Damaged	0	0	0	0	5	0	0	1
Cataract	3	3	1	6	1	1	5	4
Corneal opacity	3	0	3	2	1	2	2	1
Sum of eye findings listed above	6	3	4	8	7	3	7	6
Damaged [%]	0	0	0	0	21	0	0	5
Cataract [%]	15	15	6	21	4	4	15	19
Corneal opacity [%]	15	0	17	7	4	8	6	5
Sum of eye findings listed above [%]	30	15	22	28	29	12	21	29

Table 5.5-49 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Summary of findings in the eyes – dead and moribund sacrificed animals

	Dose group (ppm)							
	Males				Females			
	G1 0	G2 100	G3 1000	G4 10000	G1 0	G2 100	G3 1000	G4 10000
Necropsy findings in dead and moribund sacrificed rats								
No. of rats examined	30	30	32	21	26	24	17	29
Damaged	0	0	2	0	0	0	0	0
Cataract	0	1	1	1	0	3	0	0
Corneal opacity	1	1	1	0	1	2	0	1
Sum of eye findings listed above	1	2	4	1	1	5	0	1
Damaged [%]	0	0	6	0	0	0	0	0
Cataract [%]	0	3	3	5	0	13	0	0
Corneal opacity [%]	3	3	3	0	4	8	0	3
Sum of eye findings listed above [%]	3	7	13	5	4	21	0	3

Table 5.5-50 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Summary of findings in the eyes – all animals

	Dose group (ppm)							
	Males				Females			
	G1 0	G2 100	G3 1000	G4 10000	G1 0	G2 100	G3 1000	G4 10000
Necropsy findings in all rats								
No. of rats examined	50	50	50	50	50	50	50	50
Damaged	0	0	2	0	5	0	0	1
Cataract	3	4	2	7	1	4	5	4
Corneal opacity	4	1	4	2	2	4	2	2
Sum of eye findings listed above	7	5	8	9	8	8	7	7
Damaged [%]	0	0	4	0	10	0	0	2
Cataract [%]	6	8	4	14	2	8	10	8
Corneal opacity [%]	8	2	8	4	4	8	4	4
Sum of eye findings listed above [%]	14	10	16	18	16	16	14	14

Table 5.5-51 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Summary of histopathological findings (interim sacrifice group)

	Dose group (ppm)			
	Males		Females	
	G1IS 0	G4IS 10000	G1IS 0	G4IS 10000
Duodenum: Catarrh	0/10	0/20	1/10	0/20
Duodenum: Lymphoid hyperplasia	0/10	0/20	1/10	1/20
Ileum: Catarrh	0/10	0/20	1/10	1/20
Ileum: Peyers patch hyperplasia	2/10	0/20	2/10	2/20
Colon: Lymphoid hyperplasia	2/10	7/20	8/10	6/20
Colon: Parasites in lumen	1/10	0/20	0/10	1/20
Jejunum: Catarrh	1/10	3/20	2/10	2/20
Cecum: Tissue not fit for	1/10	1/20	0/10	0/19

Table 5.5-51 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Summary of histopathological findings (interim sacrifice group)

	Dose group (ppm)			
	Males		Females	
	G1IS 0	G4IS 10000	G1IS 0	G4IS 10000
examination				
Caecum: Chronic inflammation	2/10	2/20	2/10	0/19
Caecum: Tissue nit present	0/10	0/20	0/10	1/19
Rectum: Lymphoid hyperplasia	1/10	0/20	2/10	1/20
Liver: Cyst	0/10	3/20	0/10	0/20
Liver: Necrosis	2/10	1/20	3/10	0/20
Liver: Bile duct proliferation	3/10	1/20	1/10	0/20
Liver: Basophilic focus(i)	1/10	1/20	0/10	0/20
Liver: Clear cell focus	8/10	6/20	3/10	1/20
Liver: Hepatocellular adenoma	0/10	1/20	0/10	0/20
Liver: Clear cell area	0/10	1/20	1/10	0/20
Lungs: Congestion	4/10	6/20	8/10	10/20
Lungs: Haemorrhage	1/10	4/20	4/10	2/20
Lungs: Atelectasis	5/10	13/20	7/10	11/20
Lungs: Pneumonia (broncho)	0/10	1/20	0/10	1/20
Lungs: Alveolar emphysema	1/10	3/20	2/10	1/20
Lungs: Perivascular lymphocytic aggregation	10/10	13/20	8/10	14/20
Lungs: Pneumonia	0/10	1/20	1/10	0/20
Trachea: Tissue not fit for examination	0/10	1/20	1/10	1/20
Spleen: Congestion	1/10	2/20	4/10	2/20
Mesenteric lymph nodes: Tissue not fit for examination	1/10	3/20	0/10	1/18
Mesenteric lymph nodes: Oedema	1/10	0/20	0/10	0/18
Mesenteric lymph nodes: Haemorrhage	0/10	1/20	0/10	0/18
Mesenteric lymph nodes: Tissue not present	0/10	0/20	0/10	2/18
Mediastinal lymph nodes: Tissue not fit for examination	0/10	1/20	2/10	1/20
Mediastinal lymph nodes: Oedema	1/10	0/20	1/10	0/20
Mediastinal lymph nodes: Haemorrhage	1/10	4/20	2/10	3/20
Mediastinal lymph nodes: Pigmentation	1/10	0/20	0/10	2/20
Mandibular lymph node: Tissue not fit for examination	0/9	1/20	0/10	0/19
Mandibular lymph node: Lymphoid hyperplasia	1/9	0/20	0/10	0/19
Mandibular lymph node: Tissue not present	1/9	0/20	0/10	1/19
Kidneys: Lymphocytic infiltration	5/10	3/20	1/10	1/20
Kidneys: Interstitial nephritis (tubulo)	0/10	2/20	0/10	0/20
Kidneys: Nephritis (tubulo)	1/10	0/20	0/10	0/20
Urinary bladder: Tissue not fit for examination	0/10	2/19	2/10	0/20
Urinary bladder: Parasites in lumen	3/10	0/19	0/10	2/20
Urinary bladder: Tissue not present	0/10	1/19	0/10	0/20

Table 5.5-51 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Summary of histopathological findings (interim sacrifice group)

	Dose group (ppm)			
	Males		Females	
	G1IS 0	G4IS 10000	G1IS 0	G4IS 10000
Ovaries: Folicular cyst	-	-	4/10	3/20
Uterus: Tissue not fit for examination	-	-	2/10	1/20
Uterus: Suppurative inflammation	-	-	0/10	2/20
Thyroids: Tissue not fit for examination	1/10	0/20	1/10	0/20
Thyroids: Ultimobranchial cyst	1/10	1/20	0/10	1/20
Pituitary: Tissue not fit for examination	1/10	1/20	0/10	1/19
Pituitary: Cyst	0/10	1/20	1/10	1/19
Pituitary: Haemorrhage	1/10	0/20	0/10	0/19
Pituitary: Tissue not present	0/10	0/20	0/10	1/19
Eyes: Tissue not fit for examination	0/10	1/20	1/10	0/20
Eyes: Suppurative inflammation	0/10	0/20	1/10	0/20
Eyes: Keratitis	1/10	0/20	0/10	0/20
Eyes: Thickened retina	0/10	0/20	0/10	1/20
Eyes: Microphthalmia	0/10	0/20	0/10	1/20
Bone marrow (smear): Tissue not fit for examination	0/10	0/20	1/10	3/20
Skin: Tissue not fit for examination	0/10	1/20	0/10	0/20
Thymus: Tissue not fit for examination	0/9	5/9	0/9	0/20
Thymus: Haemorrhage	4/9	2/20	2/9	4/20
Thymus: Involution	0/9	2/20	0/9	2/20
Thymus: Tissue not present	1/9	0/20	1/9	0/20
Sciatic nerves: Tissue not fit for examination	0/10	0/20	2/10	1/20
Mammary gland: Tissue not fit for examination	-	-	2/8	5/18
Mammary gland: Tissue not present	-	-	2/8	2/18
Tumour/mass: Uterine polyp	0/10	0/20	0/10	1/20
Optic nerves: Tissue not fit for examination	0/10	0/20	0/10	2/19
Optic nerves: Tissue not present	0/10	0/20	0/10	1/19

Table 5.5-52 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Summary of non-neoplastic histopathological findings (dead and moribund sacrificed group)

	Dose group (ppm)							
	Males				Females			
	G1 0	G2 100	G3 1000	G4 10000	G1 0	G2 100	G3 1000	G4 10000
Salivary gland: Autolysis precludes evaluation	0/30	0/30	0/32	1/21	0/26	0/24	0/17	0/29
Salivary gland: Abscess	0/30	1/30	0/32	0/21	0/26	1/24	0/17	0/29
Salivary gland: Cyst	1/30	0/30	0/32	0/21	0/26	0/24	0/17	0/29
Salivary gland: Chronic inflammation	0/30	0/30	0/32	0/21	0/26	1/24	0/17	0/29

Table 5.5-52 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Summary of non-neoplastic histopathological findings (dead and moribund sacrificed group)

	Dose group (ppm)							
	Males				Females			
	G1 0	G2 100	G3 1000	G4 10000	G1 0	G2 100	G3 1000	G4 10000
Oesophagus: Autolysis precludes evaluation	0/30	0/30	1/32	0/21	0/26	0/24	0/17	2/29
Oesophagus: Tissue not fit for examination	1/30	0/30	0/32	0/21	0/26	0/24	0/17	0/29
Stomach: Autolysis precludes evaluation	12/30	4/30	2/32	5/21	5/26	3/24	1/17	2/29
Stomach: Tissue not fit for examination	0/30	0/30	0/32	0/21	0/26	0/24	1/17	0/29
Stomach: Haemorrhage	0/30	0/30	0/32	0/21	1/26	1/24	0/17	0/29
Stomach: Necrosis	0/30	1/30	0/32	0/21	0/26	1/24	0/17	0/29
Stomach: Pigmentation	0/30	1/30	0/32	0/21	0/26	0/24	0/17	0/29
Stomach: Ulceration	1/30	1/30	0/32	0/21	0/26	0/24	0/17	2/29
Stomach: Fibrinopurulent deposit	0/30	0/30	1/32	0/21	0/26	0/24	0/17	0/29
Stomach: Necrotic foci	0/30	2/30	0/32	0/21	0/26	0/24	0/17	0/29
Duodenum: Autolysis precludes evaluation	26/30	21/30	22/32	15/21	16/26	11/24	9/17	17/29
Duodenum: Enteritis	0/30	0/30	2/32	0/21	0/26	0/24	0/17	0/29
Duodenum: Lymphoid hyperplasia	0/30	0/30	0/32	0/21	0/26	0/24	0/17	1/29
Ileum: Autolysis precludes evaluation	26/30	21/30	22/32	15/21	16/26	11/24	9/17	17/29
Ileum: Enteritis	0/30	0/30	2/32	0/21	0/26	0/24	0/17	0/29
Ileum: Peyers patch hyperplasia	1/30	0/30	2/32	0/21	1/26	2/24	1/17	2/29
Colon: Autolysis precludes evaluation	17/30	15/30	21/32	11/21	11/26	8/24	7/17	10/28
Colon: Lamphoid hyperplasia	3/30	1/30	4/32	1/21	1/26	2/24	4/17	2/28
Colon: Parasites in lumen	1/30	4/30	1/32	1/21	1/26	0/24	1/17	1/28
Pancreas: Autolysis precludes evaluation	10/28	7/30	10/32	5/20	4/26	3/23	2/16	3/28
Pancreas: Tissue not fit for examination	0/28	1/30	1/32	0/20	0/26	0/23	0/16	0/28
Pancreas: Chronic inflammation	1/28	1/30	0/32	0/20	0/26	1/23	0/16	0/28
Pancreas: Fibrinopurulent deposit	0/28	0/30	1/32	0/20	0/26	0/23	0/16	0/28
Pancreas: Vacuolar changes	0/28	1/30	1/32	2/20	0/26	0/23	0/16	1/28
Pancreas: Vasculitis	0/28	0/30	0/32	1/20	0/26	0/23	0/16	0/28
Pancreas: Parasite	0/28	0/30	0/32	0/20	0/26	0/23	0/16	1/28
Liver: Autolysis precludes evaluation	0/30	1/30	2/32	0/21	1/26	1/23	1/17	0/29
Liver: Abscess	2/30	3/30	4/32	1/21	0/26	1/23	0/17	0/29
Liver: Cyst	0/30	0/30	1/32	0/21	1/26	0/23	0/17	0/29
Liver: Fatty changes	13/30	9/30	3/32	5/21	9/26	4/23	4/17	3/29
Liver: Haemorrhage	11/30	9/30	4/32	6/21	4/26	3/23	3/17	6/29
Liver: Necrosis	0/30	0/30	0/32	0/21	1/26	0/23	0/17	2/29
Liver: Pigmentation	1/30	0/30	0/32	0/21	0/26	0/23	0/17	0/29
Liver: Hypostatic congestion	10/30	12/30	13/32	8/21	10/26	4/23	3/17	11/29
Liver:Haematoma	0/30	0/30	0/32	0/21	0/26	0/23	0/17	2/29
Liver: Fibrinopurulent deposit	1/30	0/30	1/32	0/21	0/26	0/23	0/17	0/29
Liver: Bile duct proliferation	0/30	1/30	1/32	0/21	0/26	1/23	0/17	0/29
Liver: Nodular hyperplasia	0/30	3/30	3/32	1/21	3/26	2/23	2/17	1/29

Table 5.5-52 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Summary of non-neoplastic histopathological findings (dead and moribund sacrificed group)

	Dose group (ppm)							
	Males				Females			
	G1 0	G2 100	G3 1000	G4 10000	G1 0	G2 100	G3 1000	G4 10000
Liver: Acidophilic foci	0/30	1/30	0/32	0/21	0/26	0/23	0/17	0/29
Liver: Acidophilic area	0/30	1/30	0/32	0/21	0/26	0/23	0/17	0/29
Liver: Basophilic foci	11/30	3/30	5/32	2/21	9/26	6/23	4/17	5/29
Liver: Basophilic area	16/30	15/30	6/32	8/21	3/26	7/23	2/17	10/29
Liver: Clear cell foci	10/30	15/30	21/32	10/21	10/26	12/23	8/17	9/29
Liver: Clear cell area	2/30	8/30	7/32	3/21	4/26	3/23	2/17	5/29
Liver: Necrotic foci	6/30	4/30	3/32	1/21	1/26	1/23	1/17	1/29
Liver: Necrotic area	7/30	5/30	6/32	1/21	2/26	3/23	0/17	2/29
Liver: Cystic biliary hyperplasia	0/30	3/30	3/32	0/21	2/26	3/23	2/17	0/29
Liver: Hydropic degeneration	0/30	0/30	0/32	0/21	0/26	1/23	0/17	0/29
Liver: Atrophic hepatic cords	1/30	0/30	1/32	1/21	1/26	0/23	0/17	0/29
Liver: Recticulolistio cystic proliferation	0/30	0/30	0/32	0/21	0/26	0/23	1/17	0/29
Liver: Cirrhosis	1/30	2/30	3/32	1/21	0/26	2/23	1/17	2/29
Liver: Suppurative perihepatitis	2/30	0/30	0/32	0/21	0/26	0/23	0/17	0/29
Lungs: Autolysis precludes evaluation	0/30	0/30	0/32	0/21	1/26	0/24	0/17	0/29
Lungs: Abscess	0/30	0/30	0/32	0/21	1/26	1/24	0/17	1/29
Lungs: Oedema	0/30	0/30	0/32	1/21	0/26	0/24	0/17	0/29
Lungs: Haemorrhage	0/30	0/30	1/32	0/21	0/26	1/24	0/17	0/29
Lungs: Chronic inflammation	0/30	1/30	0/32	0/21	0/26	0/24	0/17	0/29
Lungs: Pigmentation	0/30	0/30	1/32	0/21	0/26	0/24	0/17	0/29
Lungs: Suppurative inflammation	0/30	1/30	0/32	0/21	0/26	0/24	0/17	0/29
Lungs: Hypostatic congestion	8/30	14/30	14/32	11/21	9/26	3/24	7/17	9/29
Lungs: Fibropurulent deposit	0/30	0/30	1/32	0/21	0/26	0/24	1/17	0/29
Lungs: Colony of micro organism	0/30	0/30	0/32	0/21	0/26	1/24	0/17	0/29
Lungs: Perivascular lymphocytic aggregation	0/30	0/30	0/32	0/21	0/26	1/24	0/17	0/29
Lungs: Squamous metaplasia bronchiolar epithelium	0/30	0/30	1/32	0/21	0/26	0/24	0/17	0/29
Lungs: Suppurative pneumonia	12/30	9/30	8/32	4/21	6/26	3/24	2/17	4/29
Lungs: Suppurative pleuritis	0/30	0/30	0/32	1/21	0/26	0/24	1/17	0/29
Lungs: Fibrino-purulent pleuritis	0/30	0/30	0/32	1/21	0/26	0/24	0/17	1/29
Lungs: Suppurative bronchopneumonia	3/30	5/30	6/32	4/21	5/26	4/24	4/17	9/29
Trachea: Autolysis precludes evaluation	8/29	8/30	15	6/21	3/26	4/24	4/17	6/29
Trachea: Tissue not fit for examination	1/29	1/30	1/32	0/21	1/26	1/24	0/17	0/29
Trachea: Inflammation chronic	3/29	1/30	4/32	3/21	0/26	0/24	0/17	3/29
Trachea: Suppurative inflammation	1/29	9/30	0/32	3/21	3/26	1/24	1/17	3/29
Heart: Suppurative inflammation	0/30	0/30	0/32	0/21	0/26	1/24	0/17	0/29
Heart: Myocarditis	3/30	0/30	0/32	0/21	0/26	0/24	0/17	0/29
Heart: Pericarditis	1/30	0/30	0/32	0/21	0/26	0/24	0/17	0/29
Heart: Suppurative pericarditis	1/30	0/30	0/32	1/21	0/26	0/24	2/17	0/29
Heart: Fibrinopurulent pericarditis	1/30	2/30	0/32	0/21	0/26	0/24	0/17	1/29
Heart: Chronic myocarditis	0/30	2/30	0/32	0/21	0/26	0/24	0/17	0/29
Heart: Chronic pericarditis	0/30	0/30	1/32	0/21	0/26	0/24	0/17	0/29

Table 5.5-52 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Summary of non-neoplastic histopathological findings (dead and moribund sacrificed group)

	Dose group (ppm)							
	Males				Females			
	G1 0	G2 100	G3 1000	G4 10000	G1 0	G2 100	G3 1000	G4 10000
Aorta: Autolysis precludes evaluation	2/16	0/30	0/32	0/21	2/20	0/24	0/17	0/12
Aorta: Tissue not fit for examination	1/16	0/30	0/32	0/21	1/20	0/24	0/17	0/12
Aorta: Calcification	0/16	0/30	0/32	0/21	2/20	0/24	0/17	0/12
Spleen: Autolysis precludes evaluation	13/30	14/30	17/32	10/21	10/26	4/24	4/17	8/29
Spleen: Fibrosis	1/30	0/30	0/32	0/21	0/26	0/24	0/17	0/29
Spleen: Haemosiderosis	0/30	0/30	0/32	1/21	0/26	1/24	2/17	0/29
Spleen: Lymphoid hyperplasia	0/30	1/30	1/32	3/21	2/26	7/24	1/17	1/29
Spleen: Necrosis	0/30	0/30	1/32	0/21	1/26	0/24	0/17	0/29
Spleen: Fibrinopurulent deposit	0/30	0/30	1/32	0/21	0/26	0/24	0/17	0/29
Spleen: Lymphoid atrophy	1/30	0/30	0/32	0/21	0/26	0/24	0/17	1/29
Mesenteric lymph node: Autolysis precludes evaluation	16/27	8/26	20/31	10/21	11/23	7/20	5/17	7/28
Mesenteric lymph node: Tissue not fit for examination	1/27	0/26	1/31	0/21	2/23	1/20	1/17	1/28
Mesenteric lymph node: Haemorrhage	0/27	0/26	0/31	2/21	1/23	0/20	0/17	0/28
Mesenteric lymph node: Lymphoid hyperplasia	0/27	1/26	1/31	3/21	0/23	2/20	0/17	3/28
Mediastinal lymph node: Autolysis precludes evaluation	6/28	3/28	5/30	5/20	4/26	1/22	2/16	3/29
Mediastinal lymph node: Tissue not fit for examination	1/28	3/28	3/30	0/20	1/26	4/22	1/16	3/29
Mediastinal lymph node: Abscess	1/28	1/28	1/30	0/20	1/26	0/22	0/16	0/29
Mediastinal lymph node: Cyst	0/28	0/28	0/30	0/20	0/26	1/22	0/16	0/29
Mediastinal lymph node: Oedema	3/28	0/28	1/30	1/20	2/26	0/22	0/16	2/29
Mediastinal lymph node: Haemorrhage	6/28	5/28	7/30	4/20	2/26	5/22	5/16	8/29
Mediastinal lymph node: Haemosiderosis	0/28	0/28	0/30	0/20	3/26	0/22	0/16	1/29
Mediastinal lymph node: Lymphoid hyperplasia	3/28	11/28	8/30	1/20	6/26	7/22	5/16	8/29
Mediastinal lymph node: Pigmentation	0/28	0/28	1/30	0/20	1/26	1/22	1/16	0/29
Mediastinal lymph node: Suppurative inflammation	1/28	0/28	0/30	0/20	1/26	0/22	0/16	0/29
Mediastinal lymph node: Hypostatic congestion	4/28	2/28	0/30	1/20	1/26	0/22	0/16	1/29
Mediastinal lymph node: Fibrinopurulent deposit	0/28	0/28	1/30	0/20	0/26	0/22	0/16	0/29
Mediastinal lymph node: Cystic gland	1/28	2/28	0/30	0/20	0/26	0/22	0/16	1/29
Mediastinal lymph node: Lymphoid atrophy	1/28	1/28	1/30	1/20	1/26	0/22	0/16	0/29
Mediastinal lymph node: Necrotic foci	1/28	0/28	0/30	0/20	0/26	0/22	0/16	1/29
Mandibular lymph node: Autolysis precludes evaluation	8/29	2/29	8/32	4/21	2/26	1/22	1/17	2/29

Table 5.5-52 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Summary of non-neoplastic histopathological findings (dead and moribund sacrificed group)

	Dose group (ppm)							
	Males				Females			
	G1 0	G2 100	G3 1000	G4 10000	G1 0	G2 100	G3 1000	G4 10000
Mandibular lymph node: Abscess	2/29	0/29	2/32	2/21	0/26	2/22	1/17	1/29
Mandibular lymph node: Cyst	1/29	0/29	0/32	0/21	0/26	0/22	0/17	0/29
Mandibular lymph node: Oedema	0/29	1/29	0/32	0/21	1/26	2/22	0/17	2/29
Mandibular lymph node: Granuloma	0/29	0/29	0/32	1/21	0/26	0/22	0/17	0/29
Mandibular lymph node: Haemorrhage	2/29	0/29	0/32	0/21	4/26	4/22	1/17	2/29
Mandibular lymph node: Haemosiderosis	0/29	0/29	0/32	0/21	1/26	1/22	0/17	0/29
Mandibular lymph node: Lymphoid hyperplasia	2/29	13/29	9/32	6/21	9/26	9/22	6/17	17/29
Mandibular lymph node: Pigmentation	1/29	0/29	0/32	0/21	1/26	1/22	0/17	0/29
Mandibular lymph node: Suppurative inflammation	0/29	1/29	0/32	0/21	0/26	0/22	0/17	0/29
Mandibular lymph node: Cystic gland	2/29	2/29	3/32	1/21	0/26	0/22	2/17	0/29
Kidneys: Autolysis precludes evaluation	13/30	13/30	12/32	8/21	10/26	10/24	4/17	10/29
Kidneys: Calcification	0/30	0/30	0/32	0/21	0/26	0/24	0/17	1/29
Kidneys: Cyst	0/30	1/30	0/32	0/21	1/26	1/24	0/17	0/29
Kidneys: Lymphocytic infiltration	0/30	0/30	0/32	1/21	0/26	0/24	0/17	0/29
Kidneys: Atrophy	0/30	1/30	0/32	0/21	0/26	0/24	0/17	0/29
Kidneys: Pyelonephritis	0/30	0/30	0/32	0/21	0/26	0/24	1/17	1/29
Kidneys: Interstitial nephritis (tubulo)	7/30	8/30	5/32	2/21	1/26	1/24	2/17	0/29
Kidneys: Nephritis (tubulo)	7/30	2/30	2/32	4/21	3/26	3/24	1/17	4/29
Kidneys: Interstitial nephritis	0/30	0/30	3/32	1/21	0/26	0/24	1/17	0/29
Urinary bladder: Autolysis precludes evaluation	10/27	14/30	11/32	8/20	9/26	6/24	5/16	8/28
Urinary bladder: Tissue not fit for examination	0/27	1/30	0/32	0/20	1/26	0/24	0/16	0/28
Urinary bladder: Parasites in lumen	10/27	2/30	6/32	3/20	0/26	0/24	0/16	1/28
Testes: Autolysis precludes evaluation	6/30	8/30	6/31	1/21	-	-	-	-
Testes: Calcification	2/30	2/30	0/31	0/21	-	-	-	-
Testes: Atrophy	7/30	10/30	6/31	4/21	-	-	-	-
Testes: Fibrinopurulent deposit	1/30	0/30	0/31	0/21	-	-	-	-
Prostate: Autolysis precludes evaluation	4/28	2/30	4/31	4/21	-	-	-	-
Prostate: Tissue not fit for examination	0/28	4/30	0/31	0/21	-	-	-	-
Prostate: Suppurative inflammation	1/28	0/30	0/31	0/21	-	-	-	-
Prostate: Cystic gland	16/28	13/30	18/31	12/21	-	-	-	-
Seminal vesicles: Autolysis precludes evaluation	21/29	14/30	15/31	9/21	-	-	-	-
Seminal vesicles: Atrophy	0/29	1/30	0/31	0/21	-	-	-	-
Coagulating glands: Autolysis precludes evaluation	20/28	18/26	16/31	9/21	-	-	-	-

Table 5.5-52 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Summary of non-neoplastic histopathological findings (dead and moribund sacrificed group)

	Dose group (ppm)							
	Males				Females			
	G1 0	G2 100	G3 1000	G4 10000	G1 0	G2 100	G3 1000	G4 10000
Ovaries: Autolysis precludes evaluation	-	-	-	-	1/25	0/24	0/17	0/29
Ovaries: Tissue not fit for examination	-	-	-	-	0/25	0/24	0/17	1/29
Ovaries: Abscess	-	-	-	-	0/25	0/24	1/17	1/29
Ovaries: Cyst	-	-	-	-	8/25	3/24	0/17	3/29
Ovaries: Suppurative inflammation	-	-	-	-	0/25	1/24	0/17	1/29
Ovaries: Follicular cyst	-	-	-	-	1/25	0/24	1/17	2/29
Ovaries: Bursal abscess	-	-	-	-	1/25	0/24	0/17	0/29
Ovaries: Suppurative bursitis	-	-	-	-	1/25	1/24	0/17	3/29
Uterus: Autolysis precludes evaluation	-	-	-	-	2/26	0/24	2/17	2/29
Uterus: Tissue not fit for examination	-	-	-	-	0/26	0/24	1/17	1/29
Uterus: Abscess	-	-	-	-	0/26	0/24	1/17	0/29
Uterus: Cyst	-	-	-	-	1/26	0/24	0/17	0/29
Uterus: Necrosis	-	-	-	-	0/26	1/24	0/17	0/29
Uterus: Polyps	-	-	-	-	0/26	2/24	0/17	1/29
Uterus:Haematoma	-	-	-	-	1/26	1/24	0/17	1/29
Uterus: Cystic endometrial hyperplasia	-	-	-	-	1/26	0/24	0/17	0/29
Uterus: Cystic glandular hyperplasia	-	-	-	-	1/26	0/24	0/17	0/29
Uterus: Squamous metaplasia	-	-	-	-	0/26	1/24	0/17	0/29
Uterus: Glandular atrophy	-	-	-	-	0/26	0/24	0/17	1/29
Uterus: Suppurative endometritis	-	-	-	-	0/26	1/24	1/17	4/29
Uterus: Blood filled cystic spaces	-	-	-	-	1/26	0/24	0/17	0/29
Vagina: Chronic inflammatory growth	-	-	-	-	0/26	1/24	0/17	0/29
Brain: Autolysis precludes evaluation	6/30	1/30	3/32	0/21	2/26	1/24	0/17	3/29
Brain: Tissue not fit for examination	0/30	0/30	0/32	0/21	0/26	0/24	0/17	1/29
Brain: Abscess	0/30	0/30	0/32	1/21	0/26	0/24	0/17	1/29
Brain: Cyst	0/30	1/30	0/32	0/21	0/26	0/24	0/17	0/29
Brain: Haemorrhage	0/30	0/30	0/32	0/21	0/26	0/24	1/17	0/29
Brain: Perivascular cuffing with mononuclears	0/30	1/30	0/32	0/21	0/26	1/24	0/17	0/29
Brain: Suppurative meningitis	0/30	1/30	0/32	2/21	1/26	2/24	0/17	3/29
Brain: Meningitis	0/30	0/30	0/32	0/21	0/26	1/24	0/17	0/29
Thyroid: Autolysis precludes evaluation	14/26	12/26	9/29	5/21	9/26	5/24	4/17	7/27
Thyroid: Tissue not fit for examination	0/26	1/26	1/29	1/21	0/26	0/24	1/17	1/27
Thyroid: Cyst	0/26	0/26	0/29	0/21	0/26	1/24	0/17	0/27
Thyroid: Adenomatous goitre	0/26	0/26	0/29	0/21	0/26	1/24	0/17	0/27
Thyroid: Ultimobranchial cyst	1/26	0/26	0/29	0/21	1/26	0/24	1/17	0/27
Parathyroids: Autolysis precludes evaluation	0/4	0/2	0/2	0/7	1/3	0/2	0/3	0/1
Pituitary: Autolysis precludes	15/29	9/27	9/30	9/20	11/25	3/22	5/16	11/29

Table 5.5-52 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Summary of non-neoplastic histopathological findings (dead and moribund sacrificed group)

	Dose group (ppm)							
	Males				Females			
	G1 0	G2 100	G3 1000	G4 10000	G1 0	G2 100	G3 1000	G4 10000
evaluation								
Pituitary: Tissue not fit for examination	1/29	0/27	0/30	0/20	0/25	1/22	1/16	0/29
Pituitary: Abscess	1/29	0/27	0/30	0/20	0/25	0/22	0/16	0/29
Pituitary: Cyst	0/29	2/27	1/30	0/20	0/25	0/22	0/16	0/29
Pituitary: Haemorrhage	0/29	0/27	1/30	0/20	0/25	9/22	2/16	3/29
Pituitary: Haemosiderosis	0/29	0/27	0/30	0/20	0/25	0/22	0/16	1/29
Pituitary: Pigmentation	1/29	2/27	0/30	0/20	0/25	3/22	0/16	1/29
Pituitary: Suppurative inflammation	0/29	0/27	0/30	0/20	0/25	0/22	1/16	0/29
Pituitary: Atrophy	0/29	0/27	0/30	0/20	0/25	0/22	0/16	1/29
Pituitary: Nodular hyperplasia	0/29	1/27	0/30	0/20	0/25	0/22	0/16	0/29
Adrenals: Autolysis precludes evaluation	6/30	9/30	11/32	4/21	6/26	6/24	4/17	7/29
Adrenals: Fatty changes	0/30	0/30	0/32	0/21	2/26	0/24	0/17	1/29
Adrenals: Haemorrhage	0/30	0/30	0/32	1/21	0/26	1/24	0/17	0/29
Adrenals: Lymphocytic infiltration	0/30	0/30	0/32	0/21	1/26	0/24	0/17	0/29
Adrenals: Atrophy	0/30	1/30	0/32	0/21	0/26	0/24	0/17	0/29
Adrenals: Haematoma	0/30	1/30	0/32	0/21	0/26	0/24	0/17	0/29
Adrenals: Cortical cell hyperplasia	0/30	0/30	1/32	0/21	0/26	1/24	0/17	1/29
Adrenals: Medullary cell hyperplasia	3/30	7/30	7/32	1/21	0/26	2/24	1/17	0/29
Adrenals: Nodular hyperplasia	0/30	0/30	0/32	0/21	0/26	0/24	0/17	1/29
Adrenals: Vacuolar degeneration	0/30	3/30	2/32	1/21	3/26	7/24	4/17	5/29
Adrenals: Cortical atrophy	5/30	1/30	0/32	1/21	0/26	0/24	0/17	0/29
Adrenals: Blood filled cystic spaces	1/30	3/30	0/32	0/21	7/26	4/24	1/17	5/29
Adrenals: Plasma cell infiltration	1/30	0/30	0/32	0/21	0/26	0/24	0/17	0/29
Eyes: Autolysis precludes evaluation	15/30	14/30	8/32	7/21	11/25	6/23	6/17	7/29
Eyes: Tissue not fit for examination	2/30	1/30	4/32	1/21	2/25	3/23	0/17	2/29
Eyes: Keratitis	2/30	3/30	1/32	1/21	1/25	1/23	0/17	3/29
Eyes: Epidermal plaque	0/30	0/30	1/32	0/21	0/25	0/23	0/17	0/29
Eyes: Corneal cyst	0/30	0/30	0/32	1/21	0/25	0/23	0/17	0/29
Eyes: Retinal atrophy	0/30	3/30	1/32	0/21	0/25	0/23	0/17	0/29
Bone marrow (smear): Autolysis precludes evaluation	10/27	4/28	11/28	7/21	7/21	7/23	3/17	4/26
Bone marrow (smear): Tissue not fit for examination	6/27	8/28	5/32	3/21	3/21	5/23	2/17	4/26
Skin: Autolysis precludes evaluation	0/30	0/30	1/32	0/21	0/26	0/24	0/17	0/28
Skin: Tissue not fit for examination	3/30	1/30	1/32	1/21	1/26	1/24	0/17	0/28
Skin: Chronic inflammation	0/30	0/30	0/32	0/21	0/26	1/24	0/17	0/28
Skin: Acanthosis	0/30	0/30	0/32	1/21	1/26	0/24	0/17	0/28
Skin: Hyperkeratosis	0/30	3/30	0/32	1/21	1/26	0/24	1/17	0/28
Skin: Ectoparasites	0/30	3/30	1/32	1/21	3/26	1/24	1/17	1/28
Skin: Parakeratosis	0/30	0/30	0/32	0/21	0/26	0/24	0/17	1/28
Thymus: Autolysis precludes evaluation	12/28	10/29	3/31	3/20	8/24	4/24	0/16	6/28
Thymus: Tissue not fit for	0/28	1/29	0/31	0/20	0/24	0/24	0/16	0/28

Table 5.5-52 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Summary of non-neoplastic histopathological findings (dead and moribund sacrificed group)

	Dose group (ppm)							
	Males				Females			
	G1 0	G2 100	G3 1000	G4 10000	G1 0	G2 100	G3 1000	G4 10000
examination								
Thymus: Cyst	0/28	0/29	0/31	0/20	1/24	0/24	2/16	1/28
Thymus: Oedema	0/28	0/29	0/31	0/20	0/24	1/24	0/16	0/28
Thymus: Haemorrhage	0/28	0/29	0/31	1/20	0/24	0/24	0/16	0/28
Thymus: Involution	14/28	17/29	27/31	15/20	11/24	18/24	13/16	17/28
Muscle (femoral): Autolysis precludes evaluation	0/30	0/30	0/32	0/21	1/26	0/24	0/17	0/29
Spinal chord: Autolysis precludes evaluation	4/30	0/30	1/32	0/21	3/26	0/24	0/17	2/29
Spinal chord: Suppurative meningitis	0/30	0/30	0/32	0/21	0/26	0/24	0/17	1/29
Sciatic nerves: Autolysis precludes evaluation	2/30	0/30	1/32	0/21	2/26	0/24	0/17	2/29
Sciatic nerves: Tissue not fit for examination	1/30	0/30	0/32	0/21	0/26	0/24	0/17	1/29
Sciatic nerves: Calcification	1/30	1/30	0/32	0/21	0/26	0/24	0/17	0/29
Mammary gland: Autolysis precludes evaluation	-	-	-	-	2/23	0/22	0/16	2/28
Mammary gland: Tissue not fit for examination	-	-	-	-	2/23	1/22	0/16	0/28
Mammary gland: Calcification	-	-	-	-	0/23	0/22	0/16	1/28
Mammary gland: Hyperplasia	-	-	-	-	0/23	0/22	0/16	0/28
Mammary gland: Suppurative inflammation	-	-	-	-	0/23	1/22	0/16	0/28
Mammary gland: Ductectasia	-	-	-	-	3/23	1/22	3/16	5/28
Mammary gland: Acinectasia	-	-	-	-	1/23	0/22	1/16	1/28
Mammary gland: Calcified secretion	-	-	-	-	1/23	0/22	0/16	0/28
Jejunum: Autolysis precludes evaluation	26/30	22/30	22/32	15/21	16/26	11/24	9/17	17/29
Jejunum: Enteritis	0/30	0/30	2/32	0/21	0/26	0/24	0/17	0/29
Caecum: Autolysis precludes evaluation	23/30	19/29	23/32	14/21	16/26	11/24	9/17	13/29
Caecum: Lymphoid hyperplasia	0/30	0/29	0/32	0/21	1/26	0/24	0/17	2/29
Rectum: Autolysis precludes evaluation	17/30	14/30	21/32	10/21	11/26	8/24	7/17	10/29
Rectum: Lymphoid hyperplasia	0/30	0/30	0/32	0/21	0/26	0/24	1/17	0/29
Tumour/mass: Abscess	0/30	0/30	0/31	0/21	0/26	1/24	0/17	0/29
Tumour/mass: Chronic inflammation	0/30	0/30	0/31	0/21	0/26	0/24	0/17	1/29
Tumour/mass:Chronic inflammatory growth	0/30	0/30	0/31	0/21	0/26	1/24	0/17	0/29
Tumour/mass: Fibrinopurulent deposit	0/30	0/30	1/31	0/21	0/26	0/24	0/17	0/29
Optic nerves: Autolysis precludes evaluation	5/27	3/27	1/25	0/20	1/22	1/21	0/16	3/27
Optic nerves: Tissue not fit for examination	2/27	4/27	2/25	1/20	3/22	2/21	1/16	0/27
Bone (femur) with joint: Tissue not	0/29	1/30	0/31	1/21	1/26	0/24	0/17	0/28

Table 5.5-52 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Summary of non-neoplastic histopathological findings (dead and moribund sacrificed group)

	Dose group (ppm)							
	Males				Females			
	G1 0	G2 100	G3 1000	G4 10000	G1 0	G2 100	G3 1000	G4 10000
fit for examination								
Sternum: Autolysis precludes evaluation	0/29	0/29	0/30	0/21	1/26	0/24	0/17	0/29
Sternum: Tissue not fit for examination	2/29	0/29	1/30	1/21	3/26	2/24	0/17	0/29
Lymph node (others): Lymphoid hyperplasia	0/30	0/30	0/32	0/21	0/26	1/24	0/17	0/29

Table 5.5-53 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Summary of non-neoplastic histopathological findings (terminal sacrificed group)

	Dose group (ppm)							
	Males				Females			
	G1 0	G2 100	G3 1000	G4 10000	G1 0	G2 100	G3 1000	G4 10000
Salivary gland: Abscess	0/20	-		0/29	0/24	1/1	-	0/21
Stomach: Chronic inflammation	0/20	-		0/29	0/24	-	-	1/21
Duodenum: Tissue not fit for examination	0/20	1/1	-	0/29	1/24	-	-	0/21
Duodenum: Lymphoid hyperplasia	0/20	-	-	0/29	0/24	-	-	2/21
Ileum: Tissue not fit for examination	0/20	-	-	2/29	2/24	0/1	-	1/21
Ileum: Increased goblet cells	2/20	-	-	0/29	0/24	0/1	-	0/21
Ileum: Peyers patch hyperplasia	3/20	-	-	2/29	1/24	1/1	-	5/21
Colon: Chronic inflammation	0/20	-	-	0/29	1/24	-	-	0/21
Colon: Lymphoid hyperplasia	5/20	-	-	6/29	3/24	-	-	6/21
Colon: Parasites in lumen	1/20	-	-	1/29	3/24	-	-	0/21
Pancreas: Vacuolar changes	1/20	-	-	0/29	2/24	-	-	0/21
Liver: Cyst	4/20	2/20	5/16	1/29	2/24	6/25	6/32	5/21
Liver: Fatty changes	4/20	0/20	1/16	2/29	0/24	0/25	2/32	0/21
Liver: Haemorrhage	7/20	12/20	4/16	12/29	4/24	8/25	8/32	2/21
Liver: Pigmentation	1/20	0/20	1/16	0/29	0/24	3/25	2/32	0/21
Liver: Bile duct proliferation	2/20	0/20	2/16	0/29	0/24	1/25	0/32	0/21
Liver: Extramedullary haemopoiesis	0/20	0/20	0/16	1/29	0/24	0/25	0/32	0/21
Liver: Nodular hyperplasia	3/20	2/20	2/16	6/29	2/24	1/25	0/32	1/21
Liver: Acidophilic foci	1/20	0/20	1/16	0/29	0/24	0/25	0/32	0/21
Liver: Basophilic foci	1/20	1/20	0/16	0/29	0/24	0/25	1/32	0/21
Liver: Basophilic area	13/20	17/20	7/16	18/29	19/24	17/25	16/32	12/21
Liver: Clear cell foci	16/20	20/20	14/16	25/29	19/24	22/25	23/32	18/21
Liver: Clear cell area	14/20	19/20	11/16	17/29	12/24	19/25	18/32	10/21
Liver: Necrotic foci	0/20	0/20	0/16	0/29	0/24	1/25	0/32	0/21
Liver: Necrotic area	1/20	1/20	2/16	6/29	1/24	0/25	0/32	0/21
Liver: Cystic biliary hyperplasia	4/20	9/20	7/16	7/29	3/24	9/25	9/32	3/21
Liver: Atrophic hepatic cords	1/20	0/20	1/16	0/29	0/24	0/25	1/32	0/21
Liver: Cirrhosis	1/20	0/20	0/16	0/29	0/24	1/25	2/32	0/21
Liver: Thrombus	1/20	0/20	0/16	0/29	0/24	0/25	0/32	0/21

Table 5.5-53 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Summary of non-neoplastic histopathological findings (terminal sacrificed group)

	Dose group (ppm)							
	Males				Females			
	G1 0	G2 100	G3 1000	G4 10000	G1 0	G2 100	G3 1000	G4 10000
Lungs: Abscess	0/20	0/4	0/3	0/29	2/24	1/4	1/5	1/21
Lungs: Chronic bronchitis	0/20	0/4	0/3	1/29	0/24	0/4	0/5	0/21
Lungs: Suppurative pneumonia	0/20	1/4	1/3	2/29	1/24	2/4	2/5	2/21
Lungs: Suppurative bronchopneumonia	1/20	1/4	0/3	3/29	5/24	2/4	2/5	1/21
Lungs: Necrotic area	0/20	0/4	1/3	0/29	0/24	0/4	0/5	0/21
Lungs: Granulomatous inflammation	0/20	1/4	0/3	0/29	0/24	0/4	0/5	0/21
Trachea: Tissue not fit for examination	1/20	-	-	1/29	1/24	0/1	-	1/21
Trachea: Chronic inflammation	1/20	-	-	4/29	5/24	0/1	-	4/21
Trachea: Suppurative inflammation	0/20	-	-	0/29	0/24	0/1	-	1/21
Heart: Tissue not fit for examination	0/20	-	-	0/29	1/24	0/1	0/1	0/21
Heart: Endocarditis	1/20	-	-	0/29	0/24	0/1	0/1	0/21
Heart: Myocarditis	0/20	-	-	0/29	1/24	0/1	0/1	0/21
Spleen: Lymphoid hyperplasia	0/20	-	2/3	0/29	7/24	-	1/2	2/21
Spleen: Pigmentation	0/20	-	0/3	0/29	0/24	-	1/2	0/21
Mesenteric lymph node: Haemorrhage	0/20	-	-	0/29	1/22	-	-	0/21
Mesenteric lymph node: Lymphoid hyperplasia	3/20	-	-	5/29	5/22	-	-	2/21
Mesenteric lymph node: Pigmentation	0/20	-	-	0/29	1/22	-	-	0/21
Mediastinal lymph node: Tissue not fit for examination	0/19	0/1	-	2/29	1/22	0/1	-	0/21
Mediastinal lymph node: Cyst	0/19	1/1	-	0/29	0/22	0/1	-	0/21
Mediastinal lymph node: Oedema	0/19	0/1	-	0/29	2/22	0/1	-	0/21
Mediastinal lymph node: Haemorrhage	5/19	0/1	-	5/29	4/22	0/1	-	5/21
Mediastinal lymph node: Haemosiderosis	0/19	0/1	-	0/29	1/22	0/1	-	0/21
Mediastinal lymph node: Lymphoid hyperplasia	3/19	1/1	-	2/29	3/22	0/1	-	3/21
Mediastinal lymph node: Pigmentation	2/19	0/1	-	2/29	3/22	0/1	-	3/21
Mediastinal lymph node: Lymphoid atrophy	0/19	0/1	-	0/29	0/22	1/1	-	0/21
Mandibular lymph node: Abscess	1/19	1/6	0/5	1/29	1/24	0/9	0/6	0/21
Mandibular lymph node: Cyst	0/19	3/6	0/5	3/29	0/24	6/9	5/6	0/21
Mandibular lymph node: Oedema	1/19	0/6	0/5	0/29	0/24	0/9	0/6	0/21
Mandibular lymph node: Haemorrhage	0/19	1/6	1/5	1/29	0/24	0/9	2/6	0/21
Mandibular lymph node: Lymphoid hyperplasia	9/19	5/6	4/5	19/29	15/24	9/9	6/6	16/21
Mandibular lymph node: Pigmentation	0/19	0/6	0/5	1/29	0/24	0/9	0/6	0/21
Mandibular lymph node: Cystic gland	7/19	0/6	2/5	1/29	5/24	0/9	0/6	2/21

Table 5.5-53 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Summary of non-neoplastic histopathological findings (terminal sacrificed group)

	Dose group (ppm)							
	Males				Females			
	G1 0	G2 100	G3 1000	G4 10000	G1 0	G2 100	G3 1000	G4 10000
Mandibular lymph node: Chronic lymphadenitis	1/19	0/6	0/5	0/29	0/24	0/9	0/6	0/21
Kidneys: Cyst	0/20	2/3	0/2	0/29	0/24	-	0/1	1/21
Kidneys: Lymphocytic infiltration	0/20	0/3	0/2	1/29	1/24	0/1	0/1	0/21
Kidneys: Atrophy	0/20	0/3	0/2	0/29	0/24	-	1/1	0/21
Kidneys: Dilatation on pelvis	1/20	1/3	0/2	0/29	0/24	-	0/1	0/21
Kindeys: Hydronephrosis	0/20	0/3	1/2	0/29	0/24	-	0/1	0/21
Kidneys: Interstitial nephritis (tubulo)	11/20	1/3	2/2	10/29	3/24	-	0/1	4/21
Kidneys: Nephritis (tubulo)	1/20	1/3	0/2	2/29	4/24	-	0/1	1/21
Kidneys: Interstitial nephritis	1/20	0/3	0/2	1/29	0/24	-	0/1	1/21
Kidneys: Cortical atrophy	0/20	1/3	0/2	0/29	0/24	-	0/1	0/21
Urinary bladder: Tissue not fit for examination	1/20	-	-	0/29	1/23	-	-	1/20
Urinary bladder: Parasites in lumen	4/20	-	-	10/29	1/23	-	-	2/20
Testes: Haemorrhage	0/20	0/7	0/1	1/29	-	-	-	-
Testes: Atrophy	8/20	7/7	1/1	11/29	-	-	-	-
Testes: Leydig cell hyperplasia	0/20	0/7	0/1	2/29	-	-	-	-
Prostate: Cystic gland	16/19	-	-	22/29	-	-	-	-
Seminal vesicles: Tissue not fit for examination	0/18	-	0/1	1/29	-	-	-	
Seminal vesicles: Atrophy	0/18	-	1/1	0/29	-	-	-	
Ovaries: Tissue not fit for examination	-	-	-	-	1/24	0/10	0/11	0/21
Ovaries: Cyst	-	-	-	-	1/24	4/10	5/11	1/21
Ovaries: Suppurative inflammation	-	-	-	-	1/24	0/10	0/11	2/21
Ovaries: Follicular cyst	-	-	-	-	2/24	0/10	0/11	0/21
Ovaries: Bursal cyst	-	-	-	-	1/24	0/10	0/11	1/21
Uterus: Tissue not fit for examination	-	-	-	-	0/24	0/10	0/22	1/21
Uterus: Cyst	-	-	-	-	0/24	0/10	1/22	0/21
Uterus: Haemorrhage	-	-	-	-	0/24	0/10	1/22	0/21
Uterus: Chronic inflammation	-	-	-	-	0/24	1/10	0/22	0/21
Uterus: Necrosis	-	-	-		0/24	0/10	1/22	0/21
Uterus: Polyps	-	-	-	-	1/24	2/10	4/22	1/21
Uterus: Suppurative inflammation	-	-	-	-	0/24	1/10	0/22	1/21
Uterus: Haematoma	-	-	-	-	0/24	0/10	0/22	1/21
Uterus: Endometritis	-	-	-	-	0/24	0/10	1/22	0/21
Uterus: Squamous metaplasia	-	-	-	-	0/24	0/10	0/22	1/21
Uterus: Endometrial hyperplasia	-	-	-	-	1/24	0/10	0/22	0/21
Uterus: Suppurative endometritis	-	-	-	-	3/24	1/10	8/22	3/21
Uterus: Cystic dilatation	-	-	-	-	1/24	1/10	1/22	0/21
Thyroids: Tissue not fit for examination	0/19	-	-	0/29	1/24	-	-	0/20
Thyroids: Atrophy	1/19	-	-	0/29	0/24	-	-	0/20
Thyroids: Follicular atrophy	1/19	-	-	0/29	0/24	-	-	0/20
Pituitary: Tissue not fit for examination	1/20	0/3	0/1	2/29	3/24	0/11	2/7	1/21

Table 5.5-53 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Summary of non-neoplastic histopathological findings (terminal sacrificed group)

	Dose group (ppm)							
	Males				Females			
	G1 0	G2 100	G3 1000	G4 10000	G1 0	G2 100	G3 1000	G4 10000
Pituitary: Cyst	1/20	0/3	1/1	1/29	1/24	1/11	0/7	1/21
Pituitary: Haemorrhage	4/20	3/3	0/1	4/29	3/24	9/11	3/7	3/21
Pituitary: Pigmentation	2/20	1/3	0/1	1/29	2/24	2/11	2/7	1/21
Pituitary: Vacuolar changes	0/20	0/3	0/1	1/29	0/24	0/11	0/7	0/21
Pituitary: Nodular hyperplasia	0/20	1/3	0/1	1/29	0/24	2/11	0/7	1/21
Adrenals: Tissue not fit for examination	1/20	0/3	0/4	0/29	0/24	0/4	0/7	0/21
Adrenals: Calcification	0/20	0/3	0/4	0/29	0/24	1/4	0/7	0/21
Adrenals: Oedema	0/20	0/3	0/4	0/29	0/24	1/4	0/7	0/21
Adrenals: Haemorrhage	0/20	0/3	0/4	0/29	0/24	2/4	0/7	0/21
Adrenals: Necrosis	0/20	0/3	0/4	0/29	0/24	1/4	0/7	0/21
Adrenals: Pigmentation	0/20	0/3	0/4	0/29	0/24	0/4	0/7	1/21
Adrenals: Medullary cell hyperplasia	12/20	0/3	1/4	6/29	5/24	1/4	1/7	1/21
Adrenals: Nodular hyperplasia	0/20	0/3	0/4	0/29	0/24	0/4	0/7	1/21
Adrenals: Vacuolar degeneration	2/20	1/3	0/4	3/29	4/24	1/4	2/7	6/21
Adrenals: Cortical atrophy	0/20	1/3	3/4	7/29	0/24	0/4	3/7	1/21
Adrenals: Blood filled cystic spaces	0/20	0/3	0/4	1/29	1/24	1/4	1/7	0/21
Eyes: Tissue not fit for examination	2/20	0/3	1/4	1/29	1/24	0/4	1/7	0/21
Eyes: Oedema	0/20	1/3	0/4	0/29	0/24	0/4	0/7	0/21
Eyes: Keratitis	1/20	0/3	2/4	2/29	1/24	2/4	1/7	1/21
Eyes: Retina haemorrhage	0/20	0/3	0/4	0/29	0/24	0/4	1/7	0/21
Eyes: Thickened retina	0/20	0/3	1/4	0/29	0/24	0/4	0/7	0/21
Eyes: Corneal cyst	0/20	0/3	0/4	1/29	0/24	0/4	0/7	0/21
Eyes: Retinal atrophy	1/20	0/3	0/4	4/29	0/24	1/4	1/7	1/21
Eyes: Fibrosis of lens	0/20	0/3	0/4	0/29	0/24	0/4	1/7	0/21
Eyes: Homogenous amorphous mass	0/20	0/3	0/4	0/29	0/24	0/4	1/7	0/21
Eyes: Amorphous eosinophilic mass	0/20	0/3	0/4	0/29	0/24	0/4	1/7	0/21
Eyes: Suppurative aquedate	0/20	0/3	0/4	1/29	0/24	0/4	0/7	0/21
Bone marrow (smear): Tissue not fit for examination	8/19	-	-	1/29	11/21	-	-	7/19
Skin: Tissue not fit for examination	2/20	-	-	2/29	0/24	0/4	0/2	0/21
Skin: Acanthosis	0/20	-	-	1/29	0/24	0/4	0/2	0/21
Thymus: Cyst	0/20	-	-	0/29	6/24	-	-	0/21
Thymus: Involution	14/20	-	-	19/29	11/24	-	-	15/21
Sciatic nerve: Tissue not fit for examination	0/20	-	-	1/29	0/23	-	-	0/20
Preputal glands: Abscess	0/20	-	-	1/29	-	-	-	-
Mammary gland: Tissue not fit for examination	-	-	-	-	3/17	0/8	0/17	1/20
Mammary gland: Calcification	-	-	-	-	2/17	0/8	1/17	0/20
Mammary gland: Suppurative inflammation	-	-	-	-	1/17	0/8	0/17	0/20
Mammary gland: Ductectasia	-	-	-	-	3/17	2/8	9/17	1/20
Mammary gland: Lobular hyperplasia	-	-	-	-	1/17	3/8	0/17	0/20
Mammary gland: Acinectasia	-	-	-	-	1/17	0/8	1/17	0/20

Table 5.5-53 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Summary of non-neoplastic histopathological findings (terminal sacrificed group)

	Dose group (ppm)							
	Males				Females			
	G1 0	G2 100	G3 1000	G4 10000	G1 0	G2 100	G3 1000	G4 10000
Mammary gland: Calcium spheroid	-	-	-	-	1/17	0/8	0/17	0/20
Jejunum: Tissue not fit for examination	1/20	-	-	3/29	3/24	-	-	0/21
Jejunum: Lymphoid hyperplasia	0/20	-	-	1/29	0/24	-	-	0/21
Jejunum: Peyers patch hyperplasia	0/20	-	-	1/29	0/24	-	-	0/21
Cecum: Tissue not fit for examination	2/20	-	-	1/29	1/24	-	-	0/21
Cecum: Chronic inflammation	1/20	-	-	0/29	0/24	-	-	1/21
Cecum: Lymphoid hyperplasia	1/20	-	-	2/29	0/24	-	-	1/21
TumourTumour/mass: Abscess	1/20	0/2	0/3	0/29	0/24	0/4	0/1	0/21
TumourTumour/mass: Chronic inflammation	0/20	1/2	0/3	0/29	0/24	0/4	0/1	0/21
TumourTumour/mass: Adipose tissue	0/20	0/2	0/3	0/29	0/24	1/4	1/1	1/21
Bone (femur) with joint: Tissue not fit for examination	0/20	-	-	2/29	0/24	-	-	1/21
Mesentery: Pigmentation	0/20	-	-	1/29	0/24	-	0/1	0/21
Mesentery: Haematoma	0/20	-	-	1/29	0/24	-	0/1	0/21
Sternum: Tissue not fit for examination	0/20	-	-	1/29	0/24	-	-	1/21
Lymph node (others): Abscess	0/20	1/1	-	0/29	3/24	-	-	0/21
Pelvic cavity: Abscess	0/20	-	-	0/29	0/24	1/1	-	0/21

Neoplastic changes

The historical data on neoplasm incidence for the test species indicates that the incidences of various tumours observed in the present study are within the range. The types of tumours seen were also comparable to the historical records.

No statistically significant inter group difference between the control and low, mid and high dose treatment groups has been recorded in respect of the number of rats with neoplasms, number of malignant neoplasms and incidence of metastasis either by individual sex or for combined sex.

Table 5.5-54 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Summary of neoplastic histopathological findings (dead and moribund sacrificed animals)

	Dose group (ppm)							
	Males				Females			
	G1 0	G2 100	G3 1000	G4 10000	G1 0	G2 100	G3 1000	G4 10000
Pancreas: Islet cell adenoma	1/28	0/30	0/32	0/20	0/26	0/23	0/16	0/28
Pancreas: Cholangio carcinoma (metastatic)	0/28	1/30	0/32	0/20	1/26	0/23	0/16	0/28
Pancreas: Undifferentiated sacrcoma (metastatic)	0/28	0/30	1/32	0/20	0/26	0/23	0/16	0/28
Pancreas: Histiocytic sarcoma (metastatic)	0/28	0/30	0/32	0/20	1/26	0/23	0/16	0/28

Table 5.5-54 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Summary of neoplastic histopathological findings (dead and moribund sacrificed animals)

	Dose group (ppm)							
	Males				Females			
	G1 0	G2 100	G3 1000	G4 10000	G1 0	G2 100	G3 1000	G4 10000
Liver: Cholangio carcinoma	0/30	2/30	2/32	2/21	1/26	0/23	0/17	0/29
Liver: Hepatocellular adenoma	9/30	9/30	6/32	6/21	2/26	8/23	3/17	5/29
Liver: Hepatocellular carcinoma	12/30	12/30	9/32	5/21	4/26	4/23	2/17	5/29
Liver: Intrahepatic bile duct adenoma	1/30	1/30	0/32	0/21	0/26	0/23	0/17	0/29
Liver: Histiocytic sarcoma	2/30	0/30	2/32	1/21	1/26	0/23	0/17	0/29
Liver: Tumour emboli	0/30	0/30	1/32	1/21	0/26	0/23	0/17	0/29
Liver: Fibrosarcoma	0/30	1/30	0/32	0/21	0/26	0/23	0/17	0/29
Lungs: Adenoma	0/30	0/30	0/32	0/21	0/26	1/24	0/17	0/29
Lungs: Histiocytic sarcoma (metastatic)	1/30	0/30	1/32	1/21	1/26	0/24	0/17	0/29
Lungs: Cholangio carcinoma (metastatic)	0/30	1/30	0/32	0/21	0/26	0/24	0/17	0/29
Lungs: Hepatocellular carcinoma (metastatic)	0/30	1/30	0/32	1/21	0/26	0/24	0/17	0/29
Lungs: Broncho alveolar adenoma	0/30	0/30	0/32	0/21	0/26	0/24	1/17	0/29
Lungs: Squamous cell carcinoma (metastatic)	1/30	0/30	0/32	0/21	0/26	0/24	0/17	0/29
Lungs: Giant cell tumour	0/30	0/30	0/32	1/21	0/26	0/24	0/17	0/29
Lungs: Fibroma	0/30	0/30	0/32	0/21	0/26	1/24	0/17	0/29
Lungs: Round cell sarcoma (metastatic)	0/30	0/30	0/32	0/21	0/26	0/24	1/17	0/29
Heart: Histiocytic sarcoma (metastatic)	1/30	0/30	1/32	1/21	0/26	0/24	0/17	0/29
Heart: Round cell sarcoma of pericardium	0/30	0/30	0/32	0/21	0/26	0/24	1/17	0/29
Spleen: Cholangio carcinoma (metastatic)	0/30	1/30	0/32	0/21	0/26	0/24	0/17	0/29
Mediastinal lymph node: Histiocytic sarcoma (metastatic)	1/28	0/28	1/30	0/20	1/26	0/22	0/16	0/29
Mediastinal lymph node: Cholangio carcinoma (metastatic)	0/28	1/28	0/30	0/20	1/26	0/22	0/16	0/29
Mediastinal lymph node: Hepatocellular carcinoma (metastatic)	0/28	1/28	0/30	0/20	0/26	0/22	0/16	0/29
Mediastinal lymph node: Giant cell tumour	0/28	0/28	0/30	1/20	0/26	0/22	0/16	0/29
Mediastinal lymph node: Histiocytic sarcoma	0/28	0/28	0/30	1/20	0/26	0/22	0/16	0/29
Kidneys: Cholangio carcinoma (metastatic)	0/30	1/30	0/32	0/21	0/26	0/24	0/17	0/29
Kidneys: Histosarcoma (metastatic)	0/30	0/30	1/32	0/21	0/26	0/24	0/17	0/29
Urinary bladder: Transitional cell carcinoma	0/27	0/30	0/32	0/20	0/26	0/24	0/16	1/28
Testes: Leydig cell tumour	0/30	0/30	2/31	0/21	-	-	-	-
Testes: Seminoma	0/30	0/30	1/31	0/21	-	-	-	-
Epididymides: Undifferentiated sarcoma	0/30	0/30	1/32	0/21	-	-	-	-
Uterus: Adenocarcinoma	-	-	-	-	0/26	0/24	1/17	0/29

Table 5.5-54 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Summary of neoplastic histopathological findings (dead and moribund sacrificed animals)

	Dose group (ppm)							
	Males				Females			
	G1 0	G2 100	G3 1000	G4 10000	G1 0	G2 100	G3 1000	G4 10000
Uterus: Anaplastic carcinoma	-	-	-	-	0/26	1/24	0/17	0/29
Uterus: Leiomyosarcoma	-	-	-	-	0/26	0/24	0/17	1/29
Brain: Squamous cell carcinoma (metastatic)	0/30	0/30	0/32	1/21	0/26	0/24	0/17	0/29
Thyroid: 'C' cell adenoma	0/26	0/26	1/29	0/21	0/26	0/24	1/17	0/27
Pituitary: Adenocarcinoma	0/29	1/27	0/30	0/20	0/25	0/22	0/16	0/29
Pituitary: Adenoma	2/29	2/27	3/30	1/20	5/25	8/22	3/16	5/29
Adrenals: Cortical cell adenoma	2/30	0/30	0/32	0/21	0/26	0/24	1/17	0/29
Adrenals: Pheochromocytoma	7/30	3/30	4/32	4/21	0/26	0/24	0/17	2/29
Thymus: Thymoma	0/28	0/29	0/31	0/20	0/24	1/24	0/16	0/28
Mammary gland: Adenoma	-	-	-	-	1/23	1/22	3/16	3/28
Mammary gland: Adenocarcinoma	-	-	-	-	2/23	0/22	0/16	0/28
Tumour/mass: Squamous cell carcinoma	0/30	0/30	1/31	0/21	0/26	0/24	0/17	0/29
Tumour/mass: Histiocytic sarcoma	0/30	0/30	0/31	1/21	1/26	0/24	0/17	0/29
Tumour/mass: Cholangio carcinoma (metastatic)	0/30	2/30	0/31	0/21	1/26	0/24	0/17	0/29
Tumour/mass: Giant cell tumour	0/30	0/30	0/31	1/21	0/26	0/24	0/17	0/29
Bone (femur) with joint	1/29	0/30	0/31	0/21	0/26	0/24	0/17	0/28
Sternum: Histiocytic sarcoma (metastatic)	1/29	0/29	0/30	0/21	0/26	0/24	0/17	0/29

Table 5.5-55 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Summary of neoplastic histopathological findings (terminally sacrificed animals)

	Dose group (ppm)							
	Males				Females			
	G1 0	G2 100	G3 1000	G4 10000	G1 0	G2 100	G3 1000	G4 10000
Salivary gland: Duct papilloma	1/20	-	-	0/29	0/24	0/1	-	0/21
Stomach: Papilloma (forestomach)	0/20	1/1	-	0/29	0/24	-	-	1/21
Pancreas: Islet cell adenoma	2/20	-	-	1/29	0/24	-	-	1/21
Pancreas: Lymphosarcoma (metastatic)	1/20	-	-	0/29	0/24	-	-	0/21
Liver: Cholangiocarcinoma	1/20	1/20	0/16	1/29	0/24	0/25	0/32	0/21
Liver: Hepatocellular adenoma	15/20	13/20	4/16	15/29	16/24	10/25	16/32	8/21
Liver: Hepatocellular carcinoma	9/20	16/20	9/16	19/29	6/24	11/25	12/32	4/21
Liver: Intrahepatic bile duct adenoma	1/20	0/20	0/16	0/29	0/24	1/25	0/32	0/21
Liver: Histocytic sarcoma	0/20	1/20	1/16	0/29	0/24	0/25	3/32	0/21
Liver: Tumour emboli	1/20	0/20	1/16	0/29	0/24	0/25	0/32	0/21
Liver: Lymphosarcoma	1/20	0/20	0/16	0/29	0/24	0/25	0/32	0/21
Liver: Benign mixed intrahepatic bile duct adenoma	0/20	0/20	1/16	0/29	0/24	0/25	0/32	0/21
Lungs: Bronchio alveolar adenocarcinoma	0/20	0/4	1/3	0/29	0/24	0/4	0/5	0/21
Lungs: Hepatocellular carcinoma	0/20	0/4	1/3	0/29	0/24	0/4	0/5	0/21

Table 5.5-55 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Summary of neoplastic histopathological findings (terminally sacrificed animals)

	Dose group (ppm)							
	Males				Females			
	G1 0	G2 100	G3 1000	G4 10000	G1 0	G2 100	G3 1000	G4 10000
(metastatic)								
Lungs: Bronchio alveolar adenoma	0/20	0/4	0/3	0/29	1/24	0/4	0/5	1/21
Lungs: Histocytic sarcoma	0/20	0/4	0/3	0/29	0/24	0/4	1/5	0/21
Trachea: Histocytic sarcoma (metastatic)	0/20	-	-	0/29	0/24	1/1	-	0/21
Heart: Histocytic sarcoma (metastatic)	0/20	-	-	0/29	0/24	1/1	1/1	0/21
Mesenteric lymph node: Histocytic sarcoma	1/20	-	-	0/29	0/22	-	-	0/21
Mediastinal lymph node: Histocytic sarcoma	0/19	0/1	-	0/29	0/22	1/1	-	0/21
Mandibular lymph node: Lymphoma	0/19	0/6	0/5	2/29	0/24	0/9	0/6	0/21
Kidneys: Lymphosarcoma	0/20	0/3	0/2	0/29	0/24	-	1/1	0/21
Testes: Leydig cell tumour	2/20	0/7	0/1	3/29	-	-	-	-
Uterus: Adenoma	-	-	-	-	0/24	0/10	0/22	1/21
Uterus: Adenocarcinoma	-	-	-	-	0/24	0/10	2/22	0/21
Uterus: Adenoma papillary	-	-	-	-	0/24	1/10	0/22	0/21
Uterus: Haemangioma	-	-	-	-	0/24	0/10	1/22	0/21
Brain: Pituitary adenocarcinoma (metastatic)	0/20	-	-	0/29	1/24	-	-	0/21
Thyroids: ‘C’ cell adenoma	2/19	-	-	1/29	2/24	-	-	1/20
Pituitary: Adenocarcinoma	0/20	0/3	0/1	0/29	1/24	0/11	0/7	0/21
Pituitary: Adenoma	1/20	2/3	0/1	4/29	2/24	5/11	4/7	1/21
Adrenals: Cortical cell adenoma	1/20	0/3	1/4	0/29	0/24	0/4	2/7	1/21
Adrenals: Pheochromocytoma	6/20	2/3	2/4	12/29	1/24	0/4	4/7	2/21
Adrenals: Malignant Pheochromocytoma	0/20	0/3	1/4	1/29	0/24	0/4	0/7	0/21
Thymus: Thymoma	0/20	-	-	0/29	1/24	-	-	0/21
Mammary gland: Adenoma	-	-	-	-	1/17	2/8	5/17	2/20
Mammary gland: Adenocarcinoma	-	-	-	-	1/17	0/8	0/17	0/20
Cecum: Histocytic sarcoma	0/20	1/1	-	0/29	0/24	-	-	0/21
Tumour/mass: Fibroma	0/20	1/2	1/3	0/29	1/24	0/4	0/1	0/21
Tumour/mass: Undifferentiated sarcoma	0/20	0/2	0/3	0/29	1/24	0/4	0/1	0/21

Incidentally, the number of benign tumours in the low and mid dose group males and combined sex was lower and higher in the mid dose group females. There was no dose-response relationship and therefore the occurrence of tumours was considered incidental.

The different liver tumours observed in the dead and moribund sacrificed and terminally sacrificed rats included hepatocellular adenoma, intrahepatic bile duct adenomas, cholangiocarcinoma, hepatocellular carcinoma, histiocytic sarcoma, fibrosarcoma and lymphosarcoma. Of these, hepatocellular adenomas and carcinomas occurred more frequently, as often observed in ageing rats. The occurrence of these tumours appeared to be incidental and not compound-related as their frequency of occurrence was not dose dependent. No reasons could be ascribed for the decrease in the number of benign tumours in the low and mid dose group males and for combined sex and for an increase seen in the mid group dose females (see

table above).

From this, it is concluded that the test compound at the doses tested does not cause treatment or dose related gross and histopathological changes and it is not carcinogenic under the testing conditions.

3. Assessment and conclusion

Assessment and conclusion by applicant:
Based on the study results the NOAEL in rats after chronic exposure to glyphosate technical for 24 month is 595 mg/kg bw/day for males, and 886 mg/kg bw/day for females (741 mg/kg bw/day for combined) for systemic toxicity and carcinogenicity. It is concluded that glyphosate technical is not carcinogenic in rats.

Single and inconsistent changes of clinical chemistry parameters were considered not treatment-related. (Isolated) Elevation of alkaline phosphatase activities is considered to be related to an adaptation of the metabolism rather than to damage of (liver) cells. Histopathology did not reveal any treatment-related changes in any organ further supporting the conclusion that clinical chemistry parameters changes were not related to treatment-related adverse effects.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.5/006
Report author	[redacted]
Report year	1996
Report title	Glyphosate Acid: One Year Dietary Toxicity Study in Rats
Report No	[redacted]/P/5143
Document No	Not reported
Guidelines followed in study	OECD 452, US EPA 83-1
Deviations from current test guideline (OECD 452, 2018)	Yes, mortality was observed only once per day (instead of twice per day); blood samples were not taken at the beginning of the study (but in week 14); organ weights of heart, ovaries, spleen, thyroid/parathyroid and uterus were not determined. Histopathological examination of the coagulating glands, gall bladder, lacrimal gland, mammary glands of the males and vagina was not performed. No historical control data available.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The chronic toxicity potential of glyphosate acid was assessed in a 12-month feeding study in 24 male and female Wistar rats per group with 0, 2000, 8000 and 20000 ppm (equivalent to mean achieved dose levels

of 0, 141, 560 and 1409 mg/kg bw/day for males and 0, 167, 671 and 1664 mg/kg bw/day for females).

Observations covered clinical signs, body weight, food consumption, haematology, clinical chemistry and urinalysis as well as selected organ weights, necropsy and histopathological examination.

A reduction in bodyweight was evident in animals receiving 20000 ppm glyphosate acid, together with a marginal reduction in bodyweight in rats receiving 8000 ppm. There were no toxicologically significant or treatment-related effects on haematology, blood and urine clinical chemistry or organ weights.

The treatment-related pathological finding, increased incidence of mild focal basophilia of the acinar cells of the parotid salivary gland in both sexes which had received 20000 ppm glyphosate acid, is considered an adaptive response due to oral irritation from the ingestion of glyphosate, an organic acid, in the diet.

I. MATERIALS AND METHODS

A. Materials

1. Test material:

Identification:	Glyphosate acid
Description:	White solid
Lot/Batch #:	P24
Purity:	95.6 %
Stability of test compound:	At least 1 year when stored at RT
2. Vehicle and/ or positive control:	Diet
3. Test animals:	
Species:	Rat
Strain:	Wistar (Alpk:APfSD)
Source:	[redacted]
Age:	22 - 24 days (on delivery)
Sex:	Males and females
Weight at dosing:	Males: 150.5 – 151.5 g (mean values); females: 126.7 – 133.3 g (mean values)
Acclimation period:	Approximately 2 weeks
Diet/Food:	CT1 diet (Special Diet services Ltd., Essex, UK), *ad libitum*
Water:	Mains drinking water, *ad libitum*
Housing:	Initially in litters, sexes separately, after assignment to experimental groups in group of four rats per sex per cage.
Environmental conditions:	Temperature: 21 ± 2 °C Humidity: 55 ± 15 % Air changes: at least 15/hour 12 hours light/dark cycle

B: Study design and methods

In life dates: 1995-04-03 to 1996-06-03

Animal assignment and treatment:
In a chronic toxicity study groups of 24 Wistar-derived rats per sex received daily dietary doses of 0, 2000, 8000 and 20000 ppm glyphosate acid (equivalent to mean achieved dose levels of 0, 141, 560 and 1409 mg/kg bw/day for males and 0, 167, 671 and 1664 mg/kg bw/day for females).

Test diets were prepared in either 30 or 60 kg batches by mixing the appropriate amount of the test substance with the basal diet. The stability and homogeneity of the test substance in the diet was determined in an in-house stability study at 2000 and 20000 ppm.

Observations
Rats were examined for toxic signs, ill-health or behavioural changes and pre-terminal deaths prior to the start of the study and once a day afterwards. Detailed clinical observations were conducted weekly. Ophthalmic examination was done in all animals at the start of the study. The eyes of the control and high dose group were additionally examined one week to termination.

Body weight
Individual body weights were recorded prior to start of treatment, at weekly intervals from Week 1 to 14 and every two weeks thereafter until termination.

Food consumption and compound intake
Food consumption was recorded once weekly for each cage group from Week 1 to Week 13, once in Week 16 and every fourth week thereafter.

Haematology and clinical chemistry
Blood was collected from 12 animals per sex and group at Week 14, 27 and at termination (Week 53). The following parameters were measured: Haematocrit, haemoglobin, erythrocyte count, MCV, MCH, MCHC, blood cell morphology, platelet count, total leukocyte count, differential leukocyte count, red blood cell distribution width, prothrombin time, activated partial thromboplastin time, alkaline phosphatase, aspartate amino transferase (AST), alanine aminotransferase (ALT), γ-glutamyl-transferase, creatine kinase, creatinine, urea, total protein, glucose, albumin, total bilirubin, triglycerides, total cholesterol, inorganic phosphorus, calcium, sodium, potassium, and chloride.

Urinalysis
Individual urine samples were collected from the same animals as those used for haematology analyses (except for Week 52) at Week 13, 26 and 52. The following parameters were determined: Volume, colour, appearance, specific gravity, pH, glucose, ketones, protein, urobilinogen and blood.

Sacrifice and pathology
Necropsy was conducted on all animals except for Rats 38 and 149-152, which were killed during Week 6/7 due to a sexing error. The following organ weights were determined from all animals surviving to scheduled termination: Adrenals, brain, epididymis, kidneys, liver and testes.

Tissue samples were taken from the following organs: Adrenals, aorta, bone & bone marrow (femur incl. joint), brain (cerebrum, cerebellum, brainstem), caecum, cervix, colon, duodenum, epididymis, eye, gross lesions, Harderian gland, heart, ileum, jejunum, kidneys, liver, lung, lymph nodes (cervical and mesenteric), mammary gland, nasopharyngeal cavity, sciatic nerve, oesophagus, oral cavity, ovary, pancreas, pituitary, prostrate, rectum, salivary glands, seminal vesicles, skin, spinal cord (cervical, thoracic, lumbar), spleen, sternum, stomach, testes, thymus, thyroid/parathyroid, trachea, urinary bladder, uterus and voluntary muscle.

Statistics
All data were evaluated using analysis of variance and covariance for each specified parameter using the GLM procedure in SAS (1989). Differences from control were tested statistically by comparing each

treatment group least- squares mean with the control group least-squares mean using a two-sided Student's t-test, based on the error mean square in the analysis. All statistical tests were two sided.

II. RESULTS

The mean achieved concentrations of glyphosate acid in each dietary preparation were within 8 % of the nominal concentration and the overall mean concentrations were within 4 % of nominal.

The homogeneity of glyphosate acid in diet at concentrations of 2000 and 20000 ppm was satisfactory; percentage deviations were within 7 % of the overall mean.

The stability tests determined at 2000 and 20000 ppm showed that the test substance is stable for at least 61 days when stored at room temperature.

A. MORTALITY AND CLINICAL SIGNS

There were no treatment-related deaths.

Table 5.5-56 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted], 1996): Unscheduled deaths

Dose group (ppm)							
0		2000		8000		20000	
♂	♀	♂	♀	♂	♀	♂	♀
1/24	0/24	1/24	1/24	0/24	0/24	1/24	4/24

B. CLINICAL OBSERVATIONS

There was a small increase in the number of animals in the 20000 ppm group which had urinary staining (wet or dry). All other clinical observations were of a type and incidence expected for rats of this strain.

Table 5.5-57 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted] 1996): Selected clinical observations

	Dose group (ppm)							
	0		2000		8000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
	Number of observations/number of dead animals							
Aggressive (Aggressor)	0/0	0/0	1/1	0/0	0/0	0/0	0/0	0/0
Breathing irregular	0/0	0/0	0/0	1/1	0/0	0/0	0/0	0/0
Cold	0/0	1/1	0/0	0/0	0/0	0/0	1/1	0/0
Coat stained	0/0	1/1	0/0	0/0	0/0	0/0	0/0	0/0
Dermal mas 1/more areas	4/1	15/1	0/0	0/0	0/0	0/0	0/0	0/0
Distended abdomen	0/0	0/0	0/0	0/0	0/0	0/0	0/0	11/1
Dry sores 1 or more areas	66/6	34/4	2/2	58/4	30/3	41/4	1/1	0/0
Ears swollen	9/1		0/0		0/0		0/0	
Discharge from eye	10/2	16/2	2/2	2/2	45/2	2/1	16/3	0/0
Eye pallor	0/0	0/0	5/1	3/1	0/0	0/0	0/0	0/0
Malocclusion	20/1	0/0	50/1	2/1	36/2	60/1	0/0	14/1
Hair loss	0/0	0/0	0/0	0/0	0/0	33/1	0/0	0/0
Hair loss 1 or	0/0	0/0	0/0	0/0	0/0	2/1	0/0	0/0

Table 5.5-57 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted], 1996): Selected clinical observations

	Dose group (ppm)							
	0		2000		8000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
	Number of observations/number of dead animals							
more areas								
Eyelids thickened	0/0	2/1	0/0	0/0	0/0	0/0	0/0	0/0
Hind limb swollen	0/0	0/0	0/0	0/0	2/1	0/0	0/0	0/0
Mouth ulcer on palate	0/0	0/0	0/0	1/1	1/1	0/0	0/0	1/1
Whiskers reduced in nos	0/0	0/0	0/0	18/2	0/0	0/0	0/0	2/2
Nose bleeding	0/0	0/0	0/0	1/1	1/1	0/0	0/0	0/0
Pale	0/0	1/1	0/0	0/0	0/0	0/0	0/0	0/0
Abnormal respiratory noise	0/0	0/0	0/0	1/1	0/0	0/0	0/0	0/0
Scabs 1 or more areas	15/6	10/4	10/4	5/3	22/3	7/3	17/4	0/0
Subcutaneous mass 1/more areas	27/5	2/1	54/4	23/5	0/0	4/1	17/3	0/0
Snout twisted	15/1	0/0	0/0	0/0	0/0	17/1	0/0	0/0
Stains around nose	3/1	1/1	0/0	0/0	1/1	1/1	0/0	0/0
Stained with urine – dry	0/0	0/0	11/1	0/0	0/0	1/1	15/3	35/3
Stained with urine – wet	0/0	0/0	9/2	1/1	0/0	1/1	19/2	32/5
Subdued	1/1	1/1	0/0	2/1	0/0	0/0	1/1	1/1
Swollen foot pads	0/0	0/0	0/0	0/0	5/1	0/0	0/0	0/0
Tail bleeding	2/1	1/1	0/0	0/0	0/0	0/0	0/0	0/0
Tail damaged	311/8	93/2	230/6	204/7	298/9	174/7	13/1	24/1
Scaly Tail	0/0	0/0	5/1	24/3	0/0	62/6	4/2	0/0
Teeth missing	0/0	0/0	12/1	0/0	0/0	12/1	0/0	0/0
Teeth trimmed	8/1	0/0	28/2	0/0	10/2	36/1	0/0	4/1
Tip of tail blackened	6/4	3/2	0/0	7/2	3/1	5/2	0/0	0/0
Upper incisors broken	0/0	0/0	2/1	0/0	0/0	0/0	0/0	1/1
Urine coloured	0/0	0/0	2/1	0/0	0/0	0/0	1/1	0/0
Vaginal bleeding	0/0	0/0	1/1	0/0	0/0	0/0	0/0	0/0
Wet sores 1 or more areas	10/4	1/1	0/0	0/0	0/0	0/0	0/0	0/0

C. BODY WEIGHT

Bodyweights of rats receiving 20000 ppm glyphosate acid were lower than those of controls throughout the study. Bodyweights in the intermediate group were slightly reduced throughout the study. The difference from control was not statistically significant in males and was statistically significant in females only from Week 46. As the pattern of the effect was similar to that of the high dose rats for both sexes this minor

difference in bodyweight is considered to be related to administration of glyphosate acid.

There was no effect on bodyweight in rats receiving 2000 ppm glyphosate acid.

The intergroup comparison of body weights are shown below.

D. FOOD CONSUMPTION AND COMPOUND INTAKE

Food consumption was generally lower in rats receiving 20000 ppm than in controls. The difference was most marked at the start of the study. Food consumption was generally slightly lower than controls in rats receiving 8000 ppm glyphosate acid. There was no effect on food consumption in rats receiving 2000 ppm.

Table 5.5-58 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted] 1996): Intergroup comparison of body weights

	Dose group (ppm)							
	0		2000		8000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
	Body weight [g]							
Week 1	151.5 ± 22.5 (N = 24)	128.5 ± 15.3 (N = 24)	↓150.5 ± 22.6 (N = 23)	↑130.3 ± 16.7 (N = 24)	↓150.6 ± 23.7 (N = 24)	↓126.7 ± 15.1 (N = 24)	↓151.0 ± 21.1 (N = 24)	↓128.1 ± 17.0 (N = 20)
Week 2 adjusted mean	204.1 ± 24.1 (N = 24) 203.3	158.0 ± 12.0 (N = 24) 158.0	↓202.2 ± 23.4 (N = 23) 202.6	↑158.7 ± 12.4 (N = 24) 156.5	↓202.7 ± 23.7 (N = 24) 203.1	↓153.6 ± 12.7 (N = 24) 155.6	↓198.2 ± 22.0 (N = 24) 198.1**	↓151.9 ± 16.7 (N = 20) 151.7**
Week 3 adjusted mean	252.7 ± 25.3 (N = 23) 253.5	179.5 ± 11.2 (N = 24) 179.4	↑254.4 ± 27.3 (N = 23) 254.5	↑180.8 ± 15.0 (N = 24) 178.5	↑252.9 ± 30.8 (N = 24) 252.7	↓175.6 ± 14.1 (N = 24) 177.6	↓245.0 ± 22.8 (N = 24) 244.3**	↓176.8 ± 14.8 (N = 20) 176.7
Week 4 adjusted mean	301.3 ± 36.8 (N = 24) 300.3	197.9 ± 10.8 (N = 24) 197.8	↑397.7 ± 26.9 (N = 23) 298.4	↑202.2 ± 12.5 (N = 24) 200.1	↓295.7 ± 30.1 (N = 24) 296.1	↓192.1 ± 14.8 (N = 24) 194.0	↓284.5 ± 23.1 (N = 24) 284.4**	↓189.3 ± 15.8 (N = 20) 188.9**
Week 5 adjusted mean	322.0 ± 27.5 (N = 24) 331.1	213.8 ± 11.0 (N = 24) 213.7	↑330.9 ± 28.1 (N = 23) 331.6	↑216.1 ± 13.2 (N = 24) 214.1	↑326.3 ± 30.4 (N = 24) 326.8	↓208.2 ± 13.8 (N = 24) 210.0	↓312.2 ± 23.9 (N = 24) 312.1**	↓203.5 ± 13.4 (N = 20) 203.2**
Week 6 adjusted mean	357.4 ± 30.7 (N = 24) 356.3	224.0 ± 12.6 (N = 24) 223.9	↑357.7 ± 29.8 (N = 23) 358.7	↑224.4 ± 14.9 (N = 24) 222.3	↓350.2 ± 33.1 (N = 24) 350.7	↓217.5 ± 15.8 (N = 24) 219.4	↓333.8 ± 28.3 (N = 23) 333.7**	↓215.6 ± 13.8 (N = 20) 215.4*
Week 7 adjusted mean	384.0 ± 30.3 (N = 24) 383.0	234.2 ± 13.6 (N = 24) 234.1	↓383.4 ± 30.8 (N = 23) 384.5	↑235.8 ± 14.4 (N = 24) 223.4	↓374.8 ± 36.4 (N = 24) 375.2	↓227.4 ± 17.1 (N = 24) 229.5	↓356.1 ± 30.1 (N = 24) 356.0**	↓226.7 ± 17.1 (N = 20) 226.8*
Week 8 adjusted mean	407.3 ± 32.8 (N = 24) 406.1	242.1 ± 13.0 (N = 24) 242.0	↑408.5 ± 33.8 (N = 23) 409.8	↑247.3 ± 15.3 (N = 24) 245.0	↓394.8 ± 37.6 (N = 24) 395.3	↓235.4 ± 16.9 (N = 24) 237.5	↓377.8 ± 33.9 (N = 24) 377.7**	↓233.3 ± 16.5 (N = 20) 233.3**
Week 9 adjusted mean	423.5 ± 35.5 (N = 24) 422.2	249.0 ± 15.7 (N = 24) 248.9	↑426.5 ± 36.0 (N = 23) 427.8	↑252.6 ± 14.3 (N = 24) 250.0	↓411.5 ± 38.6 (N = 24) 412.1	↓242.1 ± 16.8 (N = 24) 237.5	↓397.4 ± 33.9 (N = 24) 397.3**	↓239.5 ± 14.9 (N = 20) 239.7**
Week 10 adjusted mean	444.6 ± 36.5 (N = 24) 443.2	256.3 ± 15.6 (N = 24) 256.2	↓444.4 ± 37.5 (N = 23) 445.9	↓255.0 ± 15.2 (N = 24) 252.5	↓431.1 ± 41.2 (N = 24) 443.4*	↓248.8 ± 17.4 (N = 24) 251.1	↓427.5 ± 36.1 (N = 24) 427.4**	↓248.5 ± 17.2 (N = 20) 248.3*
Week 11	460.5 ±	262.5 ±	↓459.4 ±	↑263.8 ±	↓442.7 ±	↓253.5 ±	↓427.5 ±	↓254.1 ±

Table 5.5-58 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted] 1996): Intergroup comparison of body weights

	Dose group (ppm)							
	0		2000		8000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
	38.5	13.2	37.3	15.4	43.0	17.4	36.1	18.0
adjusted mean	(N = 24) 459.0	(N = 24) 262.4	(N = 23) 460.7	(N = 24) 261.6	(N = 24) 443.4*	(N = 24) 255.5*	(N = 24) 427.4**	(N = 20) 253.6*
Week 12	473.0 ± 39.9	266.3 ± 13.8	↑474.0 ± 38.7	↑269.3 ± 13.3	↓456.5 ± 45.0	↓258.8 ± 17.8	↓441.0 ± 38.8	↓256.3 ± 17.0
adjusted mean	(N = 24) 471.5	(N = 24) 266.2	(N = 23) 475.4	(N = 24) 267.1	(N = 24) 457.2	(N = 24) 260.8	(N = 24) 440.8**	(N = 20) 261.2**
Week 13	485.3 ± 39.8	271.2 ± 14.5	↑486.4 ± 39.1	↑271.8 ± 13.5	↓467.5 ± 45.5	↓263.2 ± 17.0	↓453.0 ± 39.9	↓261.5 ± 17.6
adjusted mean	(N = 24) 489.0	(N = 24) 271.1	(N = 23) 487.8	(N = 24) 269.7	(N = 24) 468.2	(N = 24) 265.1	(N = 24) 452.9**	(N = 20) 261.2**
Week 14	490.6 ± 39.6	270.5 ± 15.2	↑491.0 ± 40.4	↑272.8 ± 14.4	↓471.7 ± 48.1	↓263.7 ± 18.1	↓458.5 ± 39.8	↓263.6 ± 17.2
adjusted mean	(N = 24) 489.0	(N = 24) 270.4	(N = 23) 492.1	(N = 24) 270.4	(N = 24) 472.3	(N = 24) 265.8	(N = 24) 548.3**	(N = 20) 263.3*
Week 16	514.6 ± 44.0	278.8 ± 13.1	↓513.0 ± 43.9	↑281.6 ± 13.1	↓494.1 ± 51.3	↓271.8 ± 18.6	↓479.5 ± 40.3	↓278.5 ± 19.0
adjusted mean	(N = 24) 512.9	(N = 24) 278.7	(N = 23) 514.2	(N = 24) 279.4	(N = 24) 494.9	(N = 24) 273.7	(N = 24) 479.3**	(N = 20) 269.9*
Week 18	532.3 ± 45.3	287.0 ± 15.1	↓531.9 ± 44.6	↑290.6 ± 17.0	↓511.0 ± 52.0	↓276.6 ± 19.4	↓498.8 ± 43.1	↓278.5 ± 19.0
adjusted mean	(N = 24) 530.4	(N = 24) 286.9	(N = 23) 533.3	(N = 24) 287.9	(N = 24) 511.8	(N = 24) 279.0*	(N = 24) 498.7**	(N = 20) 278.2*
Week 20	543.5 ± 47.0	289.0 ± 14.9	↑543.7 ± 47.7	↑295.8 ± 16.0	↓521.7 ± 52.3	↓281.7 ± 20.2	↓511.8 ± 43.6	↓279.5 ± 18.5
adjusted mean	(N = 24) 541.6	(N = 24) 289.0	(N = 23) 545.0	(N = 24) 293.8	(N = 24) 522.5	(N = 24) 283.5	(N = 24) 511.6**	(N = 20) 279.1*
Week 22	553.3 ± 47.3	293.8 ± 15.3	↓549.1 ± 47.0	↑297.8 ± 14.9	↓529.4 ± 54.3	↓284.7 ± 21.1	↓531.9 ± 46.3	↓283.1 ± 19.0
adjusted mean	(N = 24) 551.4	(N = 24) 293.7	(N = 23) 550.4	(N = 24) 295.6	(N = 24) 541.5*	(N = 24) 286.7	(N = 24) 531.7**	(N = 20) 281.9**
Week 24	566.2 ± 49.3	296.2 ± 12.7	↓560.3 ± 48.3	↑301.5 ± 14.8	↓540.7 ± 55.1	↓287.8 ± 19.9	↓531.9 ± 46.3	↓287.1 ± 18.7
adjusted mean	(N = 24) 564.3	(N = 24) 296.1	(N = 23) 561.4	(N = 24) 299.6	(N = 24) 541.5*	(N = 24) 289.4	(N = 24) 531.7**	(N = 20) 286.9*
Week 26	574.5 ± 50.2	298.8 ± 13.8	↓568.0 ± 49.0	↑302.2 ± 14.8	↓550.6 ± 55.6	↓290.5 ± 20.3	↓540.6 ± 49.0	↓287.3 ± 20.6
adjusted mean	(N = 24) 572.6	(N = 24) 298.7	(N = 23) 569.2	(N = 24) 300.3	(N = 24) 551.5	(N = 24) 2.92.1	(N = 24) 540.4**	(N = 20) 286.6**)
Week 28	589.5 ± 49.8	300.8 ± 16.6	↓583.2 ± 49.9	↑305.5 ± 11.7	↓566.5 ± 58.1	↓293.5 ± 19.9	↓556.0 ± 53.3	↓290.6 ± 21.6
adjusted mean	(N = 24) 587.5	(N = 24) 300.8	(N = 23) 584.5	(N = 24) 303.4	(N = 24) 567.4	(N = 24) 295.4	(N = 24) 555.9*	(N = 20) 289.9**
Week 30	601.2 ± 49.7	305.7 ± 15.2	↓592.1 ± 51.2	↑308.1 ± 13.1	↓576.4 ± 58.6	↓296.4 ± 19.7	↓568.3 ± 53.2	↓292.8 ± 19.6
adjusted mean	(N = 24) 599.2	(N = 24) 305.6	(N = 23) 593.1	(N = 24) 306.0	(N = 24) 577.3	(N = 24) 298.3	(N = 24) 568.2*	(N = 20) 291.8**
Week 32	607.2 ± 50.8	307.8 ± 15.4	↓599.2 ± 51.3	↑313.7 ± 14.6	↓583.9 ± 59.5	↓300.4 ± 22.2	↓573.0 ± 55.8	↓298.2 ± 22.1
adjusted mean	(N = 24) 605.1	(N = 24) 307.7	(N = 23) 600.3	(N = 24) 311.5	(N = 24) 584.8	(N = 24) 302.4	(N = 24) 572.8*	(N = 20) 296.8*
Week 34	613.3 ±	312.9 ±	↓604.4 ±	↑317.1 ±	↓591.5 ±	↓303.5 ±	↓580.5 ±	↓303.3 ±

Table 5.5-58 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted] 1996): Intergroup comparison of body weights

	Dose group (ppm)							
	0		2000		8000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
	50.7 (N = 24)	17.1 (N = 24)	51.8 (N = 23)	16.1 (N = 24)	59.8 (N = 24)	23.3 (N = 24)	55.2 (N = 24)	24.4 (N = 20)
adjusted mean	611.2	312.8	605.2	314.8	592.5	305.6	580.4*	302.5
Week 36	624.5 ± 50.5 (N = 24)	316.2 ± 19.7 (N = 24)	↓613.9 ± 52.8 (N = 23)	↑319.0 ± 17.3 (N = 24)	↓599.6 ± 61.9 (N = 24)	↓307.8 ± 25.2 (N = 24)	↓593.9 ± 57.6 (N = 24)	↓305.4 ± 36.2 (N = 20)
adjusted mean	622.5	316.1	614.7	316.8	613.0	309.2	593.7*	304.4
Week 38	631.0 ± 52.0 (N = 24)	322.8 ± 21.1 (N = 24)	↓625.0 ± 54.5 (N = 23)	↓321.2 ± 20.7 (N = 24)	↓612.1 ± 62.6 (N = 24)	↓309.4 ± 23.9 (N = 24)	↓606.9 ± 56.7 (N = 24)	↓308.6 ± 28.0 (N = 20)
adjusted mean	628.9	322.7	626.1	318.8	613.0	311.5	606.7	306.3**
Week 40	632.5 ± 52.8 (N = 23)	325.5 ± 21.5 (N = 24)	↓629.8 ± 54.0 (N = 23)	↑326.0 ± 16.3 (N = 23)	↓615.8 ± 60.9 (N = 24)	↓312.6 ± 25.1 (N = 24)	↓610.2 ± 56.0 (N = 24)	↓313.6 ± 30.8 (N = 20)
adjusted mean	628.9	325.5	629.9	322.3	615.8	315.3	609.2	311.8*
Week 42	640.1 ± 51.0 (N = 23)	329.8 ± 25.2 (N = 24)	↓636.7 ± 56.0 (N = 23)	↓327.7 ± 18.3 (N = 23)	↓622.6 ± 63.4 (N = 24)	↓315.3 ± 26.2 (N = 24)	↓615.8 ± 56.2 (N = 24)	↓315.9 ± 31.0 (N = 20)
adjusted mean	636.4	329.8	636.8	324.0	622.7	317.8	614.8	313.5*
Week 44	642.0 ± 50.5 (N = 23)	333.3 ± 25.9 (N = 24)	↓636.3 ± 52.7 (N = 23)	↓332.2 ± 21.1 (N = 23)	↓625.1 ± 32.7 (N = 24)	↓318.4 ± 26.8 (N = 24)	↓618.4 ± 57.9 (N = 24)	↓316.2 ± 33.5 (N = 20)
adjusted mean	638.3	333.3	636.5	328.6	625.2	321.0	617.4	314.8*
Week 46	646.5 ± 52.5 (N = 23)	338.8 ± 25.7 (N = 24)	↓639.9 ± 53.6 (N = 23)	↓336.9 ± 19.9 (N = 23)	↓629.3 ± 62.1 (N = 24)	↓319.8 ± 26.3 (N = 24)	↓621.9 ± 58.5 (N = 24)	↓323.4 ± 34.4 (N = 20)
adjusted mean	643.1	338.6	640.1	333.5	529.4	321.9*	620.9	322.1*
Week 48	647.7 ± 51.0 (N = 23)	339.0 ± 24.8 (N = 24)	↓638.9 ± 52.3 (N = 23)	↓336.8 ± 19.9 (N = 23)	↓631.5 ± 62.9 (N = 24)	↓320.6 ± 27.4 (N = 24)	↓622.2 ± 60.5 (N = 24)	↓325.2 ± 36.0 (N = 20)
adjusted mean	644.4	339.1	639.0	333.1	631.6	323.0*	621.2	324.4*
Week 50	651.1 ± 53.9 (N = 23)	345.0 ± 25.8 (N = 24)	↓645.3 ± 54.6 (N = 23)	↓344.5 ± 24.4 (N = 23)	↓634.1 ± 61.3 (N = 24)	↓324.8 ± 27.0 (N = 24)	↓325.9 ± 60.8 (N = 24)	↓328.4 ± 36.3 (N = 20)
adjusted mean	647.8	345.1	645.5	340.5	634.2	327.5*	624.9	326.5*
Week 52	653.0 ± 53.2 (N = 23)	349.7 ± 26.8 (N = 24)	↓643.3 ± 55.7 (N = 23)	↓342.6 ± 27.5 (N = 23)	↓639.8 ± 63.6 (N = 24)	↓327.8 ± 28.9 (N = 24)	↓633.0 ± 59.3 (N = 23)	↓329.4 ± 35.8 (N = 20)
adjusted mean	649.1	349.7	642.7	338.6	639.4	330.4*	633.0	327.3**
Week 53	652.1 ± 53.0 (N = 23)	↓346.8 ± 26.9 (N = 24)	↓644.0 ± 56.8 (N = 23)	↓344.1 ± 26.5 (N = 23)	↓640.5 ± 62.7 (N = 24)	↓327.7 ± 30.2 (N = 24)	↓634.1 ± 60.1 (N = 23)	↓332.5 ± 40.5 (N = 20)
adjusted mean	548.5	346.8	643.2	339.3	640.1	330.8*	634.3	330.6

** Statistically significant difference from the control group mean at the 1 % level (Student's t-test, two-sided)
* Statistically significant difference from the control group mean at the 5 % level (Student's t-test, two-sided)

Table 5.5-59 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted], 1996): Intergroup comparison of food consumption

	Dose group (ppm)							
	0		2000		8000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
Body weight [g]								
Week 1	26.3 ± 2.0	21.0 ± 1.3	↓25.9 ± 1.7	↓20.9 ± 1.3	↓25.8 ± 2.2	↓20.3 ± 0.9	↓25.1 ± 1.8	↓19.5* ± 1.3
Week 2	28.7 ± 2.1	21.0 ± 0.7	↓28.5 ± 1.7	↓20.9 ± 0.9	↓28.2 ± 2.0	↑21.6 ± 2.5	↓27.2* ± 1.2	↓20.7 ± 0.8
Week 3	30.6 ± 1.7	21.4 ± 0.9	↓30.1 ± 0.8	↑21.9 ± 0.9	↓29.8 ± 1.1	↓20.6 ± 1.2	↓28.5** ± 1.1	↓20.1* ± 0.8
Week 4	30.6 ± 1.7	21.7 ± 0.2	↓30.3 ± 0.9	21.7 ± 0.7	↓29.4 ± 1.4	↓20.9 ± 1.3	↓28.0** ± 2.0	↓19.8** ± 0.5
Week 5	30.7 ± 1.7	22.3 ± 0.7	↓30.5 ± 1.6	↓21.7 ± 0.9	↓29.8 ± 1.7	↓21.0* ± 1.2	↓27.8** ± 2.0	↓20.5** ± 0.4
Week 6	30.8 ± 1.7	22.0 ± 0.6	↓30.4 ± 1.6	↓21.7 ± 1.1	↓29.9 ± 1.1	↓21.0 ± 1.2	↓28.1** ± 1.5	↓20.7* ± 1.7
Week 7	31.6 ± 1.6	22.1 ± 0.6	↓30.7 ± 1.6	22.1 ± 1.3	↓29.7** ± 1.3	↓21.2* ± 0.8	↓28.2** ± 1.6	↓20.3** ± 1.1
Week 8	30.5 ± 1.9	21.3 ± 0.9	↑30.6 ± 1.4	↑21.4 ± 0.9	↓29.2 ± 2.0	↓20.6 ± 0.7	↓28.9 ± 1.9	↓20.0* ± 0.6
Week 9	31.0 ± 2.0	20.9 ± 1.0	31.0 ± 2.1	20.9 ± 0.9	↓30.2 ± 2.0	↑21.1 ± 1.4	↓28.5** ± 1.3	↓20.6 ± 0.7
Week 10	30.7 ± 1.6	21.3 ± 0.3	↑30.9 ± 1.8	21.3 ± 1.0	↓29.2 ± 1.5	↓20.6 ± 1.1	↓28.7** ± 1.1	↓20.3 ± 1.5
Week 11	30.7 ± 2.3	21.7 ± 1.5	↑31.0 ± 2.2	↓21.2 ± 0.8	↓29.7 ± 2.1	↓20.7 ± 0.6	↓29.2 ± 1.5	↓20.3 ± 1.6
Week 12	31.2 ± 1.9	22.0 ± 0.9	↑31.3 ± 2.0	↓21.4 ± 0.7	↓29.8* ± 2.0	↓20.8* ± 0.9	↓29.9* ± 1.1	↓20.5* ± 0.8
Week 13	29.2 ± 2.0	20.3 ± 0.5	29.2 ± 1.3	↑20.5 ± 0.7	↓27.5 ± 3.9	↓19.9 ± 1.0	↓27.9 ± 1.2	↓19.5 ± 0.8
Week 16	30.1 ± 1.3	20.7 ± 0.5	↓29.8 ± 1.3	↑21.1 ± 0.8	↓29.1 ± 0.7	↓20.3 ± 1.6	↓28.8 ± 1.2	↓20.1 ± 0.9
Week 20	28.8 ± 1.3	20.1 ± 0.9	↓28.5 ± 1.4	↑20.2 ± 1.3	↓27.5 ± 1.5	↓19.6 ± 1.7	↓28.3 ± 1.3	↓19.7 ± 0.7
Week 24	30.4 ± 1.4	20.6 ± 0.9	↓29.8 ± 1.5	↓20.2 ± 0.9	↓29.3 ± 1.4	↓20.2 ± 1.4	↓29.6 ± 0.7	↓20.1 ± 1.1
Week 28	30.1 ± 1.6	20.9 ± 0.5	↓29.6 ± 1.4	20.9 ± 0.7	↓28.0 ± 3.1	↓20.3 ± 0.9	↓29.0 ± 1.0	↓20.3 ± 0.8
Week 32	29.4 ± 1.4	20.8 ± 1.1	↓29.1 ± 2.0	↑21.3 ± 1.2	↑29.6 ± 1.6	↓20.2 ± 1.2	↓28.7 ± 0.9	↑21.1 ± 1.1
Week 36	28.8 ± 1.2	20.8 ± 1.7	↑29.0 ± 1.5	↓20.2 ± 2.1	↓28.3 ± 1.4	↓19.8 ± 1.5	↓28.4 ± 0.6	↓19.6* ± 1.3
Week 40	28.7 ± 1.1	21.1 ± 1.6	↑29.0 ± 1.7	↓20.9 ± 2.2	↑28.4 ± 1.1	↓20.4 ± 1.8	↓28.3 ± 0.6	↓20.5 ± 1.5
Week 44	29.6 ± 1.4	21.8 ± 1.3	↓28.6 ± 1.4	↑22.0 ± 0.9	↓28.5 ± 1.7	↓20.4* ± 1.3	↓27.5* ± 0.7	↓20.8 ± 0.7
Week 48	28.6 ± 1.5	21.1 ± 1.4	↑29.0 ± 1.7	↑22.1 ± 1.9	↓27.7 ± 1.9	↓20.3 ± 1.8	↓28.2 ± 0.6	↓21.0 ± 0.7
Week 52	26.4 ± 1.1	19.9 ± 1.2	↑26.7 ± 2.0	↓19.6 ± 2.0	↑26.8 ± 0.9	↓19.3 ± 1.7	↓25.9 ± 0.2	↓19.4 ± 0.6

* Statistically significant from control ($p \leq 0.05$; Student's t-test, two-sided)
** Statistically significant from control ($p \leq 0.01$; Student's t-test, two-sided)

The group mean achieved doses are summarised below.

Table 5.5-60 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted], 1996): Group mean achieved dose levels

Dose group	Dietary concentration (ppm)	Mean achieved dose level (mg/kg bw/day)	
		Males	Females
1 (control)	0		
2 (low)	2000	141	167
3 (mid)	8000	560	671
4 (high)	20000	1409	1664

The results show a higher test material intake for females when compared to males for each dose level. The mean intake for each dose group is 0, 141, 560 and 1409 mg/kg bw/day for males and 0, 167, 671 and 1664 mg/kg bw/day for females for 0, 2000, 6000 and 20000 ppm, respectively.

E. OPHTHALMOSCOPY

There were no treatment-related effects observed.

Table 5.5-61 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted] 1996): Ophthalmoscopical findings

Ocular structure/observation	Dose group (ppm)							
	0		2000		8000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
	Pre-experimental							
Both eyes normal	20	20	20	19	20	19	18	20
Lens: Hazy opacity	-	-	-	1	-	-	-	-
Cornea: Opacity	-	-	-	-	-	1	-	-
Pupil: Persistent vessel	-	-	-	-	-	-	1	-
Eye general: Protruding, eyelid retracted	-	-	-	-	-	-	1	-
	Pre-terminal							
Both eyes normal	19	not examined	not examined	20	23	not examined	not examined	18
Lens: Anterior opacity	1			1	2			2
Lens: Posterior opacity	-			1	-			-
Lens: Hazy opacity	-			1	-			-
Cornea: Opacity	2			1	-			-
Retina: Pale	1			-	-			-
Dead	1			-	-			4

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

A number of statistically significant differences from control were identified but there was no evidence of a relationship to dose and the differences were small and not seen consistently at all the time points and therefore were considered to be unrelated to glyphosate acid administration.

Table 5.5-62 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted] 1996): Intergroup comparison of haematology

	Dose group (ppm)							
	0		2000		8000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
Haemoglobin [g/dL]								
Week 14	16.0 ± 0.4 (N = 12)	15.8 ± 0.3 (N = 12)	↓15.9 ± 0.6 (N = 12)	↓15.6 ± 0.5 (N = 12)	↑16.1 ± 0.6 (N = 12)	15.8 ± 0.5 (N = 12)	↑16.1 ± 0.4 (N = 12)	15.8 ± 0.6 (N = 10)
Week 27	15.6 ± 0.4 (N = 11)	15.3 ± 0.5 (N = 10)	↓15.5 ± 0.5 (N = 12)	↑15.6 ± 0.5 (N = 12)	↑15.7 ± 0.4 (N = 12)	↑15.7 ± 0.5 (N = 12)	↓15.5 ± 0.4 (N = 12)	↑15.6 ± 0.3 (N = 10)
Week 53	14.4 ± 0.7 (N = 23)	14.7 ± 0.6 (N = 24)	↑14.5 ± 0.6 (N = 23)	↑14.8 ± 0.6 (N = 23)	↑14.8 ± 0.5(N = 24)	↑14.9 ± 0.5 (N = 24)	↑14.6 ± 0.5 (N = 23)	↑14.9 ± 0.5 (N = 20)
Haematocrit [g/dL]								
Week 14	0.460 ± 0.014 (N = 12)	0.451 ± 0.009 (N = 12)	↓0.455 ± 0.020 (N = 12)	↓0.444 ± 0.013 (N = 12)	0.460 ± 0.016 (N = 12)	↓0.446 ± 0.020 (N = 11)	↓0.459 ± 0.012 (N = 12)	0.451 ± 0.014, (N = 10)
Week 27	0.450 ± 0.018 (N = 11)	0.436 ± 0.022 (N = 10)	↓0.447 ± 0.017 (N = 12)	↑0.442 ± 0.019 (N = 12)	↑0.451 ± 0.021 (N = 12)	↑0.449 ± 0.016 (N = 12)	↓0.444 ± 0.013 (N = 12)	↑0.445 ± 0.014 (N = 10)
Week 53	0.436 ± 0.022 (N = 23)	0.443 ± 0.019 (N = 24)	↑0.438 ± 0.020 (N = 23)	↑0.448 ± 0.023 (N = 23)	↑0.450* ± 0.021 (N = 24)	↑0.445 ± 0.020 (N = 24)	↑0.440 ± 0.018 (N = 23)	↑0.444 ± 0.019 (N = 20)
Red blood cell count [x1012/l]**								
Week 14	9.25 ± 0.27 (N = 12)	8.23 ± 0.25 (N = 12)	↓9.05 ± 0.39 (N = 12)	↑8.28 ± 0.26 (N = 12)	↓9.07 ± 0.34 (N = 12)	↑8.35 ± 0.28 (N = 11)	↓9.13 ± 0.30 (N = 12)	↑8.43 ± 0.34 (N = 10)
Week 27	9.35 ± 0.39 (N = 11)	8.08 ± 0.31 (N = 10)	↓9.16 ± 0.35 (N = 12)	↑8.31 ± 0.31 (N = 12)	↓9.14 ± 0.28 (N = 12)	↑8.37 ± 0.28 (N = 12)	↓9.14 ± 0.31 (N = 12)	↑8.36 ± 0.28 (N = 10)
Week 53	8.56 ± 0.54 (N = 23)	8.01 ± 0.33 (N = 24)	↑8.57 ± 0.45 (N = 23)	↑8.09 ± 0.42 (N = 23)	↑8.70 ± 0.44 (N = 24)	↑8.13 ± 0.43 (N = 24)	↑8.67 ± 0.37 (N = 23)	↑8.11 ± 0.31 (N = 20)
Mean cell volume [fL]								
Week 14	49.8 ± 1.7 (N = 12)	54.9 ± 1.4 (N = 12)	↑50.2 ± 0.8 (N = 12)	↓53.7* ± 1.3 (N = 12)	↑50.7 ± 1.1 (N = 12)	↓53.5** ± 1.5 (N = 12)	↑50.3 ± 1.3 (N = 12)	↓53.6* ± 1.3 (N = 10)
Week 27	48.3 ± 2.2 (N = 11)	53.9 ± 2.0 (N = 10)	↑48.9 ± 1.2 (N = 12)	↓53.4 ± 53.7 (N = 12)	↑49.5** ± 1.7 (N = 12)	↓53.7 ± 1.7 (N = 12)	↑48.6 ± 1.0 (N = 12)	↓51.1 ± 1.5 (N = 10)
Week 53	51.0 ± 2.0 (N = 23)	55.4 ± 2.3 (N = 24)	↑51.2 ± 1.7 (N = 23)	↑55.5 ± 2.0 (N = 23)	↑51.8 ± 2.0 (N = 24)	↓54.9 ± 2.0 (N = 24)	↓50.8 ± 1.5 (N = 23)	↓54.8 ± 1.8 (N = 20)
Mean cell haemoglobin [pg]								
Week 14	17.3 ± 0.6 (N = 12)	19.2 ± 0.4 (N = 12)	↑17.6 ± 0.3 (N = 12)	↓18.9 ± 0.4 (N = 12)	↑17.7* ± 0.4 (N = 12)	↓18.9 ± 0.5 (N = 11)	↑17.7* ± 0.5 (N = 12)	↓18.7* ± 0.4 (N = 10)
Week 27	16.7 ± 0.6 (N = 11)	18.9 ± 0.5 (N = 10)	↑17.0 ± 0.4 (N = 12)	↓18.8 ± 0.5 (N = 12)	↑17.2** ± 0.4 (N = 12)	↓18.8 ± 0.5 (N = 12)	↑17.0 ± 0.5 (N = 12)	↓18.6 ± 0.4 (N = 10)
Week 53	16.9 ± 0.5 (N = 23)	18.4 ± 0.5 (N = 24)	16.9 ± 0.4 (N = 23)	↓18.3 ± 0.5 (N = 23)	↑17.0 ± 0.7 (N = 24)	18.4 ± 0.5 (N = 24)	↓16.8 ± 0.5 (N = 23)	18.4 ± 0.4 (N = 20)
Mean cell haemoglobin concentration [g/dL]								
Week 14	34.8 ±	35.0 ± 0.2	↑35.0 ±	↑35.2 ±	↑35.0 ±	↑35.3* ±	↑35.1 ±	35.0 ± 0.5

Table 5.5-62 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted], 1996): Intergroup comparison of haematology

	Dose group (ppm)							
	0		2000		8000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
	0.6 (N = 12)	(N = 12)	0.4 (N = 12)	0.5 (N = 12)	0.5 (N = 12)	0.5 (N = 11)	0.3 (N = 12)	(N = 10)
Week 27	34.6 ± 0.7 (N = 11)	35.1 ± 0.8 (N = 10)	↑34.8 ± 0.5 (N = 12)	↑35.2 ± 0.7 (N = 12)	↑34.7 ± 0.9 (N = 12)	↓34.9 ± 0.6 (N = 12)	↑34.9 ± 0.4 (N = 12)	↓35.0 ± 0.7 (N = 10)
Week 53	33.1 ± 0.6 (N = 23)	33.2 ± 1.0 (N = 24)	↓33.0 ± 0.8 (N = 23)	↓33.1 ± 0.9 (N = 23)	↓32.8 ± 1.0 (N = 24)	↑33.5 ± 0.9 (N = 24)	↑33.1 ± 0.5 (N = 23)	↑33.6 ± 0.9 (N = 20)
Red cell distribution width [%]								
Week 14	13.4 ± 0.6 (N = 12)	11.9 ± 1.2 (N = 12)	↓13.1 ± 0.6 (N = 12)	↑12.0 ± 0.7 (N = 12)	↑13.6 ± 1.3 (N = 12)	↓11.7 ± 0.9 (N = 11)	13.4 ± 0.8 (N = 12)	↓11.5 ± 0.7 (N = 10)
Week 27	15.1 ± 0.6 (N = 11)	12.6 ± 0.4 (N = 10)	↓14.6** ± 0.6 (N = 12)	↑13.0* ± 0.5 (N = 12)	↓14.8 ± 0.7 (N = 12)	↑13.0* ± 0.6 (N = 12)	↓14.6** ± 0.6 (N = 12)	↑12.7 ± 0.4 (N = 10)
Week 53	14.2 ± 0.5 (N = 23)	12.4 ± 0.6 (N = 24)	↓13.9 ± 0.7 (N = 23)	↓12.3 ± 0.4 (N = 23)	↓14.0 ± 0.4 (N = 24)	12.4 ± 0.8 (N = 24)	↓14.0 ± 0.6 (N = 23)	↓12.2 ± 0.6 (N = 20)
Platelet count [x109/l]**								
Week 14	857 ± 84 (N = 12)	768 ± 98 (N = 12)	↓849 ± 78 (N = 12)	↑804 ± 80 (N = 12)	↓850 ± 61 (N = 12)	↓757 ± 82 (N = 12)	↓825 ± 66 (N = 12)	↑818 ± 97 (N = 10)
Week 27	893 ± 56 (N = 11)	785 ± 158 (N = 10)	↓866 ± 110 (N = 12)	↓782 ± 110 (N = 12)	↑915 ± 82 (N = 12)	↓780 ± 94 (N = 12)	↓867 ± 150 (N = 12)	↑846 ± 139 (N = 10)
Week 53	917 ± 87 (N = 23)	822 ± 104 (N = 24)	↑939 ± 121 (N = 23)	↓782 ± 102 (N = 23)	↑962* ± 109 (N = 24)	↓776 ± 98 (N = 24)	917 ± 107 (N = 23)	↑868 ± 83 (N = 20)
White blood cell count [x109/l]**								
Week 14	6.74 ± 1.54 (N = 12)	4.76 ± 0.93 (N = 12)	↑7.05 ± 1.28 (N = 12)	↑5.38 ± 2.24 (N = 12)	↓6.65 ± 1.16 (N = 12)	↓4.71 ± 1.06 (N = 12)	↓6.67 ± 0.89 (N = 12)	↓4.74 ± 0.62 (N = 10)
Week 27	6.87 ± 1.89 (N = 11)	4.27 ± 0.93 (N = 10)	↑7.01 ± 1.01 (N = 12)	↑4.43 ± 1.13 (N = 12)	↑6.99 ± 1.64 (N = 12)	↓4.05 ± 1.61 (N = 12)	↑6.89 ± 1.34 (N = 12)	↓4.24 ± 1.18 (N = 10)
Week 53	5.77 ± 1.18 (N = 23)	4.17 ± 1.02 (N = 24)	↑5.85 ± 1.30 (N = 23)	↑4.30 ± 1.06 (N = 23)	↓5.30 ± 0.94 (N = 24)	↑4.30 ± 1.26 (N = 24)	↑5.78 ± 0.62 (N = 23)	↓3.87 ± 0.78 (N = 20)
Neutrophil count [x109/l]**								
Week 14	1.49 ± 0.51 (N = 12)	1.20 ± 0.45 (N = 12)	↑1.58 ± 0.35 (N = 12)	↑1.61 ± 1.38 (N = 12)	↑1.35 ± 0.38 (N = 12)	↓1.06 ± 0.35 (N = 12)	↑1.32 ± 0.32 (N = 12)	↓0.99 ± 0.32 (N = 10)
Week 27	2.08 ± 0.72 (N = 11)	1.23 ± 0.51 (N = 10)	↑2.13 ± 0.74 (N = 12)	↑1.44 ± 0.34 (N = 12)	↑1.85 ± 0.56 (N = 12)	↓1.20 ± 0.60 (N = 12)	↑1.94 ± 0.51 (N = 12)	↓1.11 ± 0.30 (N = 10)
Week 53	2.11 ± 0.80 (N = 23)	1.29 ± 0.43 (N = 24)	↓1.99 ± 0.67 (N = 23)	↑1.37 ± 0.47 (N = 23)	↑1.39** ± 0.36 (N = 24)	↑1.36 ± 0.49 (N = 24)	↑1.80* ± 0.75 (N = 23)	↓1.14 ± 0.43 (N = 20)
Lymphocyte count [x109/l]**								
Week 14	4.81 ± 1.12 (N = 12)	3.23 ± 0.73 (N = 12)	↑5.07 ± 1.07 (N = 12)	↑3.45 ± 1.03 (N = 12)	↑4.91 ± 0.88 (N = 12)	↑3.39 ± 0.88 (N = 12)	↑4.87 ± 0.83 (N = 12)	↑3.50 ± 0.55 (N = 10)

Table 5.5-62 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted] 1996): Intergroup comparison of haematology

	Dose group (ppm)							
	0		2000		8000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
Week 27	4.24 ± 1.22 (N = 11)	2.72 ± 0.50 (N = 10)	↑4.26 ± 0.74 (N = 12)	↓2.63 ± 0.92 (N = 12)	↑4.60 ± 0.88 (N = 12)	↓2.54 ± 1.00 (N = 12)	↑4.39 ± 1.00 (N = 12)	↑2.85 ± 0.91 (N = 10)
Week 53	3.09 ± 0.61 (N = 23)	2.40 ± 0.67 (N = 24)	↑3.26 ± 0.70 (N = 23)	↑2.41 ± 0.80 (N = 23)	↑3.35 ± 0.67 (N = 24)	↑2.49 ± 0.77 (N = 24)	↑3.40 ± 0.76 (N = 23)	↓2.28 ± 0.50 (N = 20)
Monocyte count [x109/1]**								
Week 14	0.130 ± 0.051 (N = 12)	0.101 ± 0.027 (N = 12)	↓0.128 ± 0.033 (N = 12)	↑0.112 ± 0.066 (N = 12)	↓0.119 ± 0.038 (N = 12)	↓0.090 ± 0.035 (N = 12)	↑0.142 ± 0.035 (N = 12)	↓0.091 ± 0.026 (N = 10)
Week 27	0.181 ± 0.087 (N = 11)	0.118 ± 0.047 (N = 10)	↑0.240* ± 0.106 (N = 12)	↑0.138 ± 0.083 (N = 12)	↑0.207 ± 0.094 (N = 12)	↑0.121 ± 0.085 (N = 12)	↑0.221 ± 0.097 (N = 12)	↓0.113 ± 0.041 (N = 10)
Week 53	0.209 ± 0.100 (N = 23)	0.194 ± 0.102 (N = 24)	↑0.232 ± 0.135 (N = 23)	↑0.234 ± 0.156 (N = 23)	↑0.227 ± 0.154 (N = 24)	↓0.180 ± 0.109 (N = 24)	↑0.226 ± 0.138 (N = 23)	↓0.172 ± 0.109 (N = 20)
Eosinophil count [x109/1]**								
Week 14	0.222 ± 0.064 (N = 12)	0.164 ± 0.083 (N = 12)	↓0.200 ± 0.030 (N = 12)	↓0.155 ± 0.052 (N = 12)	↓0.207 ± 0.071 (N = 12)	↓0.114* ± 0.028 (N = 12)	↑0.245 ± 0.095 (N = 12)	↓0.124 ± 0.052 (N = 10)
Week 27	0.268 ± 0.123 (N = 11)	0.145 ± 0.041 (N = 10)	↓0.248 ± 0.077 (N = 12)	↑0.158 ± 0.065 (N = 12)	↓0.238 ± 0.076 (N = 12)	↓0.126 ± 0.049 (N = 12)	↓0.245 ± 0.095 (N = 12)	↓0.115 ± 0.027 (N = 10)
Week 53	0.187 ± 0.145 (N = 23)	0.092 ± 0.031 (N = 24)	↓0.156 ± 0.064 (N = 23)	↑0.113 ± 0.102 (N = 23)	↓0.141* ± 0.028 (N = 24)	↑0.108 ± 0.056 (N = 24)	↓0.161 ± 0.052 (N = 23)	↓0.072 ± 0.029 (N = 20)
Basophil count [x109/1]**								
Week 14	0.007 ± 0.005 (N = 12)	0.001 ± 0.003 (N = 12)	↓0.006 ± 0.007 (N = 12)	↑0.002 ± 0.004 (N = 12)	↓0.006 ± 0.005 (N = 12)	↓0.003 ± 0.005 (N = 12)	↓0.005 ± 0.005 (N = 12)	0.001 ± 0.003 (N = 10)
Week 27	0.008 ± 0.006 (N = 11)	0.000 ± 0.000 (N = 10)	0.008 ± 0.004 (N = 12)	↑0.002 ± 0.004 (N = 12)	↓0.005 ± 0.007 (N = 12)	↑0.001 ± 0.003 (N = 12)	↓0.006 ± 0.005 (N = 12)	↑0.001 ± 0.003 (N = 10)
Week 53	0.016 ± 0.008 (N = 23)	0.019 ± 0.016 (N = 24)	0.016 ± 0.008 (N = 23)	0.019 ± 0.010 (N = 23)	↓0.015 ± 0.008 (N = 24)	↓0.016 ± 0.010 (N = 24)	↓0.015 ± 0.007 (N = 23)	↑0.021 ± 0.020 (N = 20)
Large unstained cells [x109/1]**								
Week 14	0.067 ± 0.026 (N = 12)	0.043 ± 0.021 (N = 12)	↑0.073 ± 0.034 (N = 12)	↑0.052 ± 0.054 (N = 12)	↓0.060 ± 0.027 (N = 12)	↓0.033 ± 0.013 (N = 12)	↑0.072 ± 0.026 (N = 12)	↓0.036 ± 0.013 (N = 10)
Week 27	0.099 ± 0.032 (N = 11)	0.054 ± 0.017 (N = 10)	↑0.111 ± 0.031 (N = 12)	↓0.048 ± 0.019 (N = 12)	↓0.095 ± 0.044 (N = 12)	↓0.048 ± 0.022 (N = 12)	↓0.088 ± 0.026 (N = 12)	↓0.046 ± 0.016 (N = 10)
Week 53	0.169 ± 0.060 (N = 23)	0.173 ± 0.089 (N = 24)	↑0.181 ± 0.067 (N = 23)	↓0.157 ± 0.053 (N = 23)	↓0.163 ± 0.047 (N = 24)	↓0.143 ± 0.066 (N = 24)	↑0.173 ± 0.061 (N = 23)	↑0.185 ± 0.162 (N = 20)
Prothrombin time [sec]								
Week 53	17.9 ± 1.2 (N = 21)	18.9 ± 3.2 (N = 22)	↓17.5 ± 1.2 (N = 23)	18.9 ± 2.9 (N = 21)	↓17.5 ± 1.1 (N = 21)	↓18.8 ± 2.9 (N = 23)	↑18.1 ± 0.9 (N = 21)	↑19.6 ± 2.4 (N = 18)
Kaolin-cephalin time [sec]								
Week 53	17.1 ±	19.3 ± 2.6	↑17.2 ±	↑20.1 ±	17.1 ±	↑21.0* ±	↑17.2 ±	↑20.6 ±

Table 5.5-62 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted] 1996): Intergroup comparison of haematology

	Dose group (ppm)							
	0		2000		8000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
	1.1 (N = 21)	(N = 22)	1.3 (N = 23)	3.5 (N = 21)	1.1 (N = 21)	3.0 (N = 23)	0.6 (N = 21)	3.3 (N = 18)

* Statistically significant from control ($p \leq 0.05$; Student's t-test, two-sided)
** Statistically significant from control ($p \leq 0.01$; Student's t-test, two-sided)

Clinical chemistry
Plasma cholesterol and plasma triglycerides were marginally reduced in males receiving 20000 or 8000 ppm at Weeks 14 and 27.

Moreover, there was a treatment- and dose-related increase in plasma ALP activity throughout the study. For rats receiving 2000 ppm glyphosate acid the increase was marginal and was statistically significant only for females at Week 14. The increase in the activity of plasma ALP in animals at all dose levels was compound-related but as there was no accompanying pathological change in either the liver or bone this is considered not to be of toxicological significance.

All other differences from control were small and/or were not dose-related and are considered to be incidental to administration of glyphosate acid.

Table 5.5-63 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted] 1996): Clinical chemistry findings

	Dose group (ppm)							
	0		2000		8000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
Plasma urea [mmol/L]								
Week 14	8.2 ± 0.7 (N = 12)	9.1 ± 0.9 (N = 12)	↑8.3 ± 0.7 (N = 11)	↓8.8 ± 0.7 (N = 12)	8.2 ± 0.5 (N = 11)	↓8.8 ± 0.7 (N = 12)	↓7.8 ± 0.5 (N = 12)	↓8.8 ± 0.5 (N = 10)
Week 27	7.9 ± 0.7 (N = 12)	8.8 ± 0.9 (N = 12)	↑8.1 ± 0.8 (N = 11)	↓8.5 ± 0.6 (N = 12)	↓7.6 ± 0.7 (N = 12)	↑9.2 ± 1.0 (N = 12)	↓7.8 ± 0.7 (N = 12)	↓8.4 ± 0.5 (N = 10)
Week 53	6.5 ± 0.8 (N = 23)	7.1 ± 1.1 (N = 24)	↑6.8 ± 0.8 (N = 23)	↑7.2 ± 1.3 (N = 23)	↑6.9 ± 1.6 (N = 24)	↓7.0 ± 1.0 (N = 24)	↓6.3 ± 0.6 (N = 23)	↑7.4 ± 0.8 (N = 20)
Plasma creatinine [µmol/L]								
Week 14	102.1 ± 90.0 (N = 12)	61.8 ± 3.0 (N = 12)	↓61.8** ± 2.3 (N = 11)	↓60.7 ± 3.9 (N = 12)	↓60.6** ± 3.6 (N = 11)	↑62.0 ± 3.2 (N = 12)	↓60.7** ± 2.5 (N = 12)	↓60.8 ± 2.8 (N = 10)
Week 27	66.8 ± 6.0 (N = 12)	65.3 ± 4.1 (N = 12)	↓64.4* ± 5.0 (N = 11)	↓64.4 ± 3.1 (N = 12)	↓61.1** ± 4.7 (N = 12)	65.3 ± 2.6 (N = 12)	↓63.4** ± 3.6 (N = 12)	↓63.6 ± 4.0 (N = 10)
Week 53	62.3 ± 5.2 (N = 23)	57.9 ± 5.6 (N = 24)	↑62.9 ± 6.7 (N = 23)	↑58.8 ± 4 (N = 23)	↑63.2 ± 8.9 (N = 24)	↓55.2 ± 5.0 (N = 24)	↓59.6 ± 3.9 (N = 23)	↓55.8 ± 5.2 (N = 20)
Plasma glucose [mmol/L]								
Week 14	8.28 ± 1.69 (N = 12)	7.90 ± 1.05 (N = 12)	↓7.73 ± 0.65 (N = 11)	↑7.94 ± 1.38 (N = 12)	↓7.73 ± 0.86 (N = 11)	↓7.73 ± 0.52 (N = 12)	↓7.65 ± 0.69 (N = 12)	↑8.15 ± 0.81 (N = 10)
Week 27	7.83 ± 1.08	8.00 ± 0.94	↓7.54 ± 0.57	↑8.10 ± 1.18	↓7.51 ± 1.10	↓7.93 ± 0.88	↑7.92 ± 0.72	↑7.89 ± 0.96

Table 5.5-63 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted], 1996): Clinical chemistry findings

	Dose group (ppm)							
	0		2000		8000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
	(N = 12)	(N = 12)	(N = 11)	(N = 12)	(N = 12)	(N = 12)	(N = 12)	(N = 10)
Week 53	9.60 ± 1.70 (N = 23)	11.45 ± 2.69 (N = 24)	↑9.81 ± 2.08 (N = 23)	↑11.91 ± 2.66 (N = 23)	↑10.82* ± 2.13 (N = 24)	↓10.90 ± 2.02 (N = 24)	↓9.13 ± 1.41 (N = 23)	↑12.80 ± 3.10 (N = 20)
Plasma albumin [g/L]								
Week 14	37.1 ± 1.1 (N = 12)	37.8 ± 2.3 (N = 12)	↓36.5 ± 1.4 (N = 11)	↓37.3 ± 1.9 (N = 12)	↑37.7 ± 1.6 (N = 11)	↓37.1 ± 2.1 (N = 12)	↑37.3 ± 2.2 (N = 12)	↓36.9 ± 2.0 (N = 10)
Week 27	34.3 ± 1.8 (N = 12)	36.7 ± 1.4 (N = 12)	↓33.8 ± 1.1 (N = 11)	↓35.9 ± 1.5 (N = 12)	34.3 ± 0.9 (N = 24)	36.7 ± 2.2 (N = 12)	34.3 ± 0.9 (N = 12)	↓36.4 ± 1.8 (N =10)
Week 53	28.1 ± 2.1 (N = 23)	34.4 ± 1.9 (24)	↑28.7 ± 2.2 (N = 23)	↓33.7 ± 1.9 (N = 23)	↑29.3 ± 2.2 (N = 24)	↑34.8 ± 1.7 (N = 24)	↑29.3 ± 1.9 (N = 23)	↑34.5 ± 2.1 (N = 20)
Plasma total protein [g/L]								
Week 14	71.9 ± 1.2 (N = 12)	69.0 ± 2.6 (N = 12)	↓70.6 ± 2.1 (N = 11)	↑69.3 ± 2.3 (N = 12)	↓71.8 ± 1.8 (N = 11)	↓68.8 ± 1.8 (N = 12)	↓71.4 ± 2.4 (N = 12)	↓68.4 ± 2.4 (N = 10)
Week 27	74.3 ± 3.0 (N = 12)	73.8 ± 2.8 (N = 12)	↓72.5 ± 1.8 (N = 11)	↓72.9 ± 2.2 (N = 12)	↓74.0 ± 1.3 (N = 12)	↑74.3 ± 3.7 (N = 12)	↓73.7 ± 1.6 (N = 12)	73.8 ± 2.9 (N = 10)
Week 53	65.6 ± 2.8 (N = 23)	70.5 ± 3.5 (N = 24)	↑66.6 ± 3.2 (N = 23)	↓69.4 ± 3.9 (N = 23)	↑67.3 ± 2.7 (N = 24)	↓70.1 ± 4.6 (N = 24)	↑65.9 ± 2.6 (N = 23)	↑70.7 ± 4.2 (N = 20)
Plasma cholesterol [mmol/L]								
Week 14	2.46 ± 0.21 (N = 12)	2.13 ± 0.30 (N = 12)	↑2.53 ± 0.30 (N = 11)	↑2.28 ± 0.29 (N = 12)	↓2.31 ± 0.31 (N = 11)	↑2.26 ± 0.28 (N = 12)	↓2.28* ± 0.27 (N = 12)	↑2.21 ± 0.17 (N = 10)
Week 27	3.09 ± 0.40 (N = 12)	2.62 ± 0.38 (N = 12)	↓3.05 ± 0.47 (N = 11)	↑2.67 ± 0.39 (N = 12)	↓2.75* ± 0.32 (N = 12)	↑2.76 ± 0.34 (N = 12)	↓2.70** ± 0.30 (N = 12)	↑2.78 ± 0.44 (N = 10)
Week 53	4.53 ± 1.15 (N = 23)	3.15 ± 0.97 (N = 24)	↑4.64 ± 1.35 (N = 23)	↓2.84 ± 0.63 (N = 23)	↑4.57 ± 2.08 (N = 24)	3.15 ± 0.59 (N = 24)	↓4.07 ± 1.36 (N = 23)	↑3.22 ± 0.41 (N = 20)
Plasma triglycerides [mmol/L]								
Week 14	1.56 ± 0.34 (N = 12)	0.94 ± 0.19 (N = 12)	↑1.63 ± 0.26 (N = 11)	↓0.92 ± 0.34 (N = 12)	↓1.28** ± 0.21 (N = 11)	↓0.89 ± 0.24 (N = 12)	↓1.28** ± 0.37 (N = 12)	↑0.95 ± 0.21 (N = 10)
Week 27	1.51 ± 0.42 (N = 12)	1.07 ± 0.29 (N = 12)	↓1.43 ± 0.31 (N = 11)	↑1.10 ± 0.30 (N = 12)	↓1.15** ± 0.22 (N = 12)	↑1.13 ± 0.29 (N = 12)	↓0.97** ± 0.24 (N = 12)	↑1.10 ± 0.41 (N = 10)
Week 53	1.70 ± 0.54 (N = 23)	1.11 ± 0.54 (N = 24)	↑1.74 ± 0.60 (N = 23)	↓0.92 ± 0.20 (N = 23)	↑1.74 ± 0.92 (N = 24)	↓0.99 ± 0.28 (N = 24)	↑1.78 ± 0.83 (N = 23)	↑1.21 ± 0.54 (N = 20)
Plasma total bilirubin [mmol/L]								
Week 14	1.92 ± 0.51 (N = 12)	1.83 ± 0.58 (N = 12)	↑2.18 ± 0.60 (N = 11)	↑1.92 ± 0.29 (N = 12)	↑2.18 ± 0.87 (N = 11)	↑2.08 ± 0.51 (N = 12)	↑2.33* ± 0.65 (N = 12)	↑2.10 ± 0.57 (N = 10)
Week 27	2.17 ± 0.83 (N = 12)	2.17 ± 0.72 (N = 12)	↑2.18 ± 0.40 (N = 11)	↓1.75* ± 0.45 (N = 12)	↑2.33 ± 0.65 (N = 12)	↑2.25 ± 0.45 (N = 12)	↑2.50 ± 0.80 (N = 12)	↓2.10 ± 0.57 (N = 10)
Week 53	2.04 ±	2.46 ±	↓2.00 ±	↑2.48 ±	↓1.83 ±	↑2.58 ±	↑2.43* ±	↑2.65 ±

Table 5.5-63 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted], 1996): Clinical chemistry findings

	Dose group (ppm)							
	0		2000		8000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
	0.56 (N = 23)	0.59 (N = 24)	0.30 (N = 23)	0.79 (N = 23)	0.48 (N = 24)	0.65 (N = 24)	0.84 (N = 23)	0.67 (N = 20)
Plasma alkaline phosphatase (IU/L)								
Week 14	248 ± 35 (N = 12)	161 ± 47 (N = 12)	↑281 ± 47 (N = 11)	↑201 ± 71* (N = 12)	↑342 ± 67** (N = 11)	↑227 ± 31** (N = 12)	↑429 ± 85** (N = 12)	↑292 ± 69** (N = 10)
Week 27	221 ± 38 (N = 12)	135 ± 45 (N = 12)	↑250 ± 37 (N = 11)	↑171 ± 81 (N = 12)	↑306 ± 55** (N = 12)	↑200 ± 39** (N = 12)	↑412 ± 108** (N = 12)	↑254 ± 66** (N = 10)
Week 53	232 ± 89 (N = 23)	87 ± 42 (N = 12)	↑258 ± 61 (N = 23)	↑100 ± 34 (N = 12)	↑291 ± 54** (N =24)	↑114 ± 38 (N = 24)	↑379 ± 101** (N = 23)	↑160 ± 77** (N = 19)
Plasma gamma-glutamyl transferase (IU/L)								
Week 14	1.5 ± 1.2 (N = 12)	0.8 ± 0.9 (N = 12)	↓1.1 ± 0.8 (N = 11)	↑1.1 ± 0.9 (N = 12)	↓1.1 ± 0.5 (N = 11)	↑1.3 ± 1.0 (N = 12)	↓0.5** ± 0.5 (N = 12)	0.8 ± 1.0 (N = 10)
Week 27	1.1 ± 0.8 (N = 12)	0.6 ± 0.5 (N = 12)	↓0.7 ± 0.5 (N = 11)	0.6 ± 0.7 (N = 12)	↓0.8 ± 0.8 (N = 12)	↑0.9 ± 0.5 (N = 12)	↓0.4* ± 0.5 (N = 12)	↑0.9 ± 0.9 (N = 10)
Week 53	3.4 ± 2.1 (N = 23)	2.0 ± 1.2 (N = 23)	↑3.9 ± 2.5 (N = 23)	↓1.8 ± 1.5 (N = 23)	↓2.6* ± 1.6 (N = 24)	↓1.9 ± 1.1 (N = 24)	↓2.7 ± 2.0 (N = 23)	↓1.6 ± 1.0 (N = 20)
Plasma alanine aminotransferase (IU/L)								
Week 14	84.3 ± 12.0 (N = 12)	66.2 ± 16.9 (N = 12)	↑92.8 ± 13.6 (N = 11)	↑79.3 ± 38.9 (N = 12)	↑110.9** ± 10.1 (N = 11)	↑88.2** ± 16.5 (N = 12)	↑109.6** ± 23.1 (N = 12)	↑90.5** ± 20.2 (N = 10)
Week 27	98.3 ± 14.6 (N = 12)	87.3 ± 33.3 (N = 12)	↑94.1 ± 25.9 (N = 12)	↓83.8 ± 19.3 (N = 12)	↑100.8 ± 29.8 (N = 12)	↑87.4 ± 27.6 (N = 12)	↑110.5 ± 18.6 (N = 23)	↑93.4 ± 18.3 (N = 10)
Week 53	87.3 ± 49.4 (N = 23)	74.8 ± 42.6 (N = 24)	↑89.0 ± 36.2 (N = 23)	↑89.7 ± 46.5 (N = 23)	↑91.8 ± 26.3 (N = 24)	↑104.6* ± 54.1 (N = 24)	↑102.1 ± 49.2 (N = 23)	↑80.1 ± 44.8 (N = 20)
Plasma aspartate aminotransferase (IU/L)								
Week 14	97.5 ± 17.8 (N = 12)	90.4 ± 18.4 (N = 12)	↓92.4 ± 15.2 (N = 11)	↑103.4 ± 47.4 (N = 12)	↓91.6 ± 7.6 (N = 11)	↓89.4 ± 13.8 (N = 12)	↑101.9 ± 17.7 (N = 12)	↑92.7 ± 10.0 (N = 10)
Week 27	120.0 ± 29.7 (N = 12)	157.1 ± 85.3 (N = 12)	↓105.3 ± 27.0 (N = 11)	↓130.4 ± 58.4 (N = 12)	↓108.4 ± 38.0 (N = 12)	↓116.9* ± 49.1 (N = 12)	↓113.3 ± 22.3 (N = 12)	↓132.8 ± 37.4 (N = 10)
Week 53	122.1 ± 57.8 (N = 23)	133.9 ± 64.2 (N = 24)	↑123.0 ± 47.5 (N = 23)	↑178.7* ± 99.4 (N = 23)	↓118.3 ± 28.9 (N = 24)	↑198.3** ± 103.7 (N = 24)	↑132.0 ± 86.3 (N = 23)	↑138.3 ± 86.7 (N = 20)
Plasma creatine kinase (IU/L)								
Week 14	118.2 ± 14.9 (N = 12)	96.7 ± 16.5 (N = 12)	↑123.5 ± 18.9 (N = 11)	↑107.5 ± 24.6 (N = 12)	↑127.3 ± 21.5 (N = 11)	↑107.3 ± 15.4 (N = 12)	↑143.7** ± 32.9 (N = 12)	↑124.1** ± 19.0 (N = 10)
Week 27	96.6 ± 25.7 (N = 12)	77.3 ± 17.7 (N = 12)	↓89.1 ± 8.3 (N = 11)	↑79.7 ± 13.6 (N = 12)	↑103.4 ± 35.4 (N = 12)	↑85.4 ± 16.0 (N = 12)	↓93.1 ± 13.7 (N = 12)	↑107.9** ± 20.9 (N = 10)
Week 53	118.3 ± 40.8 (N = 23)	101.3 ± 27.6 (N = 24)	↓116.7 ± 23.2 (N = 23)	↑117.5 ± 48.8 (N = 23)	↑138.2 ± 50.5 (N = 24)	↑281.6* ± 828.1 (N = 24)	↑133.1 ± 33.2 (N = 23)	↑139.4 ± 101.7 (N = 20)

Table 5.5-63 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted] 1996): Clinical chemistry findings

	Dose group (ppm)							
	0		2000		8000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
Plasma sodium (mmol/L)								
Week 14	142.5 ± 1.9 (N = 12)	142.4 ± 1.7 (N = 12)	↓142.1 ± 0.8 (N = 11)	↑143.4 ± 1.8 (N = 12)	↓142.2 ± 1.7 (N = 11)	↑143.0 ± 1.5 (N = 12)	↑143.8* ± 1.6 (N = 12)	↓142.1 ± 1.1 (N = 10)
Week 27	142.6 ± 0.9 (N = 12)	142.3 ± 1.5 (N = 12)	↓141.9 ± 1.2 (N = 11)	↓142.2 ± 1.0 (N = 12)	142.6 ± 1.4 (N = 12)	↓142.2 ± 1.5 (N = 12)	142.6 ± 0.7 (N = 12)	↓142.2 ± 1.3 (N = 10)
Week 53	146.0 ± 1.9 (N = 23)	143.8 ± 1.6 (N = 22)	↑146.1 ± 2.3 (N = 23)	↑145.0** ± 2.1 (N = 22)	↑146.3 ± 1.5 (N = 12)	↑144.0 ± 1.9 (N = 22)	↓145.6 ± 2.2 (N = 22)	↑144.4 ± 2.2 (N = 17)
Plasma potassium (mmol/L)								
Week 14	4.60 ± 0.26 (N = 12)	4.55 ± 0.25 (N = 12)	↑4.85 ± 0.34 (N = 11)	↓4.50 ± 0.35 (N = 12)	↑4.63 ± 0.18 (N = 11)	↓4.47 ± 0.37 (N = 12)	↑4.64 ± 0.35 (N = 12)	↓4.36 ± 0.21 (N = 10)
Week 27	4.43 ± 0.36 (N = 12)	4.19 ± 0.31 (N = 12)	↑4.53 ± 0.29 (N = 11)	↓4.08 ± 0.38 (N = 12)	↑4.48 ± 0.26 (N = 12)	↓4.13 ± 0.26 (N = 12)	↑4.46 ± 0.40 (N = 12)	↓4.07 ± 0.39 (N = 10)
Week 53	4.53 ± 0.84 (N = 23)	4.75 ± 0.78 (N = 22)	↑4.63 ± 0.65 (N = 23)	↑4.82 ± 0.69 (N = 22)	↑4.87 ± 0.82 (N = 23)	↓4.34 ± 0.76 (N = 22)	↑4.67 ± 0.81 (N = 22)	↓4.51 ± 0.87 (N = 17)
Plasma chloride (mmol/L)								
Week 14	101.3 ± 1.9 (N = 12)	103.7 ± 1.1 (N = 12)	↑101.8 ± 1.8 (N = 11)	↑104.4 ± 1.3 (N = 12)	↑101.8 ± 1.3 (N = 11)	↑103.9 ± 0.9 (N = 12)	↑101.9 ± 1.1 (N = 12)	↓102.8 ± 1.1 (N = 10)
Week 27	101.2 ± 1.5 (N = 12)	102.5 ± 1.8 (N = 12)	↑101.6 ± 1.0 (N = 11)	↑102.7 ± 1.7 (N = 12)	↓101.0 ± 2.0 (N = 23)	↑102.9 ± 1.6 (N = 12)	↑101.3 ± 1.2 (N = 12)	↓102.3 ± 1.3 (N = 10)
Week 53	106.2 ± 2.7 (N = 23)	104.6 ± 2.6 (N = 22)	↓105.9 ± 2.3 (N = 23)	↑105.4** ± 2.9 (N = 22)	↓105.5 ± 2.0 (N = 23)	↑105.4* ± 2.4 (N = 22)	↓105.5 ± 2.1 (N = 22)	↓104.3 ± 2.4 (N = 17)
Plasma calcium (mmol/L)								
Week 14	2.88 ± 0.04 (N = 12)	2.79 ± 0.06 (N = 12)	↓2.84 ± 0.04 (N = 11)	↓2.78 ± 0.05 (N = 12)	↓2.81** ± 0.05 (N = 11)	2.79 ± 0.05 (N = 12)	↓2.86 ± 0.04 (N = 12)	↑2.75* ± 0.04 (N = 10)
Week 27	2.82 ± 0.07 (N = 12)	2.81 ± 0.06 (N = 12)	↓2.78* ± 0.05 (N = 11)	↓2.75** ± 0.04 (N = 12)	↓2.78 ± 0.04 (N = 12)	↓2.76* ± 0.07 (N = 12)	↓2.80 ± 0.05 (N = 12)	↓2.74** ± 0.07 (N = 10)
Week 53	2.86 ± 0.10 (N = 23)	2.99 ± 0.16 (N = 23)	↑2.90 ± 0.11 (N = 23)	↓2.94 ± 0.14 (N = 23)	↑2.94** ± 0.14 (N = 24)	↓2.88** ± 0.13 (N = 24)	↓2.85 ± 0.09 (N = 23)	↓2.92 ± 0.16 (N = 20)
Plasma phosphorus (mmol/L)								
Week 14	1.96 ± 0.17 (N = 12)	1.33 ± 0.18 (N = 12)	↓1.88 ± 0.26 (N = 11)	↑1.59** ± 0.24 (N = 12)	↓1.82 ± 0.26 (N = 11)	↑1.48 ± 0.31 (N = 12)	↑2.09 ± 0.26 (N = 12)	↑1.74** ± 0.21 (N = 10)
Week 27	1.48 ± 0.16 (N = 12)	1.29 ± 0.13 (N = 12)	↑1.50 ± 0.11 (N = 11)	↓1.18 ± 0.20 (N = 12)	↑1.55 ± 0.10 (N = 12)	↓1.15* ± 0.28 (N = 12)	↑1.73** ± 0.21 (N = 12)	↑1.39* ± 0.20 (N = 10)
Week 53	1.37 ± 0.36 (N = 23)	1.94 ± 0.42 (N = 24)	↑1.49 ± 0.36 (N = 23)	↑1.97 ± 0.59 (N = 23)	↑1.64** ± 0.39 (N = 24)	↓1.62** ± 0.26 (N = 24)	↑1.56* ± 0.26 (N = 23)	↓1.82 ± 0.26 (N = 20)

* Statistically significant from control ($p \leq 0.05$; Student's t-test, two-sided)
** Statistically significant from control ($p \leq 0.01$; Student's t-test, two-sided)

G. URINALYSIS

There were no consistent treatment- and dose-related effects seen in the any urinary parameters.

Table 5.5-64 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted], 1996): Urine clinical chemistry

	Dose group (ppm)							
	0		2000		8000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
Urine volume [mL]								
Week 13	8.75 ± 1.91 (N = 12)	4.71 ± 1.54 (N = 12)	↓7.55 ± 2.25 (N = 11)	↓4.38 ± 1.52 (N = 12)	↓6.83** ± 1.63 (N = 12)	↓3.71 ± 1.36 (N = 12)	↓5.63** ± 1.72 (N = 12)	↓3.95 ± 1.50 (N = 10)
Week 26	9.50 ± 2.27 (N = 12)	4.04 ± 1.59 (N = 12)	↓7.36** ± 1.96 (N = 12)	↑4.21 ± 1.45 (N = 12)	↓6.63** ± 1.46 (N = 12)	↓3.38 ± 1.55 (N = 12)	↓6.21** ± 1.75 (N = 12)	↓3.65 ± 1.33 (N = 10)
Week 52	7.88 ± 2.06 (N = 12)	5.29 ± 1.70 (N = 12)	↓7.79 ± 2.78 (N = 12)	↓4.54 ± 1.81 (N = 12)	↓6.58 ± 1.59 (N = 12)	↓4.25 ± 1.41 (N = 12)	↓5.88* ± 1.86 (N = 12)	↓4.05 ± 1.48 (N = 10)
Urine specific gravity								
Week 13	1.038 ± 0.006 (N = 12)	1.047 ± 0.014 (N = 12)	↑1.041 ± 0.009 (N = 11)	↑1.048 ± 0.013 (N = 12)	↑1.041 ± 0.005 (N = 12)	↑1.048 ± 0.012 (N = 12)	↑1.049* ± 0.011 (N = 12)	↑1.049 ± 0.009 (N = 10)
Week 26	1.038 ± 0.005 (N = 12)	1.053 ± 0.017 (N = 12)	↑1.042 ± 0.009 (N = 11)	↑1.048 ± 0.010 (N = 12)	↑1.046 ± 0.010 (N = 12)	↓1.051 ± 0.013 (N 0 12)	↑1.043 ± 0.008 (N = 12)	↓1.049 ± 0.004 (N = 10)
Week 52	1.044 ± 0.006 (N = 12)	1.044 ± 0.009 (N = 12)	↓1.041 ± 0.006 (N = 12)	↑1.051 ± 0.015 (N = 12)	↑1.048 ± 0.012 (N = 12)	↑1.047 ± 0.009 (N = 12)	↑1.049 ± 0.009 (N = 12)	↑1.047 ± 0.009 (N = 10)
Urine [pH]								
Week 13	6.42 ± 0.51 (N = 12)	5.75 ± 0.45 (N = 12)	↓6.36 ± 0.50 (N = 12)	↑5.92 ± 0.29 (N = 12)	↓6.33 ± 0.49 (N = 12)	↑5.92 ± 0.67 (N = 12)	↓6.00* ± 0.00 (N = 12)	↓5.40 ± 0.52 (N = 10)
Week 26	6.08 ± 0.29 (N = 12)	5.08 ± 0.29 (N = 12)	↑6.18 ± 0.40 (N = 11)	↑5.50* ± 0.52 (N = 12)	6.08 ± 0.29 (N = 12)	↑5.67** ± 0.49 (N = 12)	↓5.92 ± 0.29 (N = 12)	↑5.30 ± 0.48 (N = 10)
Week 52	6.00 ± 0.00 (N = 12)	5.67 ± 0.49 (N = 12)	↑6.33 ± 0.49 (N = 12)	↓5.58 ± 0.51 (N = 12)	↑6.08 ± 0.67 (N = 12)	5.67 ± 0.49 (N = 12)	↓5.75 ± 0.62 (N = 12)	↓5.40 ± 0.52 (N = 10)

* Statistically significant from control ($p \leq 0.05$; Student's t-test, two-sided)
** Statistically significant from control ($p \leq 0.01$; Student's t-test, two-sided)

H. NECROPSY

Gross pathology

There were no treatment-related macroscopic effects.

Organ weights

There were no treatment- and dose-related effects on organ weights when corrected for bodyweight.

Table 5.5-65 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted] 1996): Organ weights

Dose group	Nominal dose (ppm)	Weight	ORG/TBW [%]	Organ weight adjusted for bodyweight
Adrenal glands (males)				
1 (control)	0	0.0063 ± 0.011 (N = 23)	0.010 ± 0.002	0.063
2 (low)	2000	↓0.058 ± 0.011 (N = 23)	0.009 ± 0.002	0.058
3 (mid)	8000	↓0.062 ± 0.012 (N = 24)	0.010 ± 0.002	0.062
4 (high)	20000	0.063 ± 0.011 (N = 23)	0.010 ± 0.002	0.063
Adrenal glands (females)				
1 (control)	0	0.080 ± 0.012 (N = 24)	0.023 ± 0.004	0.080
2 (low)	2000	↑0.084 ± 0.011 (N = 23)	0.024 ± 0.004	0.084
3 (mid)	8000	↓0.074 ± 0.013 (N = 24)	0.023 ± 0.004	0.075
4 (high)	20000	↓0.075 ± 0.014 (N = 20)	0.023 ± 0.005	0.075
Brain (males)				
1 (control)	0	2.21 ± 0.10 (N = 23)	0.34 ± 0.03	2.21
2 (low)	2000	↓2.19 ± 0.08 (N = 23)	0.34 ± 0.03	2.20
3 (mid)	8000	↓2.17 ± 0.13 (N = 24)	0.34 ± 0.02	2.17
4 (high)	20000	2.21 ± 0.07 (N = 23)	0.35 ± 0.03	2.22
Brain (females)				
1 (control)	0	2.02 ± 0.08 (N = 23)	0.59 ± 0.05	2.01
2 (low)	2000	2.02 ± 0.07 (N = 23)	0.59 ± 0.04	2.01
3 (mid)	8000	↓1.98 ± 0.07 (N = 24)	0.61 ± 0.05	1.99
4 (high)	20000	↓1.99 ± 0.08 (N = 20)	0.61 ± 0.06	2.00
Epididymides (males)				
1 (control)	0	1.507 ± 0.138 (N = 23)	0.232 ± 0.022 (N = 23)	1.515
2 (low)	2000	↓1.437* ± 0.149 (N = 23)	0.225 ± 0.032 (N = 23)	1.433*
3 (mid)	8000	↓1.448 ± 0.210 (N = 24)	0.227 ± 0.035 (N = 24)	1.449
4 (high)	20000	↓1.469 ± 0.159 (N = 23)	0.234 ± 0.035 (N = 23)	1.469
Kidneys (males)				
1 (control)	0	4.17 ± 0.59 (N = 24)	0.64 ± 0.09	4.11
2 (low)	2000	↓4.09 ± 0.60 (N = 23)	0.64 ± 0.08	4.10
3 (mid)	8000	↓4.06 ± 0.60	0.63 ± 0.08	4.07

Table 5.5-65 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted] 1996): Organ weights

Dose group	Nominal dose (ppm)	Weight	ORG/TBW [%]	Organ weight adjusted for bodyweight
		(N = 24)		
4 (high)	20000	↓4.02 ± 0.44 (N = 23)	0.64 ± 0.05	4.05
Kidneys (females)				
1 (control)	0	2.48 ± 0.16 (N = 24)	0.72 ± 0.06	2.46
2 (low)	2000	↑2.52 ± 0.14 (N = 23)	0.73 ± 0.05	2.49
3 (mid)	8000	↓2.26* ± 0.20 (N = 24)	0.72 ± 0.06	2.38
4 (high)	20000	↓2.44 ± 0.26 (N = 20)	0.74 ± 0.07	2.45
Liver (males)				
1 (control)	0	22.7 ± 2.8 (N = 23)	3.5 ± 0.4	22.3
2 (low)	2000	↓22.2 ± 3.5 (N = 23)	3.4 ± 0.4	22.3
3 (mid)	8000	↓22.4 ± 3.3 (N = 24)	3.5 ± 0.5	22.4
4 (high)	20000	↓21.9 ± 2.5 (N = 23)	3.5 ± 0.2	22.2
Liver (females)				
1 (control)	0	12.3 ± 1.7 (N = 24)	3.5 ± 0.4	12.0
2 (low)	2000	↓12.1 ± 1.3 (N = 23)	3.5 ± 0.3	11.9
3 (mid)	8000	↓11.3* ± 1.4 (N = 24)	3.4 ± 0.3	11.6
4 (high)	20000	↓12.1 ± 1.9 (N = 20)	3.7 ± 0.3	12.3
Testis (males)				
1 (control)	0	3.68 ± 0.21 (N = 23)	0.57 ± 0.06	3.68
2 (low)	2000	↓3.60 ± 0.11 (N = 23)	0.56 ± 0.11	3.58
3 (mid)	8000	↓3.52 ± 0.4 (N = 24)	0.55 ± 0.07	3.52
4 (high)	20000	↑3.72 ± 0.28 (N = 23)	0.59 ± 0.07	3.70

Histopathology

An increased incidence and severity of focal basophilia of the acinar cells of the parotid salivary gland were seen in both sexes receiving 20000 ppm glyphosate acid. This effect was also evident in females receiving 8000 ppm. This change was considered to be related to treatment and consequently the salivary glands of the 8000 ppm dose group were examined. The examples of focal parotid basophilia seen at this dose were all of minimal severity and the incidence was comparable to that in the control group.

All other observed differences in the incidence of findings are considered to be unrelated to the treatment with glyphosate acid in view of the spontaneous incidence in this strain. No treatment-related neoplasms

were found.

Table 5.5-66 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted] 1996): Non-neoplastic findings (intercurrent)

Effect		Dose group (ppm)							
		0		2000		8000		20000	
		♂	♀	♂	♀	♂	♀	♂	♀
Epididymides									
Reduced spermatozoa	Total	0	-	0	-	0	-	1	-
	Marked	0	-	0	-	0	-	1	-
Increased sperm precursor cells	Total	0	-	0	-	0	-	1	-
	Slight	0	-	0	-	0	-	1	-
Kidneys									
Chronic progressive glomerulo-nephropathy	Total	0	0	0	0	0	0	1	0
	Minimal	0	0	0	0	0	0	1	0
Liver									
Proliferative cholangitis	Total	0	0	0	0	0	0	1	0
	Minimal	0	0	0	0	0	0	1	0
Congestion	Total	1	0	0	0	0	0	0	0
	Moderate	1	0	0	0	0	0	0	0
Hepatocyte necrosis	Total	1	0	0	0	0	0	0	0
	Slight	1	0	0	0	0	0	0	0
Increased pigmentation	Total	0	0	0	0	0	0	1	0
	Slight	0	0	0	0	0	0	1	0
Pituitary gland									
Cyst/s	Total	1	0	0	0	0	0	0	0
	Marked	0	0	0	0	0	0	1	0
Tail									
Trauma		1	0	0	0	0	0	0	0
Testis									
Bilateral tubular degeneration	Total	0	-	0	-	0	-	1	-
	Marked	0	-	0	-	0	-	1	-
Interstitial oedema	Total	0	-	0	-	0	-	1	-
	Marked	0	-	0	-	0	-	1	-

Table 5.5-67 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted], 1996): Non-neoplastic findings (terminal)

Effect		Dose group (ppm)							
		0		2000		8000		20000	
		♂	♀	♂	♀	♂	♀	♂	♀
Adrenal gland									
Vascular ectasia	Total	1	14	0	0	0	0	0	6
	Minimal	1	3	0	0	0	0	0	1
	Slight	0	6	0	0	0	0	0	2
	Moderate	0	4	0	0	0	0	0	3
	Marked	0	1	0	0	0	0	0	0
Cortical congestion/haemorrhage	Total	1	4	0	0	0	0	0	4
	Slight	1	1	0	0	0	0	0	2
	Moderate	0	3	0	0	0	0	0	2
Cortical fat vacuolation	Total	4	0	0	0	0	0	0	0
	Minimal	2	0	0	0	0	0	0	0

Table 5.5-67 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted] 1996): Non-neoplastic findings (terminal)

Effect		Dose group (ppm) 0 ♂	0 ♀	2000 ♂	2000 ♀	8000 ♂	8000 ♀	20000 ♂	20000 ♀
	Slight	2	0	0	0	0	0	0	0
Cortical pigmentation	Total	1	0	0	0	0	0	0	0
	Minimal	1	0	0	0	0	0	0	0
Cervix									
Increased mucification	Total	-	1	-	0	-	0	-	1
	Slight	-	1	-	0	-	0	-	1
Duodenum									
Lymphoid proliferation	Total	0	0	0	0	0	0	1	0
	Slight	0	0	0	0	0	0	1	0
Ear/Zymbals gland									
Fatty nodule pinna		0	0	0	0	0	0	1	0
Harderian gland									
Mononuclear cell infiltration	Total	3	4	0	0	0	0	2	5
	Minimal	3	4	0	0	0	0	2	5
Acinar dilatation	Total	1	0	0	0	0	0	0	0
	Minimal	1	0	0	0	0	0	0	0
Heart									
Degenerative cardiomyopathy	Total	4	0	0	0	0	0	4	0
	Minimal	4	0	0	0	0	0	4	0
Endocrinal cell proliferation	Total	1	0	0	0	0	0	0	0
	Moderate	1	0	0	0	0	0	0	0
Kidneys									
Unilateral hydronephrosis	Total	3	0	0	0	0	0	2	0
	Minimal	1	0	0	0	0	0	1	0
	Slight	2	0	0	0	0	0	1	0
Chronic progressive glomerulonephropathy	Total	23	6	0	0	0	0	22	7
	Minimal	9	5	0	0	0	0	10	7
	Slight	9	1	0	0	0	0	8	0
	Moderate	5	0	0	0	0	0	4	0
Eosinophilic casts	Total	0	7	0	0	0	0	0	4
	Minimal	0	6	0	0	0	0	0	4
	Slight	0	1	0	0	0	0	0	0
Intratubular microlithiasis	Total	0	22	0	0	0	0	0	18
	Minimal	0	4	0	0	0	0	0	11
	Slight	0	10	0	0	0	0	0	5
	Moderate	0	8	0	0	0	0	0	2
Pelvic urolithiasis	Total	2	0	0	0	0	0	0	0
	Slight	1	0	0	0	0	0	0	0
	Moderate	1	0	0	0	0	0	0	0
Transitional epithelial hyperplasia	Total	2	0	0	0	0	0	0	0
	Slight	2	0	0	0	0	0	0	0
Tubular atrophy	Total	0	1	0	0	0	0	0	0
	Minimal	0	1	0	0	0	0	0	0
Tubular pigmentation	Total	0	0	0	0	0	0	0	2
	Minimal	0	0	0	0	0	0	0	2
Interstitial mononuclear cell infiltration	Total	0	1	0	0	0	0	0	0
	Minimal	0	1	0	0	0	0	0	0
Cyst/s		2	0	0	0	0	0	0	0
Liver									
Proliferative cholangitis	Total	12	10	0	0	0	0	11	19
	Minimal	12	10	0	0	0	0	10	18

Table 5.5-67 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted], 1996): Non-neoplastic findings (terminal)

Effect		Dose group (ppm) 0 ♂	0 ♀	2000 ♂	2000 ♀	8000 ♂	8000 ♀	20000 ♂	20000 ♀
	Slight	0	0	0	0	0	0	1	1
Hepatitis	Total	1	0	0	0	0	0	0	0
	Minimal	1	0	0	0	0	0	0	0
Spongiosis hepatis	Total	2	0	0	0	0	0	0	0
	Minimal	2	0	0	0	0	0	0	0
Hepatocyte necrosis	Total	14	4	0	0	0	0	16	4
	Minimal	8	4	0	0	0	0	10	2
	Slight	6	0	0	0	0	0	6	1
	Moderate	0	0	0	0	0	0	0	1
Altered hepatocytes – clear cell	Total	2	0	0	0	0	0	1	0
	Minimal	2	0	0	0	0	0	1	0
Increased pigmentation	Total	1	0	0	0	0	0	1	2
	Minimal	1	0	0	0	0	0	1	2
Infarction lobe/s		1	0	0	0	0	0	0	0
Lung									
Lymphoid proliferation	Total	1	1	0	0	0	0	0	0
	Slight	1	1	0	0	0	0	0	0
Alveolar macrophage infiltration	Total	1	0	0	0	0	0	0	0
	Minimal	1	0	0	0	0	0	0	0
Alveolitis	Total	0	1	0	0	0	0	0	0
	Slight	0	1	0	0	0	0	0	0
Haemorrhage	Total	2	0	0	0	0	0	1	1
	Minimal	1	0	0	0	0	0	1	1
	Slight	1	0	0	0	0	0	0	0
Vascular mineralisation	Total	0	1	0	0	0	0	0	1
	Slight	0	1	0	0	0	0	0	1
Pleurisy	Total	1	0	0	0	0	0	0	0
	Slight	1	0	0	0	0	0	0	0
Lymph node - cervical									
Cystic degeneration	Total	1	2	0	0	0	0	4	0
	Slight	0	0	0	0	0	0	2	0
	Moderate	1	1	0	0	0	0	1	0
	Marked	0	1	0	0	0	0	1	0
Blood filled sinuses	Total	0	2	0	0	0	0	0	0
	Slight	0	1	0	0	0	0	0	0
	Moderate	0	1	0	0	0	0	0	0
Pigmented macrophages	Total	0	1	0	0	0	0	0	0
	Slight	0	1	0	0	0	0	0	0
Lymph node - mesenteric									
Blood filled sinuses	Total	1	0	0	0	0	0	0	0
	Moderate	1	0	0	0	0	0	0	0
Dilated sinuses	Total	0	1	0	0	0	0	0	0
	Slight	0	1	0	0	0	0	0	0
Haemangiomatous change	Total	0	0	0	0	0	0	1	0
	Moderate	0	0	0	0	0	0	1	0
Mammary gland									
Secretory activity	Total	-	5	-	0	-	0	-	2
	Slight	-	1	-	0	-	0	-	2
	Moderate	-	4	-	0	-	0	-	0
Diffuse hyperplasia	Total	-	2	-	0	-	0	-	0
	Slight	-	2	-	0	-	0	-	0

Table 5.5-67 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted] 1996): Non-neoplastic findings (terminal)

Effect		Dose group (ppm) 0 ♂	0 ♀	2000 ♂	2000 ♀	8000 ♂	8000 ♀	20000 ♂	20000 ♀
Oesophagus									
Luminal dilatation	Total	0	1	0	0	0	0	0	0
	Moderate	0	1	0	0	0	0	0	0
Oral cavity									
Malocclusion (macroscopic observation)		1	0	0	0	0	0	0	0
Ovary									
Cystic bursa/e		-		-		-		-	
Pancreas									
Mononuclear cell infiltration	Total	1	1	0	0	0	0	1	1
	Minimal	1	1	0	0	0	0	0	0
	Slight	0	0	0	0	0	0	1	1
Increased pigmented macrophages	Total	1	0	0	0	0	0	2	0
	Minimal	1	0	0	0	0	0	1	0
	Slight	0	0	0	0	0	0	1	0
Exocrine atrophy	Total	0	1	0	0	0	0	1	2
	Minimal	0	1	0	0	0	0	0	1
	Slight	0	0	0	0	0	0	1	1
Dilated ducts	Total	1	0	0	0	0	0	0	0
	Slight	1	0	0	0	0	0	0	0
Pituitary gland									
Focal cellular alteration		2	1	0	0	0	0	1	1
Cyst/s		10	3	0	0	0	0	5	4
Vascular ectasia with pigmentation	Total	0	0	0	0	0	0	0	2
	Slight	0	0	0	0	0	0	0	1
	Moderate	0	0	0	0	0	0	0	1
Prostate gland									
Prostatitis	Total	2	-	0	-	0	-	9	-
	Minimal	1	-	0	-	0	-	4	-
	Slight	0	-	0	-	0	-	4	-
	Marked	1	-	0	-	0	-	1	-
Rectum									
Luminal dilatation	Total	0	1	0	0	0	0	0	0
	Moderate	0	1	0	0	0	0	0	0
Inflammation	Total	1	0	0	0	0	0	0	0
	Moderate	1	0	0	0	0	0	0	0
Salivary glands									
Mononuclear cell infiltration	Total	0	2	0	0	0	0	1	1
	Minimal	0	2	0	0	0	0	1	1
Atrophy	Total	0	0	0	0	0	0	1	0
	Marked	0	0	0	0	0	0	1	0
Interstitial fibrosis	Total	0	0	0	0	0	0	1	0
	Marked	0	0	0	0	0	0	1	0
Basophilia of parotid acinar cells	Total	2	2	0	0	3	6	13	15
	Minimal	2	2	0	0	3	6	10	8
	Slight	0	0	0	0	0	0	3	5
	Moderate	0	0	0	0	0	0	0	2
Sciatic nerve									
Demyelination	Total	13	5	0	0	0	0	10	4
	Minimal	13	5	0	0	0	0	10	4
Skin									

Table 5.5-67 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted] 1996): Non-neoplastic findings (terminal)

Effect		Dose group (ppm) 0 ♂	0 ♀	2000 ♂	2000 ♀	8000 ♂	8000 ♀	20000 ♂	20000 ♀
		0	0	0	0	0	0	0	1
Granuloma/ta		1	0	0	0	0	0	0	0
Reduced hair follicles	Total	1	0	0	0	0	0	0	1
	Slight	0	0	0	0	0	0	0	1
	Moderate	1	0	0	0	0	0	0	0
Spleen									
Spindle cell proliferation	Total	0	0	0	0	0	0	1	0
	Moderate	0	0	0	0	0	0	1	0
Siderofibrosis	Total	1	0	0	0	0	0	0	0
	Slight	1	0	0	0	0	0	0	0
Stomach									
Hyperplasia non-glandular epithelium	Total	1	1	0	0	0	0	1	0
	Slight	1	0	0	0	0	0	1	0
	Moderate	0	1	0	0	0	0	0	0
Subcutaneous tissue									
Cyst/s		0	0	0	0	0	0	1	0
Tail									
Acanthosis/hyperkeratosis	Total	1	0	0	0	0	0	0	0
	Moderate	1	0	0	0	0	0	0	0
Dermatitis	Total	2	0	0	0	0	0	0	0
	Moderate	2	0	0	0	0	0	0	0
Inflammation	Total	1	2	0	0	0	0	0	0
	Moderate	0	2	0	0	0	0	0	0
	Marked	1	0	0	0	0	0	0	0
Malformation (macroscopic observation)		2	1	0	0	0	0	0	1
Trauma (macroscopic observation)		4	0	0	0	0	0	0	1
Testis									
Interstitial oedema	Total	0	-	0	-	0	-	1	-
	Minimal	0	-	0	-	0	-	1	-
Thymus									
Congestion/haemorrhage	Total	0	0	0	0	0	0	1	0
	Slight	0	0	0	0	0	0	1	0
Cyst/s		2	6	0	0	0	0	0	4
Epithelial hyperplasia	Total	0	1	0	0	0	0	0	0
	Minimal	0	1	0	0	0	0	0	0
Thyroid gland									
Squamous cyst/s		0	0	0	0	0	0	1	0
Urinary bladder									
Transitional epithelial hyperplasia	Total	1	0	0	0	0	0	0	0
	Slight	1	0	0	0	0	0	0	0
Uterus									
Glandular dilatation	Total	-	1	-	0	-	0	-	3
	Slight	-	0	-	0	-	0	-	2
	Moderate	-	1	-	0	-	0	-	1
Luminal dilatation	Total	-	6	-	0	-	0	-	4
	Slight	-	3	-	0	-	0	-	1
	Moderate	-	3	-	0	-	0	-	3
Squamous metaplasia glands	Total	-	0	-	0	-	0	-	1
	Marked	-	0	-	0	-	0	-	1
Endometritis	Total	-	0	-	0	-	0	-	3

Table 5.5-67 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted] 1996): Non-neoplastic findings (terminal)

Effect		Dose group (ppm) 0 ♂	0 ♀	2000 ♂	2000 ♀	8000 ♂	8000 ♀	20000 ♂	20000 ♀
	Minimal	-	0	-	0	-	0	-	2
	Slight	-	0	-	0	-	0	-	1
Endometrial hyperplasia	Total	-	1	-	0	-	0	-	0
	Slight	-	1	-	0	-	0		0
Cystic endometrial hyperplasia	Total	-	0	-	0	-	0	-	1
	Minimal	-	0	-	0	-	0	-	1

Table 5.5-68 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted] 1996): Neoplastic findings (intercurrent)

Effect	Dose group (ppm) 0 ♂	0 ♀	2000 ♂	2000 ♀	8000 ♂	8000 ♀	20000 ♂	20000 ♀
Adrenal gland								
Cortical adenocarcinoma (malignant)	1	0	0	0	0	0	0	0
Brain								
Astrocytoma (malignant)	0	0	0	0	0	0	1	0

Table 5.5-69 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted] 1996): Neoplastic findings (terminal)

Effect	Dose group (ppm) 0 ♂	0 ♀	2000 ♂	2000 ♀	8000 ♂	8000 ♀	20000 ♂	20000 ♀
Lymph node - mesenteric								
Haemangioma (benign)	0	0	0	0	0	0	1	0
Pituitary gland								
Adenoma (benign)	0	6	0	0	0	0	1	2
Spleen								
Haemangioma (benign)	1	0	0	0	0	0	0	0
Subcutaneous tissue								
Fibroma (benign)	0	0	0	0	0	0	1	0
Lipoma (benign)	1	0	0	0	0	0	0	0
Tail								
Fibrosarcoma (malignant)	1	0	0	0	0	0	0	0
Thyroid gland								
Follicular cell adenoma (benign)	0	0	0	0	0	0	0	1
Parafollicular cell adenoma (benign)	1	0	0	0	0	0	0	0
Uterus								
Stromal cell polyp (benign)	-	1	-	0	-	0	-	2

3. Assessment and conclusion

Assessment and conclusion by applicant:

Based on body weight and salivary gland effects at 20000 ppm, the NOAEL for toxicity for glyphosate acid was 8000 ppm equivalent to 560 mg/kg bw/day in males and 671 mg/kg bw/day in females. There was no evidence of carcinogenicity.At the mid dose, effects on the acinar cells of the parotid salivary gland were slightly increased (minimal severity) in females only. These effects when occurring at a minimal severity are generally considered adaptive being due to pH changes of the glyphosate acid. The body weight reduction was neither dose nor time-dependent. The effects on body weight was furthermore rather low at the mid dose level (<10 %) at most time points. The higher alkaline phosphatase activity at 8000 and 20000 ppm is not considered an adverse effect as no other enzyme activities were affected indicative of organ damage. Furthermore, histopathology did not indicate any damage of organs, e.g. liver. No indication of neoplastic changes was observed.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.5/007 CA 5.5/008 CA 5.5/009
Report author	[redacted]
Report year	1993
Report title	Glyphosate – 104 week combined chronic feeding/oncogenicity study in rats with 52 week interim kill (results after 104 weeks)
Report No	7867
Document No	153-GLY
Guidelines followed in study	US-EPA Pesticide Assessment Guidelines Subdivision F, 83-5 (1982); in general compliance with OECD 453
Deviations from current test guideline (OECD 453, 2018)	Yes, haematological examination of the mean corpuscular volume (MCV), the mean corpuscular haemoglobin (MHC), mean corpuscular haemoglobin concentration (MCHC), prothrombin time and activated partial thromboplastin time (APTT) were not performed; urinalysis was performed without determining glucose; organ weight of the thyroid/parathyroid was not determined; histopathological examinations were performed without Harderian gland, cervix, coagulating glands, gall bladder, gross lesions, lacrimal glands, upper respiratory tract, seminal vesicles and vagina.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The chronic toxicity and carcinogenic potential of glyphosate technical was assessed in a 104-week feeding study in male and female Sprague-Dawley rats. Groups of 50 rats per sex received daily dietary doses of 0,

10, 100, 300 or 1000 mg/kg bw/day glyphosate technical for 24 months. In addition, five groups of 35 rats/sex, receiving daily dietary doses of, 0, 10, 100, 300 or 1000 mg/kg bw/day, were included for interim sacrifice at the 12th month for evaluation of chronic toxicity. Observations covered clinical signs, body weight, food and water consumption, ophthalmoscopy, haematology, clinical chemistry and urinalysis, as well as organ weights, necropsy and histopathological examination.

Achieved doses throughout the study period were generally close to nominal. There were no treatment-related deaths or clinical signs in any of the dose-groups. Ophthalmoscopic examinations showed no inter-group differences. At 1000 mg/kg bw/day males and females had statistically significant reductions in body weight throughout the study. Reductions started at week one of dosing and were still apparent at week 104. The high-dose group males displayed the greatest reduction in body weights. Food and water consumption did not differ significantly from the controls. Moreover, there were no treatment-related changes in haematological parameters. Clinical chemistry evaluation indicated a treatment-related increase of ALP in males of the 1000 mg/kg bw/day dose group and females of the 100, 300 and 1000 mg/kg bw/day dose groups, as well as reduced urinary pH in males at 1000 mg/kg bw/day.

At necropsy no treatment-related gross lesions were observed. Organ weight data showed reduced liver weights in females at 100, 300 and 1000 mg/kg bw/day at interim kill in week 52, but not after 104 weeks. At week 52 salivary gland weights were increased in 100, 300 and 1000 mg/kg bw/day dose group males. Combined sublingual and submaxillary gland weights were also increased in males and females treated with 1000 mg/kg bw/day. However there were no significant inter-group differences by week 104. Histopathological examination noted cellular alteration of in submaxillary and parotid salivary glands in males and females of the 300 and 1000 mg/kg bw/day dose groups (week 52) and the 100, 300 and 1000 mg/kg bw/day dose groups of both sexes at week 104. These changes followed a dose-related pattern and are considered treatment related. However, these cellular alterations are similar to those seen occasionally in other subchronic or long-term studies and are considered to be an adaptive response to acidic diet and are of no adverse consequence.

No treatment-related neoplastic lesions were observed at termination.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate technical
Description:	White powder
Lot/Batch #:	229-JaK-5-1; 229-Jak-142-6
Purity:	98.9 %; 98.7 %
Stability of test compound:	At least two years at ambient temperature in the dark
2. Vehicle and/ or positive control:	Diet
3. Test animals:	
Species:	Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	Approx. 4 weeks upon arrival at testing facility
Sex:	Males and females
Weight at dosing:	Males: 208 ± 1.9 g, females: 148.5 ± 1.6 g

Acclimation period:	14 days
Diet/Food:	SQC Expanded (Fine Ground) Rat and Mouse Maintenance Diet No. 1 (Special Diet Services Limited, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	In groups of five per sex in suspended polypropylene cages with stainless steel wire grid tops and bottoms
Environmental conditions:	Temperature: 20 ± 2 °C Humidity: 55 ± 10 % Air changes: 15 - 20 / hour 12 hours light/dark cycle

B: Study design and methods

In life dates: 1990-02-16 to 1992-03-09

Animal assignment and treatment:
In a combined chronic toxicity and carcinogenicity study groups of 50 Sprague-Dawley rats per sex received daily dietary doses of 0, 10, 100, 300 or 1000 mg/kg bw/day glyphosate technical. An additional five groups with 35 rats per sex receiving daily dietary doses of 0, 10, 100, 300 or 1000 mg/kg bw/day were included for the toxicity study. Fifteen rats per sex and per dose of the toxicity study were scheduled for interim sacrifice after 12 months. The dose levels were selected based on the results of a 13-week dietary toxicity study in rats.

Test diets were prepared once per week for the first 13 weeks and at least once every two weeks thereafter by direct admixture of the test substance to the plain diet and mixing for 20 minutes.

Analyses for achieved concentrations of the test substance in the diet were conducted from formulated diets at approximately fortnight intervals for the first 12 weeks and in intervals of 2 month thereafter.

The stability and homogeneity of the test substance in the diet was determined prior to the start of the study.

Clinical observations
A check for mortality was made twice daily on all animals throughout the study. In addition, all animals were examined for clinical signs during each day. A detailed clinical examination and check for palpable masses were done once each week on every animal. An ophthalmoscopic examination was conducted on 20 rats per sex of each group of the oncogenicity study before the start of the study and on 20 rats per sex of the control and high-dose group of the oncogenicity study at weeks 24 and 50. In addition, an ophthalmoscopic examination was conducted on all control and high-dose rats of the oncogenicity and toxicity study at week 102.

Body weight
Individual body weights were recorded for each animal before dosing, at weekly intervals until the end of week 13 and approximately every 4 weeks thereafter until termination.

Food and water consumption and compound intake
Food consumption was recorded once weekly for each cage group starting one week before treatment until Week 13 and subsequently every 4 weeks until termination. Water consumption was monitored by visual inspection throughout the study period.

Achieved dosages were calculated from nominal dietary concentration, taking into account actual food consumption and body weight data.

Haematology and clinical chemistry
Individual blood samples for haematology and clinical chemistry evaluations were collected from the orbital sinus of 10 rats/sex of each study group of the toxicity study after approximately 14, 25, 51, 78 and 102 weeks. Samples were taken where possible, on the same animals at each time point. Individual blood smears for differential blood counts were taken from the tail vein after approximately 52, 78, and 103 weeks of dosing from all surviving animals of the oncogenicity study.

Haematology
The following parameters were measured: Haemoglobin, haematocrit, total erythrocyte count total leukocyte count, differential leukocyte count, platelets, and clotting time. Absolute indices were calculated. Differential blood counts were evaluated with blood smear samples from all control and high-dose animals of the oncogenicity study at weeks 53 and 79. In addition, differential blood cell counts were evaluated on all surviving animals of the oncogenicity study at week 104.

Blood chemistry
The following parameters were measured: Total proteins, albumin, albumin-globulin ratio, ALT, AST, ALP, blood urea nitrogen, blood glucose, sodium, potassium, chloride, cholesterol, creatinine, calcium, phosphate, total bilirubin, plasma cholinesterase, creatine phosphokinase and red blood cell cholinesterase.

Brain cholinesterase activity determination
Brain cholinesterase activity was determined from 10 rats per sex from each dose group at the week 52 and 104 necropsies. Approximately 0.5 g of brain was removed at the week 52 and 104 necropsies and stored at -20 °C until analysis.

Urinalysis
Individual urine samples were collected from 10 rats/sex of each study group of the toxicity study after approximately 14, 25, 51, 78 and 102 weeks. Samples were taken where possible, on the same animals at each time point. Samples were collected over a period of 4 hours of food and water deprivation in metabolism cages. The following measurements were made: volume, specific gravity, pH, urobilinogen, bilirubin, blood pigments, protein, glucose, ketones, microscopy of sediments.

Sacrifice and pathology
At interim kill after 52 weeks 15 rats per sex from each toxicity study group were sacrificed and necropsied. All remaining toxicity study and surviving oncogenicity study animals were killed and necropsied after 104 weeks. All pre-terminally dead and moribund sacrificed rats were also necropsied.

The following organs were weighed from all interim kill animals of the toxicity study and from 10 rats per sex per group of the oncogenicity study: adrenals, brain, heart, kidneys, liver, lungs, ovaries (with fallopian tubes), parotid salivary glands, pituitary, prostrate, sublingual and submaxillary salivary glands (weighed together), spleen, testes including epididymides, thymus and uterus.

The following organs were collected: adrenals, aortic arch, any abnormal tissue, bladder, bone and bone marrow (sternum and rib), brain, ears, eyes, intestine (duodenum, jejunum, ileum, caecum, colon, rectum), kidneys, liver, lungs, mammary gland, lymph nodes (mesenteric and submandibular), muscle (thigh), nasal cavity (oncogenicity study only), oesophagus, optic nerve, ovaries (with fallopian tubes), pancreas, parotid salivary glands, pituitary, prostrate, sciatic nerve, seminal vesicles, skin, spinal cord (cervical, thoracic and lumbar), spleen, stomach (glandular and non-glandular), sublingual salivary glands, submaxillary salivary glands, testes with epididymides, thymus, thyroid/parathyroid, tongue, trachea, uterus and vagina.

A detailed histopathological examination was performed on all tissues collected from the control and high-dose animals at interim kill, all oncogenicity study animals, and all animals that died or were killed in extremis. In addition, a histopathological examination of the liver, kidneys and lungs was performed on all other toxicity study animals at interim kill and all oncogenicity study animals. Furthermore, the salivary glands of all low- and mid-dose animals at interim kill and the oncogenicity study were examined.

Statistics
Haematology, clinical chemistry, organ weight and body weight data were analysed for homogeneity of variance using the F-max test. If the group variances appeared homogeneous a parametric ANOVA was used and pair wise comparisons made *via* Student's t-test using Fisher´s F-protected LSD. If the variances were heterogeneous log or square root, transformations were used. If the variances remained heterogeneous a non-parametric test (e.g. Kruskal-Wallis ANOVA) was used. Organ weights were also analysed conditional on body weight (i.e. ANOVA). Differences in survival between the control and test substance groups from the oncogenicity study were assessed graphically using Kaplan-Meier plots and tested formally using the Gehan-Wilcoxon test. Because no notable survival differences were evident, histological lesion incidences were analysed using Fisher Exact test.

II. RESULTS

A. ANALYSIS OF DOSE FORMULATIONS
Analyses for achieved concentrations showed that the diet preparations of all dose groups were within an acceptable degree of accuracy (± 10 %).

B. MORTALITY
There were 336 pre-terminal deaths throughout the study. There was no evidence to suggest that any of these deaths were treatment-related. There were also no significant treatment-related effects on the survival times in the oncogenicity study.

The numbers of pre-terminal deaths are summarised in the table below.

Table 5.5-70 Glyphosate – 104 week combined chronic feeding / oncogenicity study in rats with 52 week interim kill (results after 104 weeks) ([redacted] 1993): Cumulated mortalities after 104-week dietary exposure to glyphosate technical

Sex	Dose group (mg/kg bw/day)*				
	0	10	100	300	1000
Male	27/85	32/85	25/85	26/85	26/85
Female	42/85	41/85	42/85	40/85	35/85

* Number of dead / total number

C. CLINICAL OBSERVATIONS
The only notable clinical sign was pale faeces, from weeks 16-104, the majority or all the cages of animals (males and females) in the 300 and 1000 mg/kg /day dose groups had pale faeces. However, this clinical sign was not considered to be toxicologically significant. There were no other notable clinical signs considered to be treatment related.

Ophthalmoscopy examinations demonstrated no inter-group differences.

D. BODY WEIGHT
The high-dose group males and females had statistically significant reductions in body weight throughout the study. Reductions started at week one of dosing and were still apparent at week 104. The high-dose group males displayed the greatest reduction in body weights and body weight gains. The mean body weight gain data are summarised in the table below.

Table 5.5-71 Glyphosate – 104 week combined chronic feeding / oncogenicity study in rats with 52 week interim kill (results after 104 weeks) ([redacted] 1993): Body weight development (mean values) after 52 and 104-week dietary exposure to glyphosate technical – oncogenicity study

	Dose group (mg/kg bw/day)									
	0		10		100		300		1000	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Weight gain (g) (0-52 weeks)	514	265	↓498	↑285	↑523	↑270	↓500	↑274	↓450	↓243
% of control	--	--	97	108	102	102	97	103	88	92
Weight gain (g) 0-104 weeks	635	376	↓609	↑445	↑644	↑391	↓623	↑405	↓549	↓333
% of control	--	--	96	118	101	104	98	108	86	89

E. FOOD AND WATER CONSUMPTION AND COMOUND INTAKE

There were no treatment-related effects on food and water consumption for either sex noted during the study.
The overall group mean achieved doses are summarised in the table below.

Table 5.5-72 Glyphosate – 104 week combined chronic feeding / oncogenicity study in rats with 52 week interim kill (results after 104 weeks) ([redacted] 1993): Group mean achieved dose levels – oncogenicity study

Dose group	Nominal dose (mg/kg bw/day)	Mean achieved dose level (mg/kg bw/day)		Mean achieved dose level (% of nominal)	
		Males	Females	Males	Females
1 (control)	0	--	--	--	--
2 (low)	10	10	10	100	100
3 (mid I)	100	101	103	101	103
4 (mid II)	300	306	311	102	104
5 (high)	1000	1007	1018	101	102

Over the entire study duration the mean achieved dosages in all groups were close to the nominal.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

Haemoglobin, haematocrit and mean corpuscular haemoglobin were occasionally increased in 100 and 1000 mg/kg bw/day dose group males. Haemoglobin was also increased in males from the 300 mg/kg bw/day dose group and females from the 1000 mg/kg bw/day group. Females of the 1000 mg/kg bw/day dose group also had increased levels of mean corpuscular haemoglobin.

The haematological changes were not considered to be treatment related due to the lack of a clear dose–response relationship. In addition, the differences observed were rather small and no consistent trend became obvious throughout the study. In the absence of any histopathological change these small increases are not considered to be of toxicological significance (see table below).

Table 5.5-73 Glyphosate – 104 week combined chronic feeding / oncogenicity study in rats with 52 week interim kill (results after 104 weeks) ([redacted] 1993): Haematology findings (group mean values)

	Dose group (mg/kg bw/day)									
	0		10		100		300		1000	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Haemoglobin (g/dL)										
Week 14/15	15.6	15.5	↑15.8	↓15.0*	↑16.2	↓15.0*	↑16.2	15.5	↑16.2	↑15.9
Week 25/26	15.3	15.2	↑15.5	↓14.9	↑16.1***	↓14.9	↑15.9*	↑15.4	↑16.4***	↑15.6
Week 51/52	15.3	14.7	↑15.5	↓14.6	↑15.9	↓14.5	↑15.4	14.7	↑15.6	↑15.3*
Week 78/79	15.1	14.1	↓14.3	↓13.8	↑15.7	↑14.4	↓14.6	↑14.4	↑15.4	↑15.1
Week 102/103	14.0	12.1	↓13.1	↑13.6	↑14.3	↑13.1	↓13.8	↑13.3	↑14.6	↑12.9
Haematocrit (L/L)										
Week 14/15	0.397	0.396	↑0.405	↓0.386	↑0.406	↓0.387	↑0.407	↓0.395	↑0.411	↑0.407
Week 25/26	0.388	0.392	↑0.389	↓0.389	↑0.409**	↓0.384	↑0.399	↑0.398	↑0.409**	↑0.403
Week 51/52	0.406	0.394	↑0.415	↓0.388	↑0.415	↓0.386	↑0.410	↓0.392	↑0.414	↑0.408
Week 78/79	0.405	0.382	↓0.386	↓0.375	↑0.415	0.382	↓0.392	↑0.387	↑0.411	↑0.406
Week 102/103	0.392	0.343	↓0.365	↑0.381	↑0.394	↑0.367	↓0.387	↑0.369	↑0.401	↑0.363
MCH (pg)										
Week 14/15	21.3	22.6	↓21.1	↓22.5	↑21.7	↓22.4	↑21.9	↓22.4	↑21.8	↑22.8
Week 25/26	21.2	22.4	↑21.4	22.4	↑21.9	22.4	↑21.9	↓22.2	↑22.0	↑22.8
Week 51/52	20.2	22.1	↓20.1	↑22.3	↑21.1*	22.1	↑20.8	↑22.2	↑20.9*	↑22.7
Week 78/79	20.1	22.3	↓19.7	↑22.4	↑20.8*	↑22.4	↑20.6	↑23.0	↑20.9*	↑23.1**
Week 102/103	20.4	22.3	↓20.1	22.3	↑20.1	↓22.0	↑20.9	↑22.6	↑20.6	↑22.7
WBC (x 10-9/L)										
Week 14/15	14.0	12.0	↑14.5	↑13.3	↓13.4	12.0	↓13.7	↓11.1	↑14.2	↓12.0
Week 25/26	13.4	8.8	↓13.2	↑10.3	↓11.8	↑9.9	↓12.2	↑8.9	↓12.7	↑10.5
Week 51/52	12.8	7.9	↑13.7	↑9.1	↓11.7	↓7.7	↑12.9	↓7.4	↓12.4	↑8.8
Week 78/79	12.4	7.7	↑13.6	↓7.3	↓10.9	↑8.1	↑13.6	↓6.8	↓10.6	↓7.0
Week 102/103	10.5	10.1	↑12.2	↓7.1*	↓10.3	↓6.4**	↑11.6	↓7.3*	↓9.5	↓8.4
Lymphocytes (x 10-9/L)										
Week 14/15	11.7	10.8	↑12.6	↑11.9	↑12.0	↑10.9	↑11.8	↓9.2	↑12.2	↓10.7
Week 25/26	10.7	7.1	↑10.8	↑8.2	↓9.6	↑8.1	↓10.1	↑7.4	↓10.3	↑8.6
Week 51/52	10.9	6.5	↑11.0	↑7.4	↓9.7	↑6.6	↓10.8	↓6.0	↓10.3	↑7.5
Week 78/79	10.0	5.7	↑10.3	↓5.6	↓8.7	↑6.4	↑10.1	↓4.8	↓8.5	↓5.6
Week 102/103	7.6	5.7	↑8.0	↓4.8	↓7.3	↓4.3**	↑7.8	↓4.7*	↓6.7	↓5.6

*: $p < 0.05$
**: $p < 0.01$
***: $p < 0.001$

Clinical chemistry

Clinical chemistry measurements showed significant increased alkaline phosphatase levels in males at 1000 mg/kg bw/day and in females at 100, 300 and 1000 mg/kg bw/day. Although the increases were of small magnitude they were consistent and might be treatment-related. However, in the absence of any histopathological changes these small changes are not considered to be of toxicological significance. All other changes in clinical chemistry parameters were not considered to be treatment-related.

Table 5.5-74 Glyphosate – 104 week combined chronic feeding / oncogenicity study in rats with 52 week interim kill (results after 104 weeks) ([redacted] 1993): Clinical chemistry findings (group mean values)

	Dose group (mg/kg bw/day)									
	0		10		100		300		1000	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
ALP (IU/L)										
Week 14	287	182	↑329	↓158	↑320	↑213	↑334	↑223	↑461***	↑244*
Week 25	251	148	↑272	↑152	↑267	↑201*	↑306	↑227**	↑367**	↑225**
Week 51	308	144	↓293	↓143	↑310	↑190*	↑353	↑195*	↑403	↑221**
Week 78	258	124	↑286	↑139	↑284	↑172	↑351*	↑207**	↑414***	↑186*
Week 102	212	190	↑265	↓161	↑287*	↑193	↑267	↑228	↑365***	↑286*

*: $p < 0.05$
**; $p < 0.01$
***: $p < 0.001$

G. URINALYSIS

Urinary pH was slightly reduced in males at 1000 mg/kg bw/day. This change was consistent with that found in a previously conducted 13-week toxicity study with glyphosate.

H. NECROPSY

Gross pathology

There were no treatment-related macroscopic findings observed at the interim and terminal kill necropsies.

Organ weights

At the interim kill (week 52) absolute liver weights were reduced in males and females at doses of 100 mg/kg bw/day and above. However, for males this finding was not confirmed by the sensitive means of covariance analysis, i.e. with correction for final body weight. Absolute adrenal weights were reduced in males at 300 and 1000 mg/kg bw/day. However, this finding was also not confirmed by the sensitive means of covariance analysis, i.e. with correction for final body weight.

At the terminal kill (week 104) no statistical significant decrease in liver and adrenal weights was noted in any dose group. Absolute kidney weight was reduced in males at 100 and 1000 mg/kg bw/day after 104 weeks, but a clear dose relationship was lacking.

At 52 weeks parotid salivary gland weight was increased in males at 100, 300 and 1000 mg/kg bw/day. Combined sublingual and submaxillary gland weight was increased in high-dose males and females. However, salivary gland weights were not affected at week 104 at any dose level.

Histopathology

The most notable histological finding was seen in the salivary glands where cellular alteration was seen in submaxillary and parotid salivary glands in males and females at 300 and 1000 mg/kg bw/day at week 52, and in both sexes at 100, 300 and 1000 mg/kg bw/day at week 104. These changes followed a dose-related pattern and are considered to be treatment related; however, these cellular alterations are similar to those seen occasionally in other subchronic or long-term dietary studies and are considered an adaptive response due to oral irritation from the ingestion of glyphosate, an organic acid, in the diet and are of no adverse consequence (see discussion at the beginning of the section and CA 5.8.2/002; CA 5.8.2/003).

Another histopathological finding was a decreased incidence of nephropathy in males at 100, 300 and 1000 mg/kg bw/day at interim kill. This finding was also noted in high-dose males at 104 weeks, but with reduced severity. Nephropathy is a common finding in old rats and as the incidence is decreased this finding is not considered as toxicologically significant.

In addition, the decreased incidence of urothelial hyperplasia in high-dose females at week 52 and 104, as well as in females at 300 mg/kg bw/day at week 104, is also not considered to be of toxicological significance.

Neoplastic changes
Neoplastic lesions were seen in all dose groups, however there was no dose relationship in the incidence of any individual tumour or in the incidence of animals with tumours.

It is concluded that the test compound at dose levels up to and including 1000 mg/kg bw/day produced no carcinogenic effect.

3. Assessment and conclusion

Assessment and conclusion by applicant:
In conclusion, glyphosate technical was not carcinogenic in male and female Sprague-Dawley rats following continuous dietary exposure of up to 1000 mg/kg bw/day (the limit dose for this type of study) for 104 weeks. Based on the study results and the lack of toxicological significance of the salivary gland findings, as well as a slight and isolated increase of plasma alkaline phosphatase observed at 300 mg/kg bw/day, the NOAEL in rats after chronic exposure to glyphosate technical for 104 weeks is considered to be 300 mg/kg bw/day.

Cellular alteration of the parotid/mandibular salivary gland was considered an adaptive response based on the addition of glyphosate acid to the diet. This effect is in concordance with findings in other studies with glyphosate acid as well as with citric acid (see CA 5.8.2/002). Furthermore, the effect on the salivary glands was not clearly dose related but may be following a threshold effect potentially related to pH changes. This assumption is also supported by the fact that the effects on the salivary glands were already observed after 52-weeks and did not increase in incidence or severity. Increased ALP activity is not considered an adverse effect as the increase was rather low, not accompanied but other liver enzymes and not related to any treatment-related histopathological changes in the liver. Furthermore, the liver weight was comparable between control and treatment groups.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.5/010
Report author	[redacted]
Report year	1990
Report title	Chronic study of glyphosate administered in feed to Albino rats
Report No	[redacted]-10495
Document No	M-651388-02-1
Guidelines followed in study	US-EPA Pesticide Assessment Guidelines Subdivision F, 83-5 (1982); in general accordance with OECD 453
Deviations from current test guideline (OECD 453, 2018)	Animals were approximately 8 weeks old at study begin (not >8 weeks); the top dose of the females exceeded 1000 mg/kg bw/day; haematological examinations were performed without prothrombin time and activated partial thromboplastin time; clinical chemistry was performed without gamma glutamyl transpeptidase or ornithine decarboxylase; volume of the urine was not determined; organ weights of adrenals, heart, ovaries, spleen, thyroid/parathyroid and uterus were

	not recorded; histopathological examinations of the coagulating glands, gall bladder, lacrimal glands, upper respiratory tract, mammary glands and vagina were not performed; overall survival at termination was below 50 %). Historical control attached for histopathological examinations.
Previous evaluation	Yes, accepted in the RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The chronic toxicity and carcinogenic potential of glyphosate was assessed in a 24-month feeding study in 50 male and 50 female Sprague-Dawley rats with 0, 2000, 8000 and 20000 ppm (equivalent to mean achieved dose levels of 0, 89, 362 and 940 mg/kg bw/day for males and 0, 113, 457 and 1183 mg/kg bw/day for females). In addition, 10 rats per sex per dose were included for interim sacrifice after 12 month. Observations covered clinical signs, ophthalmic examinations, body weight, food consumption, haematology, clinical chemistry and urinalysis as well as organ weights, necropsy and histopathological examination.

There were no treatment-related effects on survival, clinical signs, food consumption, and haematology and clinical chemistry parameters. Observed increased urine specific gravity, decreased urinary pH, as well as increased absolute and relative liver weights in high-dose males were not considered to be of toxicological significance, since there were no correlated findings in clinical chemistry or histopathology. Increased incidences of inflammation of the stomach mucosa in mid-dose females, and pancreatic islet cell adenomas in low-dose males were not dose-related and considered incidental findings.

Treatment-related findings in this study were statistically reduced body weights in high-dose females, as well as increased incidences of cataractous lens changes in high-dose males. There was no evidence of a carcinogenic effect observed in any dose group of either sex.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate
Description:	White solid
Lot/Batch #:	XLH-264
Purity:	96.5 %
Stability of test compound:	Guaranteed for the study period. Confirmed by analysis.

2. Vehicle and/or positive control: Diet

3. Test animals:

Species:	Albino Rat
Strain:	Sprague-Dawley (CD)
Source:	[redacted]
Age:	Approx. 8 weeks (at start of study)

Sex:	Males and females
Weight at dosing:	Males: approx. 284 g; females: approx. 221 g
Acclimation period:	29 days
Diet/Food:	Purina Mills certified Rodent Chow #5002 (Purina Mills), *ad libitum*
Water:	Mains drinking water, *ad libitum*
Housing:	In stainless steel cages with wire mesh bottoms suspended over paper bedding
Environmental conditions:	Animal housing & husbandry were in accordance with the provisions of ´Guide to the Care and Use of Laboratory Animal´; USPHS-NIH Publ. No. 85-23 Temperature: 17.8 - 21.1 °C Humidity: 40 - 70 % Air changes: not specified 12 hours light/dark cycle

B: Study design and methods

In life dates: 1987-08-05 to 1989-08-10

Animal assignment and treatment:
In a combined chronic toxicity and carcinogenicity study groups of 50 Sprague-Dawley rats per sex received daily dietary doses of 0, 2000, 8000 and 20000 ppm glyphosate (equivalent to mean achieved dose levels of 0, 89, 362 and 940 mg/kg bw/day for males and 0, 113, 457 and 1183 mg/kg bw/day for females) for 24 months.

A further ten animals per sex were added to each group and were designated for interim kill after 12 month to study chronic toxicity and non-neoplastic histopathological changes.

	Dose (ppm)							
	0		2000		8000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
Compound intake (mg/kg bw/d)	0	0	89	113	362	457	940	1183
Main group (# of animals)	50	50	50	50	50	50	50	50
Interim kill (# of animals)	10	10	10	10	10	10	10	10

Test diets were prepared in approximately weekly intervals by mixing a known amount of the test substance with basal diet. The stability of the dietary formulations were determined by analysis of samples of the low- and high-dose levels after storage at room temperature for 7 and 14 days, and frozen after storage for 35 days. The homogeneity of the test substance in the diet was determined for the low- and high-dose level preparations in the first and 88th week of the study. Analyses for achieved concentrations were done for all dose levels for the first six weeks, and for at least one dose level in weekly intervals thereafter. The stability of the neat test substance was verified by analysis before the start of the study, during month 8, 14 and 21, and after termination.

Clinical observations
All rats were examined for mortality and clinical signs of toxicity twice daily. Detailed clinical observations were conducted weekly. An ophthalmic examination was done in all animals before the start of the study, and prior to termination.

Body weight
Individual body weights were recorded prior to start of treatment, at weekly intervals from Week 1 to 13 and every four weeks thereafter until termination.

Food consumption and compound intake
Food consumption was recorded at weekly intervals for the first 13 weeks, and every fourth week thereafter.

Haematology and clinical chemistry
Blood was collected from 10 fasted animals per sex and group at Months 6, 12, 18 and at termination. The following parameters were measured: haematocrit, haemoglobin, total erythrocyte count, MCV, MCH, MCHC, platelet count, total leukocyte count, differential leukocyte count, reticulocyte count, alkaline phosphatase, aspartate amino transferase (AST), alanine aminotransferase (ALT), creatinine, blood urea nitrogen, total protein, glucose, albumin, globulin, total bilirubin, direct bilirubin, total cholesterol, inorganic phosphorus, calcium, sodium, potassium, and chloride.

Urinalysis
Individual urine samples were collected from the same animals as those used for haematology analyses at Month 6, 12, 18 and prior to termination. Sampling was done over a period of about 18-hours *via* metabolism trays. The following parameters were determined: appearance, specific gravity, pH, glucose, ketones, protein, bilirubin, urobilinogen and blood. In case that blood and / or protein in excess of the control urine samples were found, the sediment was examined for the presence of bacteria, epithelial cells, erythrocytes, leukocytes, casts or abnormal crystals.

Sacrifice and pathology
A gross necropsy was conducted on all surviving animals at scheduled sacrifice after 12 and 24 month. The following organ weights were determined: brain, kidneys, liver and testes with epididymides.

Tissue samples were taken from the following organs and subjected to a histopathological examination: adrenals, aorta, bone & bone marrow, brain, caecum, colon, duodenum, eyes, gross lesions including palpable masses, Harderian gland, heart, ileum, jejunum, kidneys, liver, lung (with main stem bronchi), lymph nodes (mesenteric and submandibular), muscle, nasal turbinates, oesophagus, ovaries, pancreas, pituitary, prostrate, rectum, sciatic nerve, seminal vesicles, skin (with mammary tissue), spinal cord (cervical, thoracic, lumbar), spleen, stomach, submaxillary salivary gland, testes with epididymides, thymus, thyroid/parathyroid, trachea, urinary bladder, uterus (corpus and cervix).

Statistics
Dunnett's Multiple Comparison Test (two-tailed) was used for body weights, cumulative body weight changes, food consumption, absolute leukocyte counts, reticulocyte counts, urine pH, urine specific gravity and clinical chemistry data obtained at Months 6, 12 and 18. Fisher´s exact test (one-tailed) was used for incidence of selected ocular lesions, as well as in combination with Bonferroni inequality procedure for incidences of non-neoplastic (at $p \leq 0.01$) and neoplastic lesions (at $p \leq 0.01$ and ≤ 0.05). EHL decision tree analysis was used for evaluation of terminal haematology, clinical chemistry, body weight, absolute and relative organ weight data and organ to brain weight ratios. Depending on the results either parametric (Dunnett´s Test and linear regression) or nonparametric (Kruskal-Wallis, Jonckheere´s and / or Mann-Whitney Tests) were applied. Mortality data were analysed by SAS lifetable procedure, and Peto Analysis was used for evaluation of histopathological data.

II. RESULTS

A. ANALYSIS OF DOSE FORMULATIONS
The stability analyses proved the neat test substance to be stable throughout the study period.

The stability and homogeneity of glyphosate in diet at concentrations of 2000 and 20000 ppm was satisfactory. The mean achieved concentrations of glyphosate in each dietary preparation were 95 % of the nominal concentration.

B. MORTALITY

There were no statistically significant differences in the group survival rates. The percentage of survival in each of the dose groups are summarised below.

Table 5.5-75 Chronic study of glyphosate administered in feed to Albino rats ([redacted] 1990): Percentage survival at termination after 24-month dietary exposure to glyphosate

Sex	Dose group (ppm)			
	0	2000	8000	20000
Male	29	38	34	34
Female	44	44	34	36

C. CLINICAL OBSERVATIONS

There were no treatment-related clinical sings noted except the ophthalmological findings (see below).

Table 5.5-76 Chronic study of glyphosate administered in feed to Albino rats ([redacted] 1990): Clinical sings

		Number of animals affected / number of occurrences							
		Males				Females			
ppm		0	2000	6000	20000	0	2000	6000	20000
Number of animals		60	60	60	60	60	60	60	60
Activity	Hypoactivity	5/10	5/9	5/10	2/2	6/16	3/17	4/7	4/8
	Prostrate	-	-	-	-	-	-	1/1	-
Movement/Posture	Head Tilt	1/13	1/2	-	-	5/21	2/24	5/19	3/8
	Tremor(s)	-	-	1/17	-	-	-	-	-
	Misuse of limbs	-	-	-	1/4	1/3	-	1/2	1/5
	Disuse of limbs	1/26	2/13	1/17	3/5	1/1	-	2/7	-
Respiration	Laboured	-	1/1	-	-	-	-	-	-
	Gasping	-	-	-	-	-	1/1	-	1/1
	Rattling sounds	-	-	-	1/1	-	-	-	-
Nose	Red/pink discharge	1/10	-	-	-	1/2	1/4	1/1	1/1
	Blood-like discharge	2/2	1/1	-	-	-	-	-	-
	Red/brown perinasal encrustation	3/14	-	1/1	2/2	4/7	4/10	8/19	5/12
Mouth	Salivating	-	1/1	-	-	-	-	-	-
	Perioral wetness	1/1	-	-	-	-	-	-	-
	Swollen mouth	3/9	-	-	-	-	1/1	1/12	-
	Overgrown teeth	11/141	7/32	10/181	9/70	9/107	5/98	7/79	3/45
	Mass	1/10	1/5	-	2/25	-	-	-	-
Eye(s)	Red discharge	9/52	8/13	9/48	5/13	10/32	10/39	8/40	6/8
	Blood-like discharge	-	-	1/1	-	-	-	-	-
	Lacrimation	-	-	-	-	-	-	1/12	-
	Periorbital wetness	15/273	10/59	12/135	10/31	10/162	22/144	15/82	14/42
	Eyelid(s) partially/completel	-	-	1/1	-	2/3	1/3	-	1/3

Table 5.5-76 Chronic study of glyphosate administered in feed to Albino rats ([redacted] 1990): Clinical sings

		Number of animals affected / number of occurrences							
		Males				Females			
ppm		0	2000	6000	20000	0	2000	6000	20000
Number of animals		60	60	60	60	60	60	60	60
	y closed								
	Opacity	2/34	4/7	3/5	2/66	2/3	-	-	1/3
	Pale	2/6	-	2/15	1/3	1/3	-	1/1	2/5
	Pitted/raised corneal surface	-	-	1/1	-	1/2	-	-	-
	Necrosis/rupture of globe	-	-	-	1/12	2/4	-	-	1/4
	Loss of eye	-	-	-	-	2/46	-	-	1/6
	Perorbital encrustation	7/24	3/23	5/47	2/3	6/32	8/54	5/54	3/7
Ear(s)	Pale	-	-	1/13	2/4	-	-	-	1/1
	Swollen	6/76	3/82	7/178	7/189	5/110	5/151	5/242	5/136
Integument	Focal loss of hair	6/57	5/77	11/66	4/73	8/35	7/111	5/69	11/59
	Generalised loss of hair	-	-	-	1/19	2/67	-	-	1/9
	Piloerection	17/64	14/76	16/56	20/73	16/149	25/155	12/86	22/138
	Abrasion(s)	-	-		1/4	3/9	1/2	1/9	1/9
	Scab(s)	8/21	6/39	9/13	6/36	4/93	2/16	3/13	3/16
	Ulceration	22/176	23/228	20/138	20/182	9/41	6/38	6/39	6/38
	Pale	3/6	3/10	3/14	5/17	5/15	6/8	1/1	3/11
	Urine-stained hair	2/8	1/1	2/2	5/9	3/10	4/12	3/7	3/5
	Faeces-stained hair		-	-	1/1	-	-	-	-
	Anogenital staining	4/13	6/10	6/10	6/12	3/5	5/16	2/2	4/12
Feet and limbs	Focal loss of hair		-	1/7	-	2/9	2/15	2/13	3/61
	Swollen	15/274	22/339	17/202	16/227	3/11	-	1/24	2/106
	Abrasion(s)	-	-	-	1/6	-	-	-	-
	Sore(s) on foot	13/242	18/228	14/207	13/207	1/11	3/8	1/1	3/45
Genitalia	Yellow/brown discharge	-	-	-	-	-	-	-	1/22
	Blood-like discharge	-	-	-	-	1/1	-	-	2/3
	Swollen	-	1/2	3/10	1/11	-	-	1/7	-
	Protrusion of tissue	-	-	1/2	-	-	-	-	1/24
	Abnormal penile erection	-	1/2	1/1	1/2	-	-	-	-
Excreta	Unusual urine colour	1/1	2/9	4/22	-	-	-	-	-
	Blood-like urine colour	2/7	3/26	6/59	1/4	1/1	-	2/26	1/1
	Decreased urination	1/1	-	-	-	-	-	-	-
	Black stool	1/1	1/1	-	-	-	-	-	-
	Soft stool	1/2	-	-	5/29	-	-	-	-
	Diarrhoea	2/3	1/1	1/1	-	-	2/2	-	1/1
	Decreased	20/35	15/34	17/29	13/18	11/21	15/52	12/3	15/39

Table 5.5-76 Chronic study of glyphosate administered in feed to Albino rats ([redacted] 1990): Clinical sings

		Number of animals affected / number of occurrences							
		Males				Females			
ppm		0	2000	6000	20000	0	2000	6000	20000
Number of animals		60	60	60	60	60	60	60	60
	defecation								
	Hard stool	1/1	-	-	-	1/1	-	2/4	1/1
General appearance	Emaciated	16/27	5/6	3/8	4/5	6/27	16/30	10/28	9/17
	Dehydrated	10/18	3/5	5/11	4/6	3/11	6/8	7/12	3/7
	Distended abdomen	-	-	-	-	-	-	1/1	-
Miscellaneous	Missing tag	-	-	-	1/2	-	-	-	-
	Blood-stained appearance under/in cage	-	2/5	2/2	1/7	-	-	-	-
	Intra-abdominal swelling(s)	1/11	1/2	-	2/4	-	1/7	-	-

D. BODY WEIGHT

There were no effects on body weight noted in males of any dose group. In high-dose females body weights were statistically significantly reduced from Week 7 through approximately the 20th month. During this time, absolute body weights gradually decreased to 14 % below the control value. The maximum difference in body weights was observed at 20th month. At this time-point the cumulative body weight gain in high-dose females was 23 % lower as compared to controls

There were no treatment-related effects in females fed 2000 or 8000 ppm glyphosate.

Table 5.5-77 Chronic study of glyphosate administered in feed to Albino rats ([redacted] 1990): Summary of cumulative body weight changes

		Males				Females			
ppm		0	2000	6000	20000	0	2000	6000	20000
Number of animals		60	60	60	60	60	60	60	60
Week 1	Mean body weight (g)	35.1	↑41.9*	↓38.7	↑41.0*	17.0	↑17.4	↑20.1	↓10.1
	Std. Dev.	16.75	7.37	18.59	8.06	7.45	7.63	6.92	7.06
Week 2	Mean body weight (g)	72.5	↑78.6*	↑74.9	↑74.3	29.1	↑29.3	↑30.9	↓26.7
	Std. Dev.	12.48	11.94	14.21	10.57	8.98	9.25	8.15	8.91
Week 3	Mean body weight (g)	103.6	↑110.8*	↑104.2	↓102.7	40.1	↑41.9	↑43.3	↓37.7
	Std. Dev.	16.05	16.48	15.58	14.10	10.10	10.42	8.12	9.61
Week 4	Mean body weight (g)	127.3	↑133.5	↓126.2	↓124.5	48.3	↑50.0	↑50.6	↓43.3
	Std. Dev.	20.19	20.82	16.95	17.17	11.34	11.50	22.32	10.41
Week 5	Mean body weight (g)	150.9	↑158.7	150.9	↓149.7	58.1	↑61.1	↑61.7	↓55.1
	Std. Dev.	24.73	25.04	21.24	20.23	12.55	12.50	12.83	11.93
Week 6	Mean body weight (g)	171.8	↑180.9	↓171.3	↓168.0	64.5	↑71.0*	↑70.5	↓59.9
	Std. Dev.	26.94	27.68	23.90	22.19	12.97	15.17	17.27	13.86
Week 7	Mean body weight	194.0	↑205.2	↓186.5	↓182.1	75.9	↓70.2	↑78.6	↓67.0**

Table 5.5-77 Chronic study of glyphosate administered in feed to Albino rats ([redacted] 1990): Summary of cumulative body weight changes

		Males				Females			
ppm		0	2000	6000	20000	0	2000	6000	20000
Number of animals		60	60	60	60	60	60	60	60
	(g)								
	Std. Dev.	29.46	30.96	26.86	23.49	13.58	16.80	14.43	14.60
Week 8	Mean body weight (g)	209.0	↑219.3	↓207.7	↓203.6	79.4	↑83.6	↑84.6	↓71.3**
	Std. Dev.	32.58	33.34	27.96	25.25	12.87	16.03	16.49	14.23
Week 9	Mean body weight (g)	223.0	↑235.4	↓222.7	↑215.9	87.4	↑89.4	↑90.6	↓79.4*
	Std. Dev.	35.93	35.69	30.05	26.44	14.89	17.30	17.93	15.17
Week 10	Mean body weight (g)	237.3	↑248.7	↓235.8	↓229.2	88.0	↑90.0	↑90.4	↓76.8**
	Std. Dev.	37.84	38.23	31.80	28.42	14.83	17.10	19.03	16.12
Week 11	Mean body weight (g)	246.0	↑257.8	↓244.6	↓237.1	96.2	↑100.4	↑100.2	↓85.4**
	Std. Dev.	37.76	38.44	34.03	29.19	15.20	19.84	18.20	17.63
Week 12	Mean body weight (g)	253.8	↑265.0	↑254.4	↓243.1	100.8	↑103.7	↑105.0	↓90.0**
	Std. Dev.	39.78	39.76	36.99	30.44	16.29	19.67	19.45	15.29
Week 13	Mean body weight (g)	265.9	↑273.2	↓260.5	↓262.3	105.1	↑107.2	↑108.3	↓94.4**
	Std. Dev.	40.54	40.70	36.74	30.82	17.13	20.72	21.40	14.88
Week 14	Mean body weight (g)	300.6	↑308.6	↓292.1	↓288.4	121.7	↑122.2	↑125.6	↓107.6**
	Std. Dev.	48.53	48.49	45.04	35.28	22.45	23.89	28.39	20.67
Week 18	Mean body weight (g)	331.8	↑346.1	↓323.3	↓318.7	134.3	↑137.2	↑140.0	↓116.6**
	Std. Dev.	50.35	55.01	49.68	41.73	28.49	27.77	32.75	24.65
Week 22	Mean body weight (g)	362.7	↑379.7	↓361.1	↓348.8	151.6	↑153.8	↑155.6	↓131.0**
	Std. Dev.	60.61	61.12	56.33	45.49	31.99	31.48	40.65	28.40
Week 26	Mean body weight (g)	381.9	↑399.6	↓368.2	↑390.5	161.6	↑164.2	↑167.7	↓141.5*
	Std. Dev.	64.48	67.06	61.08	66.49	35.29	36.42	44.02	33.66
Week 30	Mean body weight (g)	404.1	↑425.4	↓390.5	↓389.2	171.8	↑177.0	↑179.6	↓148.5*
	Std. Dev.	65.33	71.48	66.49	52.59	41.45	40.88	50.12	36.48
Week 34	Mean body weight (g)	410.5	↑446.0	↓408.4	↓406.4	186.8	↑195.8	↑195.2	↓163.0*
	Std. Dev.	70.22	74.30	71.21	55.23	44.46	48.32	55.43	39.63
Week 38	Mean body weight (g)	427.9	↑455.6	↓420.4	↓419.5	197.8	↑199.7	↑202.9	↓167.8**
	Std. Dev.	76.37	78.44	76.32	57.79	47.95	52.43	58.64	44.96
Week 42	Mean body weight (g)	442.1	↑471.6	↓431.3	↓433.1	211.0	↑218.3	↑217.5	↓181.7*
	Std. Dev.	76.53	84.48	81.80	6353	51.51	59.26	68.67	45.79
Week 44	Mean body weight (g)	460.6	↑483.8	↓446.0	↓450.8	220.8	↑232.2	↑230.3	↓193.2
	Std. Dev.	75.52	98.68	91.76	68.34	55.94	65.34	72.31	51.55
Week 48	Mean body weight (g)	468.4	↑600.7	↓455.8	↓458.4	226.4	↑236.3	↑237.1	↓203.3
	Std. Dev.	79.93	87.92	94.24	72.38	59.39	68.82	79.06	58.10
Week 52	Mean body weight	475.0	↑528.9*	↓465.2	↑476.2	246.6	↑255.4	↓243.8	↓212.8*

Table 5.5-77 Chronic study of glyphosate administered in feed to Albino rats ([redacted] 1990): Summary of cumulative body weight changes

		Males				Females			
ppm		0	2000	6000	20000	0	2000	6000	20000
Number of animals		60	60	60	60	60	60	60	60
	(g)								
	Std. Dev.	93.76	80.24	107.40	77.58	59.01	72.99	78.73	59.83
Week 56	Mean body weight (g)	488.0	↑539.0*	↓479.9	↓485.7	260.6	↑268.4	↑262.6	↓223.1
	Std. Dev.	91.47	95.96	103.31	94.40	65.83	80.27	87.96	70.48
Week 60	Mean body weight (g)	490.7	↑550.0*	↓478.8	↑498.1	274.2	↑285.5	↑278.0	↓242.3
	Std. Dev.	103.36	94.21	98.66	106.40	72.93	88.61	94.97	71.25
Week 64	Mean body weight (g)	506.6	↑550.3	↓484.4	↑508.8	292.0	↓289.1	↓286.9	↓250.1
	Std. Dev.	115.23	99.83	109.50	106.48	74.09	94.98	101.26	79.97
Week 68	Mean body weight (g)	502.9	↑547.0	↓490.8	↑515.6	296.3	↑296.5	↑304.9	↓251.1*
	Std. Dev.	102.30	111.34	118.03	11.69	74.63	99.55	93.88	79.20
Week 72	Mean body weight (g)	485.3	↑560.0*	↑498.3	↑503.7	314.6	↓306.1	↓312.4	↓250.3**
	Std. Dev.	110.31	101.33	116.27	116.06	71.87	85.82	92.97	77.59
Week 76	Mean body weight (g)	467.7	↑556.8*	↑480.1	↑490.2	321.8	↓302.5	↓318.4	↓249.5**
	Std. Dev.	120.19	118.93	139.08	123.46	84.05	88.54	101.13	80.91
Week 80	Mean body weight (g)	476.6	↑545.2	↑486.3	↑492.7	324.1	↓302.2	↓321.4	↓249.5**
	Std. Dev.	107.30	122.98	136.68	132.80	101.58	103.09	114.46	80.68
Week 84	Mean body weight (g)	438.2	↑555.9*	↑481.6	↑491.2	304.9	↑311.1	↑335.8	↓248.6
	Std. Dev.	146.32	104.6	146.97	136.6	96.78	104.06	110.08	90.06
Week 88	Mean body weight (g)	473.6	↑538.0	↓460.9	↑485.6	302.6	↑326.4	↑328.5	↓254.8
	Std. Dev.	104.96	131.14	161.73	146.12	106.26	102.44	122.50	79.07
Week 92	Mean body weight (g)	432.3	↑495.9	↑449.4	↑462.5	294.0	↑310.2	↑317.3	↓243.0
	Std. Dev.	107.59	146.74	139.82	126.14	101.12	116.87	139.84	82.58
Week 96	Mean body weight (g)	424.3	↑488.9	↑453.6	↑463.1	288.1	↑326.4	↑322.2	↓241.1
	Std. Dev.	95.94	132.81	108.3	136.39	120.43	106.88	137.60	106.47
Week 100	Mean body weight (g)	395.3	↑466.7	↑436.0	↑440.6	267.3	↑315.4	↑323.6	↓250.3
	Std. Dev.	94.79	153.01	113.98	143.77	125.90	115.46	117.01	109.49

*/** Significantly different from the control at 5 % and 1 % level of probability, respectively.

E. Food consumption and compound intake

There were no statistically significant decreases in food consumption in any group of either sex during the study period. However, significant increased food consumption was noted frequently in high-dose males, and on some occasions in low-dose males. The group mean achieved doses are summarised below.

Table 5.5-78 Chronic study of glyphosate administered in feed to Albino rats ([redacted] 1990): Summary of food consumption data

		Males				Females			
ppm		0	2000	6000	20000	0	2000	6000	20000
Week 1	Mean GM/day (g)	26.0	↑26.7	↓25.8	↓25.2	20.6	↓20.2	↑20.8	↓19.4*
	Std. Dev.	3.69	2.21	3.58	2.31	2.69	2.10	2.57	2.69
Week 2	Mean GM/day (g)	27.2	↓27.1	↓26.7	↓26.6	21.6	↓20.4	↓20.8	↓20.1
	Std. Dev.	2.76	2.40	2.03	2.11	3.59	2.23	2.97	2.37
Week 3	Mean GM/day (g)	28.1	↓27.9	↓27.3	↓27.9	21.0	↑21.3	↑21.1	↓20.7
	Std. Dev.	2.95	2.41	2.12	2.50	2.87	3.86	2.88	2.11
Week 4	Mean GM/day (g)	27.4	↓27.3	↓27.0	↑27.7	20.4	↓20.3	↑20.7	↓20.2
	Std. Dev.	2.84	2.62	2.06	2.63	2.96	1.68	2.62	2.47
Week 5	Mean GM/day (g)	26.4	↑27.0	↑26.7	↑27.1	20.5	↑20.7	↑20.9	↓20.2
	Std. Dev.	2.68	2.62	2.60	2.49	3.24	2.42	2.80	2.46
Week 6	Mean GM/day (g)	26.2	↑26.8	↑26.4	↑26.8	19.6	↑19.9	↑19.9	↑19.7
	Std. Dev.	2.72	2.72	2.88	2.43	2.15	1.86	2.17	2.12
Week 7	Mean GM/day (g)	26.1	↑26.6	↑26.7	↑27.6**	19.8	↑20.3	↑20.2	19.8
	Std. Dev.	2.39	2.63	2.29	2.24	2.71	2.78	2.22	2.03
Week 8	Mean GM/day (g)	26.0	↑26.3	↑26.1	↑26.4	19.5	↑20.1	↑20.1	19.5
	Std. Dev.	2.45	2.45	2.60	2.13	2.13	2.80	2.34	2.36
Week 9	Mean GM/day (g)	25.8	↑26.3	↑26.3	↑26.6	20.1	↓19.8	↑20.3	20.1
	Std. Dev.	3.47	2.60	2.35	2.18	1.86	1.78	1.91	3.57
Week 10	Mean GM/day (g)	26.7	↑27.0	26.7	↑27.1	20.7	↓20.4	↓20.6	↓20.3
	Std. Dev.	2.79	2.61	2.38	2.24	1.63	1.91	1.97	3.29
Week 11	Mean GM/day (g)	26.5	↓26.3	↓26.1	↑27.0	20.3	↓20.0	↑20.4	↓19.6
	Std. Dev.	2.52	2.34	2.33	2.32	2.62	2.13	2.81	3.68
Week 12	Mean GM/day (g)	26.2	↑26.7	↑26.5	↑27.0	20.4	↑20.6	↑20.8	↓19.5
	Std. Dev.	2.39	2.38	2.22	2.48	2.75	2.51	2.86	1.57
Week 13	Mean GM/day (g)	26.0	↑26.3	↑26.3	↑27.4**	20.7	↓20.4	20.7	↓19.8
	Std. Dev.	2.36	2.63	2.61	2.54	2.86	2.92	2.80	2.25
Week 14	Mean GM/day (g)	23.5	↑24.0	↓22.9	↑24.6*	18.7	↓18.3	↓18.3	↓18.0
	Std. Dev.	2.63	2.39	2.42	1.86	2.41	1.79	2.40	1.86
Week 15	Mean GM/day (g)	23.6	↑24.6	↑24.1	↑24.9*	19.1	↓18.5	19.1	↓18.4
	Std. Dev.	3.03	2.60	2.18	2.11	3.26	2.58	2.53	2.65
Week 16	Mean GM/day (g)	24.2	↑24.7	↓23.9	↑24.8	18.9	↓18.4	↓18.7	↓18.7
	Std. Dev.	2.99	2.40	2.35	2.36	2.01	1.88	2.36	2.25
Week 17	Mean GM/day (g)	25.5	↑26.3	↓24.7	↑26.6	20.2	↓19.8	20.2	↓19.8
	Std. Dev.	3.62	2.79	2.73	2.47	1.92	2.38	2.39	2.41
Week 18	Mean GM/day (g)	25.4	25.4	↓24.9	↑26.4	19.3	↑19.8	↑20.3	↑20.0
	Std. Dev.	2.60	2.64	2.23	2.20	2.86	2.29	2.98	2.71
Week 19	Mean GM/day (g)	25.1	↑25.5	↓25.0	↑26.2	20.3	↑20.9	20.3	↓20.0
	Std. Dev.	2.95	2.62	2.62	2.32	2.22	2.52	2.60	2.33
Week 20	Mean GM/day (g)	23.7	↑24.2	↑24.4	↑25.5**	19.5	↑19.6	↑19.9	19.5
	Std. Dev.	2.91	2.69	2.48	2.07	2.13	2.20	2.61	3.15
Week 21	Mean GM/day (g)	24.8	↑25.7	↑24.9	↑26.2*	20.7	↑21.2	↑20.8	↓20.5
	Std. Dev.	4.03	3.18	2.50	2.52	2.02	2.45	3.13	2.75
Week 22	Mean GM/day (g)	26.5	26.5	↓25.8	↑28.2*	21.3	↑22.2	↑21.6	↑21.9
	Std. Dev.	2.63	4.26	3.45	2.46	3.07	2.90	3.09	3.53
Week 23	Mean GM/day (g)	27.0	↑28.0	↓26.7	↑28.3*	21.9	↑22.4	↓21.6	↓21.7
	Std. Dev.	2.90	2.98	2.93	2.74	2.50	3.02	3.66	2.04
Week 24	Mean GM/day (g)	26.3	↑28.1*	↑26.5	↑27.6	22.1	↑23.0	↑21.4	↑22.1
	Std. Dev.	4.92	2.74	3.25	2.55	3.46	3.09	4.26	2.83
Week 25	Mean GM/day (g)	25.4	↑26.2	↓25.1	↑27.1*	21.2	↑21.7	↑21.4	↓20.8
	Std. Dev.	3.34	2.55	2.73	3.40	2.84	3.96	3.08	3.01
Week 26	Mean GM/day (g)	23.9	↑24.9	↑24.1	↑25.1	21.0	↓20.9	↓20.1	↓19.7
	Std. Dev.	4.76	2.89	4.16	4.71	2.42	2.83	3.35	2.51

Table 5.5-78 Chronic study of glyphosate administered in feed to Albino rats ([redacted] 1990): Summary of food consumption data

		Males				Females			
ppm		0	2000	6000	20000	0	2000	6000	20000
Week 27	Mean GM/day (g)	23.9	↓23.3	↓23.5	↑24.9	21.3	↓19.7	↓19.6	↓19.5
	Std. Dev.	4.19	3.56	3.34	2.72	2.28	5.40	3.58	2.87
Week 28	Mean GM/day (g)	24.5	↑24.6	↑24.8	↑26.8*	21.7	↓20.5	↓20.3	↓20.4
	Std. Dev.	3.98	4.48	4.60	3.53	3.52	4.37	4.17	3.73
Week 29	Mean GM/day (g)	25.6	↑27.0	↑26.6	↓26.5	21.9	↑22.1	↓21.3	↓21.1
	Std. Dev.	4.25	3.62	3.48	4.17	2.57	3.42	3.90	3.37
Week 30	Mean GM/day (g)	24.5	↑28.4*	↑26.0	↑28.5*	24.5	↓23.1	↓24.0	↓22.1
	Std. Dev.	8.22	4.97	6.72	4.93	3.08	5.62	5.76	4.91
Week 31	Mean GM/day (g)	27.1	↓25.8	↓26.5	↑28.2	23.6	↓21.9	↓22.3	↓22.5
	Std. Dev.	5.71	9.61	5.76	4.44	4.69	6.43	6.97	4.81
Week 32	Mean GM/day (g)	23.0	↑28.6**	↑25.9	↑27.7*	22.8	↑23.9	↑24.3	↓22.6
	Std. Dev.	10.03	4.61	5.37	5.07	6.54	5.58	5.47	5.27
Week 33	Mean GM/day (g)	25.3	↓24.6	↓22.9	↑25.8	21.9	↑22.4	↑21.7	↓19.7
	Std. Dev.	4.34	8.49	7.50	5.99	5.72	5.14	4.50	7.11
Week 34	Mean GM/day (g)	25.0	↓24.5	↓24.8	↓24.6	22.3	↓21.4	↓22.2	↓22.0
	Std. Dev.	4.52	7.93	6.25	8.61	5.54	5.75	6.48	6.11
Week 35	Mean GM/day (g)	26.5	↑26.9	↑26.8	↑28.2	23.1	↑24.6	↑23.2	↓21.8
	Std. Dev.	4.62	7.10	6.64	5.61	5.83	3.40	6.04	8.09
Week 36	Mean GM/day (g)	24.0	↓23.9	↑26.3	↑25.3	21.2	↓21.0	↓21.1	↑21.3
	Std. Dev.	3.94	8.98	5.25	7.16	5.20	4.21	4.61	5.38

* Dunnett test ($p \leq 0.05$)
** Dunnett test ($p \leq 0.01$)

Table 5.5-79 Chronic study of glyphosate administered in feed to Albino rats ([redacted] 1990): Group mean achieved dose levels

Dose group	Dietary concentration (ppm)	Mean achieved dose level (mg/kg bw/day)	
		Males	Females
1 (control)	0	0	0
2 (low)	2000	89	113
3 (mid)	8000	362	457
4 (high)	20000	940	1183

F. OPHTHALMOSCOPY

There were no treatment-related ocular effects observed in females of any dose group, as well as of males of the low-, and mid-dose group. In high-dose males a statistically increased incidence ($p \leq 0.05$) of cataractous lens changes were observed at the ophthalmic examination prior to termination. However, the observed incidence of 25 % was within the historical control range of 0-33 %. A second independent ophthalmic examination also performed prior to termination confirmed a statistically significant increase ($p \leq 0.05$) in the incidence of cataractous lens changes in high-dose males (1/14 (control) compared to 8/19 (high dose)). The results are summarised in the table below.

Table 5.5-80 Chronic study of glyphosate administered in feed to Albino rats [redacted] 1990): Incidences of cataract and lens fibre degeneration in males observed during ophthalmic examinations

	Dose group (ppm in diet)*			
	0	2000	8000	20000
1st examination	0/15 (0)	↑1/22 (5)	↑3/18 (17)	↑5/20** (25)

Table 5.5-80 Chronic study of glyphosate administered in feed to Albino rats ([redacted] 1990): Incidences of cataract and lens fibre degeneration in males observed during ophthalmic examinations

	Dose group (ppm in diet)*			
	0	2000	8000	20000
2nd examination	1/14 (7)	↑2/22 (9)	↑3/17 (18)	↑8/19** (42)
HCD (range) [%]	0 - 33			

* Number of rats affected/ number of rats examined (% affected);
** Statistically significant from control ($p \leq 0.05$)

The histopathological examination confirmed a slightly, but not statistically, increased incidence of degenerative lens changes (i.e. cataract and/or lens fibre degeneration) in high-dose males (see table below).

Table 5.5-81 Chronic study of glyphosate administered in feed to Albino rats ([redacted] 1990): Histopathological confirmed incidences of cataract and lens fibre degeneration in males

	Dose group (ppm in diet)*			
	0	2000	8000	20000
Terminal sacrifice	2/14	↑3/19	↑3/17	↑5/17
All animals	4/60	↑6/60	↑5/60	↑8/60

* Number of rats affected / number of rats examined

To summarise, ophthalmic examinations performed at the end of the study revealed a statistically significant increase in the incidence of degenerative lens changes in high dose males; however, the incidence was within the historical control range in the first evaluation. Histopathological examination also indicated a slightly increased incidence of degenerative lens changes in high dose males, although the difference was not statistically significant. Interpretation of these data are difficult since the numbers of animals examined ophthalmologically and affected at the end of the study were small. Nonetheless, the occurrence of degenerative lens changes in high dose male rats appears to have been exacerbated by treatment. There is no indication of treatment-related ocular effects in low or mid dose males or in any group of treated females.

G. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology and clinical chemistry evaluations noted various changes in the examined parameters. However, the changes were not consistently noted at more than one time point, were within historical control ranges, small in magnitude, and/or did not occur in a dose-related manner. Therefore, they were considered to be either unrelated to treatment or toxicologically insignificant.

The statistically increased alkaline phosphatase level observed in high-dose females at termination was mostly due to an extremely high value for one animal. However, this finding is in line with observation made in other long-term studies in rats.

Table 5.5-82 Chronic study of glyphosate administered in feed to Albino rats ([redacted] 1990): Summary of haematological data

ppm		Males				Females			
		0	2000	6000	20000	0	2000	6000	20000
WBC									
Day - 191	Mean [1000/mm²]	9.7500	9.7500	↑11.0636	↓9.4464	8.8393	↑9.0536	↓7.0640	↑9.1786
- 192	Std. Dev.	2.7267	1.8340	2.5870	1.0663	2.7319	2.5617	0.8317	2.4196
	% control	-	100	113	97	-	102	79	104

Table 5.5-82 Chronic study of glyphosate administered in feed to Albino rats ([redacted] 1990): Summary of haematological data

ppm		Males				Females			
		0	2000	6000	20000	0	2000	6000	20000
Day 370 - 374	Mean [1000/mm²]	11.0179	↓10.9286	↓10.1607	↓9.4464	7.9365	↓7.9286	↑8.2143	↓7.8760
	Std. Dev.	2.5240	2.9890	2.8453	1.6419	2.0596	1.8921	5.2805	1.2834
	% control	-	99	92	86	-	100	104	99
Day 569 - 570	Mean [1000/mm²]	10.6944	↓9.3651	↓10.1429	↓9.8250	7.7500	↑8.6071	↓7.2500	↑8.8750
	Std. Dev.	1.3902	2.1708	2.3582	1.9678	1.4802	3.4388	1.9401	3.4411
	% control	-	88	95	90	-	111	94	115
Day 734 - 735	Mean [1000/mm²]	16.1071	↓12.1964	↓12.1429	↓14.8036	11.8383	↓9.7321	↑11.8571	↑15.8214
	Std. Dev.	5.2831	2.9403	1.8842	10.0253	3.1565	1.8293	4.9230	8.2797
	% control	-	76	75	92		82	100	134
RBC									
Day 191 - 192	Mean [10^6/mm²]	8.8054	↓8.7839	↓8.3054	↓8.0196	7.9250	↑8.1964	↑8.0159	↑8.3679
	Std. Dev.	0.9199	0.6651	1.2732	1.7048	0.4517	0.6335	0.5570	0.4893
	% control	-	100	94	91	-	103	101	106
Day 370 - 374	Mean [10^6/mm²]	8.3536	↑8.4321	↓8.1964	↓7.8250	7.4385	↑7.6875	↓6.4518	↑7.7339
	Std. Dev.	0.5558	0.3910	0.4997	0.5477	0.5010	0.3567	1.7354	0.4289
	% control	-	101	98	94	-	103	87	104
Day 569 - 570	Mean [10^6/mm²]	7.1671	↑8.9484**	↑7.8607	↑8.1786	8.1429	↓7.3607	↓7.2911*	↓7.4625*
	Std. Dev.	1.5571	0.4913	0.6785	0.6557	0.6838	1.6918	0.8798	0.6544
	% control	-	128	111	115	-	90	90	92
Day 734 - 735	Mean [10^6/mm²]	6.8714	↓5.8946	↑7.9536	↓6.5571	6.3857	↓6.0839	↑6.4589	↓5.3911
	Std. Dev.	1.5015	1.4206	1.8201	1.7326	1.0665	1.1168	0.5965	1.4334
	% control	-	86	116	95	-	95	101	84
HGB									
Day 191 - 192	Mean [g/dL]	16.8571	↓16.8214	↓15.8214	↓15.5000	16.0714	↑16.8571	↑16.4881	↑17.2857*
	Std. Dev.	1.0487	0.8367	2.4603	2.8865	0.7192	1.0950	1.1573	0.7520
	% control		98	97	92	-	105	103	108
Day 370 - 374	Mean [g/dL]	16.2500	↓15.9107	↓15.8214	↓15.2143*	15.5754	↑16.8214	↓13.6786	↑16.1786
	Std. Dev.	0.7761	0.5549	0.5905	1.1869	1.0891	0.8996	2.8412	0.8213
	% control	-	98	97	94	-	102	88	104
Day 569 - 570	Mean [g/dL]	13.6508	↑16.8452**	↑15.7679*	↑16.1260*	16.6429	↓15.5000	↓15.7321	↓16.2321
	Std. Dev.	2.3113	0.6973	0.7809	1.0243	0.8325	2.9207	1.4688	1.2007
	% control	-	90	117	100	-	100	105	92
Day	Mean	13.3036	↓12.0179	↑15.6071	↓13.2857	13.535	↑13.600	↑14.214	↓12.4821

Table 5.5-82 Chronic study of glyphosate administered in feed to Albino rats ([redacted] 1990): Summary of haematological data

ppm		Males				Females			
		0	2000	6000	20000	0	2000	6000	20000
y	[g/dL]					7	0	3	
734	Std. Dev.	2.4358	2.9439	2.5200	2.6409	1.6297	1.8485	1.3101	2.3198
-	% control	-	90	117	100	-	100	105	92
735									
HCT									
Da	Mean [%]	48.8393	↓48.4464	↓45.6250	↓45.4643	46.196	↑47.964	↑47.242	↑49.5357
y						4	3	1	*
191	Std. Dev.	3.7127	3.1509	6.1100	8.8209	2.7626	3.4485	32453	1.9673
-	% control	-	99	93	93	-	104	102	107
192									
Da	Mean [%]	47.1071	↓47.0893	↓46.2500	↓44.2500	44.484	45.3929	39.2143	46.2143
y						1			
370	Std. Dev.	2.7585	2.2225	2.4943	4.1902	3.2477	2.6684	8.4267	2.6147
-	% control	-	100	98	94	-	102	88	104
374									
Da	Mean [%]	40.4960	↓49.3452**	↑44.4286	↑45.4464	47.714	↓44.196	↓44.178	↓43.8571
y						3	4	6	*
569	Std. Dev.	6.9710	2.8202	4.1869	3.1274	3.2240	8.1277	3.7136	3.3674
-	% control	-	122	110	112	-	93	93	92
570									
Da	Mean [%]	38.1429	↓34.1429	↑45.5000	↓37.7679	38.375	↓36.839	↑39.089	↓34.4107
y						0	3	3	
734	Std. Dev.	7.3782	7.7766	9.4973	8.6534	4.8126	5.3253	2.8228	7.1770
-	% control	-	91	119	99	-	96	102	90
735									
MCV									
Da	Mean [fL]	55.6000	↓55.1100	↓55.0800	↑57.0900	58.240	↑58.530	↑58.900	↑59.2000
y						0	0	0	
191	Std. Dev.	2.8284	1.5293	1.8534	3.1939	1.2429	1.2338	1.1192	2.2647
-	% control	-	99	99	103	-	100	101	102
192									
Da	Mean [fL]	56.4400	↓55.8200	↑56.5160	↓56.4100	59.766	↓58.940	↑61.940	↓59.7100
y						7	0	0	
370	Std. Dev.	2.5457	0.7510	2.2353	1.8040	0.9631	1.3930	5.2103	1.7966
-	% control	-	99	100	100	-	99	104	100
374									
Da	Mean [fL]	57.6333	↓55.1778	↓56.4100	↓55.5800	58.670	↑60.920	↑60.860	↑58.7800
y						0	0	0	
569	Std. Dev.	4.4003	2.4101	1.9712	1.7753	2.3195	4.6901	4.3247	1.7962
-	% control	-	96	98	96	-	104	104	100
570									
Da	Mean [fL]	55.9400	↑59.2200	↑57.6800	↑57.9700	60.560	↑61.080	↑60.620	↑64.9500
y						0	0	0	
734	Std. Dev.	3.7230	4.4382	6.4020	2.6953	4.3247	4.3078	2.4489	6.7531
-	% control	-	106	103	104	-	101	100	107
735									
MCH									
Da	Mean [pg]	19.2300	19.2300	↓19.0700	↑19.5200	20.310	↑20.600	↑20.544	↑20.6500
y						0	0	4	
191	Std. Dev.	1.0446	0.8945	0.9129	1.1104	0.5646	0.4967	0.4391	0.6621
-	% control	-	100	99	102	-	98	103	100
192									
Da	Mean	19.5300	↓18.9200	↓19.3400	↓19.4800	20.966	↓20.580	↑21.660	↓20.9400

Table 5.5-82 Chronic study of glyphosate administered in feed to Albino rats ([redacted] 1990): Summary of haematological data

ppm		Males				Females			
		0	2000	6000	20000	0	2000	6000	20000
y 370 - 374	[pg]					7	0	0	
	Std. Dev.	0.9129	0.5534	0.8592	0.5007	0.5000	0.6106	1.9614	0.6484
	% control	-	97	99	100	-	98	103	100
Day 569 - 570	Mean [pg]	19.5111	↓18.8667	↑20.1600	↑19.7400	20.5000	↑21.3700	↑21.6400*	↑21.7700**
	Std. Dev.	1.6534	0.8170	1.5657	0.7276	1.1086	1.5326	1.3142	0.9393
	% control	-	97	103	101	-	104	106	106
Day 734 - 735	Mean [pg]	19.5600	↑20.4100	↑19.9400	↑20.5300	21.3900	↑22.4000	↑22.0500	↑23.7800
	Std. Dev.	1.3906	1.0386	1.9968	1.8655	1.6650	1.7951	0.7472	2.8790
	% control	-	104	102	105	-	105	103	111
MCHC									
Day 191 - 192	Mean [g/dL]	34.5900	↑34.8400	34.5900	↓34.1800	34.8600	↑35.1500	↓34.8333	34.8600
	Std. Dev.	0.8543	0.9095	1.5495	0.8080	1.0146	0.7457	0.4359	0.6186
	% control	-	101	100	99	-	101	100	100
Day 370 - 374	Mean [g/dL]	34.6000	↓33.9100	↓34.2400	↓34.5500	35.1333	↓34.8700	↓34.9500	↓35.0400
	Std. Dev.	0.8832	0.8425	1.1247	1.1128	1.2903	0.4668	1.1414	0.8579
	% control	-	98	99	100	-	99	99	100
Day 569 - 570	Mean [g/dL]	33.8333	↑34.1889	↑35.7000	↑35.4500	34.9200	↑35.0400	↑35.5200	↑37.0400**
	Std. Dev.	1.1413	1.4243	2.6658	0.8410	1.1622	0.5358	1.2770	1.7430
	% control	-	101	106	105	-	100	102	106
Day 734 - 735	Mean [g/dL]	34.9900	↓34.5400	↑34.6100	↑35.4000	35.3400	↑36.6900	↑36.3600	↑36.5700
	Std. Dev.	2.5484	2.5439	2.3483	2.2420	2.3220	2.6710	1.8001	2.8418
	% control	-	99	99	101	-	104	103	103
PLT									
Day 191 - 192	Mean [1000/mm²]	794.1071	↑830.3571	↓316.9643	↑794.8214	821.0714	↓759.6429	↓818.4524	↓810.7143
	Std. Dev.	129.4230	100.2371	104.5867	212.9330	113.8285	88.3811	127.0276	174.4830
	% control	-	105	103	100	-	93	100	99
Day 370 - 374	Mean [1000/mm²]	765.8928	↑835.5357	↑825.7143	↑767.1428	774.6031	↓760.7143	↑857.3214	↓763.9286
	Std. Dev.	182.1671	75.6840	119.3069	166.2584	73.7771	71.1004	161.4499	98.1865
	% control	-	109	108	100	-	98	111	99
Day 569 - 570	Mean [1000/mm²]	1180.3572	↓847.4207**	↓855.3571**	↓857.1429**	733.5714	↓727.5000	↓733.2143	↑735.0000
	Std. Dev.	320.4399	160.6674	136.1548	148.8952	171.3310	114.1405	79.9713	122.7554

Table 5.5-82 Chronic study of glyphosate administered in feed to Albino rats ([redacted] 1990): Summary of haematological data

ppm		Males				Females			
		0	2000	6000	20000	0	2000	6000	20000
	% control	-	72	72	73	-	99	100	100
Day 734 - 735	Mean [1000/mm²]	1300.1984	↓1142.0635	↓1025.3572	↓1166.4286	804.6428	↓781.6071	↑837.8571	↑835.6548
	Std. Dev.	354.3073	312.2577	295.7942	304.2532	188.888	123.2532	1209778	174.9873
	% control	-	88	79	90	-	86	93	92

*/** Significantly different from the control at 5 % and 1 % level of probability, respectively.

Table 5.5-83 Chronic study of glyphosate administered in feed to Albino rats ([redacted] 1990): Summary of serum chemistry data

ppm		Males				Females			
		0	2000	6000	20000	0	2000	6000	20000
Glucose									
6 month	group means	97.2	↓90.5	↑105	↑98.9	97.2	↓87.9	↓93.5	↓93.8
12 month		166	↑191	↑182	↑195	192	↑194	↓187	↓189
18 month		101	↑108	↓98.7	↑108	103	↓101	↑104	↓89.4
Bun									
6 month	group means	15.7	↑15.8	↑16.2	↓14.9	18.4	↓17.3	↓17.7	↓16.3
12 month		14.0	↑14.9	↑14.5	↑15.6	15.5	↓14.8	↓15.3	↓15.4
18 month		17.0	↓15.8	↓14.7	↓15.2	13.5	↑17.9	↑14.2	↓13.1
Dir Bili									
6 month	group means	0.06	↓0.03	↓0.05	↓0.03	0.04	↓0.03	↑0.06	↓0.02
12 month		0.00	↑0.01	↑0.04	↑0.03	0.01	↓0.00	↓0.00	0.01
18 month		0.00	↑0.03	↑0.07	↑0.01	0.00	0.00	↑0.01	0.00
Chloride									
6 month	group means	107	107	↓105	↓105	105	107	↓104	↑106
12 month		103	103	103	103	99.5	↑99.7	↑100	↓98.7
18 month		107	↓104*	↓105*	↓102**	97.2	↑99.8	↑97.3	97.2
Tot Bili									
6 month	group means	0.11	0.11	0.11	↓0.10	0.15	↑0.17	0.15	↑0.18
12 month		0.09	↑0.10	↑0.12	↑0.12	0.17	↓0.11*	↓0.13	↓0.14
18 month		0.13	↑0.19	↑0.29	↑0.16	0.16	↓0.13	↑0.17	↓0.13
Tot Protein									
6 month	group means	7.9	↓7.8	↓7.7	↓7.5	8.9	8.9	8.9	↓8.4
12 month		7.0	↓6.7	↓6.7	↓6.7	8.1	↓7.7	↓7.4*	↓7.8
18 month		7.2	↑7.9*	↑7.9*	↑7.7	7.9	↓7.7	↓7.4	↓7.4
Albumin									
6 month	group means	4.0	↓3.8	↓3.8	↓3.8	4.9	↓4.8	4.9	↓4.5
12 month		3.4	↓3.2	↓3.3	♫3.3	4.4	♫4.1	↓4.0*	↓4.2
18 month		2.9	↑3.2*	3.1	3.2	4.0	3.9	3.8	3.9
Calcium									
6 month	group means	11.9	↓11.7	↓11.4	↓11.4	12.3	↑12.4	12.3	12.3
12 month		11.3	↑11.4	11.3	11.3	12.0	↓11.9	↓11.9	↑12.1
18 month		10.9	↑11.3	↑11.3	↑11.2	11.4	↓11.2	↓11.0	↓11.1
Phos									
6 month	group means	8.4	↓7.8	↓7.4	↓7.4	7.1	↑7.9	↓7.0	↑7.5
12 month		6.9	↑7.6	↑7.5	↓6.6	6.1	↑6.4	↓6.0	↑6.2
18 month		6.4	↓6.0	↓5.8	↓5.6**	5.6	↓5.4	↓5.3	↓5.2

Table 5.5-83 Chronic study of glyphosate administered in feed to Albino rats ([redacted] 1990): Summary of serum chemistry data

		Males				Females			
ppm		0	2000	6000	20000	0	2000	6000	20000
Alk Phos									
6 month	group means	209	↓201	↑262	↑248	80.5	↑83.7	↓78.6	↑89.7
12 month		169	↑177	↑199	↑209	55.2	↑62.5	↑71.6	↑67.0
18 month		205	↑225	↑210	↑254	56.4	↑94.9	↑101	↑75.8
SGOT									
6 month	group means	134	↓121	↓123	↓124	153	↓115	↑171	↑159
12 month		107	↑110	↓96.3	↓105	136	↓129	↓99.7	↑183
18 month		132	↑158	↑170	↑166	146	↓126	↓134	↓119
SGPT									
6 month	group means	35.6	↑44.7	↓35.0	↓35.1	49.4	↓31.4	↑55.2	↓46.0
12 month		39.9	↑50.4	↑42.8	↑45.4	42.2	↑47.0	↓30.7	↑67.1
18 month		45.1	↑66.2	↑50.3	↓34.4	43.7	↓42.7	↑44.3	↓33.2
Creat									
6 month	group means	0.52	↓0.48	0.52	↓0.45	0.72	↓0.68	↓0.68	↓0.71
12 month		0.47	0.47	0.47	↑0.50	0.54	↓0.50	↓0.43*	↓0.52
18 month		0.64	↓0.63	↑0.66	↓0.53	0.53	↑0.54	↓0.52	↓0.50
Globulin									
6 month	group means	3.9	3.9	3.9	↓3.7	4.1	↑4.2	↓4.0	↓3.9
12 month		3.6	↓3.5	↓3.4	↓3.4	3.7	↓3.6	↓3.5	↓3.6
18 month		4.3	↑4.7	↑4.7	↑4.5	3.9	↓3.8	↓3.6	↓3.5
Chol									
6 month	group means	42.5	↓35.8	↑43.3	↓37.1	59.2	↓55.1	↑64.9	↓49.1
12 month		49.1	↓40.8	↓44.3	↑56.6	49.6	↑53.3	↑68.5	↑56.2
18 month		87.4	↑88.1	↓81.3	↑98.0	104	↓69.7*	↓77.8	↓60.9**
Sodium									
6 month	group means	153	153	↑155	↑154	150	↑152	150	↑152
12 month		148	148	↓147	148	146	↑147	146	146
18 month		153	153	↑155	↑154	148	↑150	↑150	↑150
Potassium									
6 month	group means	6.7	↑6.8	↓6.6	6.7	6.4	6.4	↓6.0	↑6.6
12 month		6.6	↑7.6	↑7.7	↑6.9	6.3	↓5.9	↓6.0	↑6.4
18 month		6.0	6.0	↑6.2	↑6.4	4.6	↑5.2	↑5.5**	↑5.1

*/** Significantly different from the control at 5 % and 1 % level of probability, respectively.

H. URINALYSIS

Urine specific gravity was statistically significant increased at the Month 6 examination. The observed statistically significant decreased urinary pH at 6, 18 and 24 months might be related to the renal excretion of glyphosate, which is an acid.

Table 5.5-84 Chronic study of glyphosate administered in feed to Albino rats ([redacted] 1990): Summary of urinalysis data

		Males				Females			
ppm		0	2000	6000	20000	0	2000	6000	20000
Specific gravity									
6 month	Group mean	1.043	↑1.046	↑1.047	↑1.061*	1.056	↓1.053	↓1.049	↑1.064
	Std. Dev.	0.011	0.008	0.014	0.022	0.014	0.022	0.012	0.027
12 month	Group mean	1.038	↓1.029	↓1.033	↑1.039	1.035	↑1.037	↑1.036	↑1.040
	Std. Dev.	0.011	0.008	0.004	0.010	0.007	0.012	0.006	0.012
18 month	Group mean	1.053	↑1.057	↓1.052	↑1.056	1.034	↑1.041	↓1.032	↑1.040

Table 5.5-84 Chronic study of glyphosate administered in feed to Albino rats ([redacted] 1990): Summary of urinalysis data

		Males				Females			
ppm		0	2000	6000	20000	0	2000	6000	20000
Specific gravity									
	Std. Dev.	0.018	0.011	0.012	0.013	0.006	0.011	0.009	0.026
24 month	Group mean	1.034	1.034	↑1.036	↑1.039	1.031	↑1.032	↑1.032	↑1.034
	Std. Dev.	0.013	0.009	0.008	0.012	0.005	0.009	0.007	0.012
pH									
6 month	Group mean	6.9	↓6.5	↓6.8	↓6.0*	5.6	↑5.9	5.6	↓5.4
	Std. Dev.	0.4	0.4	0.6	1.1	0.4	0.6	0.3	0.4
12 month	Group mean	6.6	↓6.5	6.6	↓6.2	5.8	↑6.0	5.8	↑6.0
	Std. Dev.	0.4	0.5	0.5	0.4	0.4	0.4	0.3	0.3
18 month	Group mean	6.8	↓6.2	↓6.4	↓5.8**	6.2	↑6.4	6.2	↓5.8
	Std. Dev.	0.9	0.4	0.6	0.5	0.5	0.4	0.4	0.5
24 month	Group mean	6.4	6.0	6.0	↓5.7*	5.8	↑6.2	↑6.0	5.8
	Std. Dev.	0.6	0.5	0.6	0.4	0.4	0.5	0.6	0.5

*/** Significantly different from the control at 5 % and 1 % level of probability, respectively.

I. NECROPSY

Gross pathology

There were no treatment-related gross pathological findings observed at necropsy.

Organ weights

At interim kill after 12 months relative liver weights were slightly, but statistically significantly increased in high-dose males. At terminal sacrifice absolute liver weights, as well as liver to brain weight ratios were also statistically increased in high-dose males. Relative liver weights when compared to bodyweight were not significantly different compared to the control. There were no other significant and dose-related effects on organ weights.

Table 5.5-85 Chronic study of glyphosate administered in feed to Albino rats ([redacted] 1990): Summary of organ weights

ppm		Males				Females			
		0	2000	6000	20000	0	2000	6000	20000
Terminal body weight									
Day 370 - 374	Mean	720.3800	↑722.0500	↑726.7900	↓691.4000	403.8200	↑455.0000	↑437.2250	↓386.3400
	Std. Dev.	78.0037	125.5382	85.4748	85.0447	84.0449	70.7029	89.8582	52.0690
	% control	-	100	101	96	-	113	108	95
Day 734 - 737	Mean	632.9286	↑750.4526*	↑672.5059	↑705.6647	476.0409	↑522.2500	↑512.4824	↓437.9667
	Std. Dev.	93.8606	114.6882	120.9860	148.6255	116.0952	93.2104	117.9763	109.1727
	% control	-	119	106	111	-	110	108	92
Brain									
Day 370 - 374	Mean	2.1759	↑2.2187	↑2.2036	↑2.2053	2.1211	↓2.0820	↓2.0764	↓2.0486
	Std. Dev.	0.1138	0.1260	0.1148	0.0890	0.0654	0.0815	0.0669	0.1143
	% control	-	102	101	101	-	98	98	97

Table 5.5-85 Chronic study of glyphosate administered in feed to Albino rats ([redacted] 1990): Summary of organ weights

ppm		Males				Females			
		0	2000	6000	20000	0	2000	6000	20000
Day 734 - 737	Mean	2.3390	↓2.3046	↑2.3514	↓2.3131	2.1345	↓2.1302	↑2.1484	↓2.1022
	Std. Dev.	0.1146	0.1589	0.1407	0.1344	0.1024	0.0924	0.0999	0.1600
	% control	-	99	101	99	-	100	101	98
Kidney(s)									
Day 370 - 374	Mean	4.2045	↑4.5684	↑4.2684	↑4.5197	2.9643	↑3.0975	↑3.5243*	↑3.0254
	Std. Dev.	0.4871	0.7267	0.3856	0.7576	0.4435	0.3446	0.6136	0.5009
	% control	-	109	102	107	-	104	119	102
Day 734 - 737	Mean	5.5255	↑6.3361	↓5.3061	↓5.3587	3.9219	↓3.7465	↓3.5072	↓3.6081
	Std. Dev.	1.4593	4.2875	1.0977	1.2354	0.8817	0.6391	0.2821	0.7295
	% control	-	115	96	97	-	96	89	92
Liver									
Day 370 - 374	Mean	17.4555	↑18.1414	↑18.3410	↑18.6718	11.6039	↑12.4752	↑13.8455	↓11.3471
	Std. Dev.	3.2018	3.4751	2.4805	2.2841	2.2199	1.9673	2.7471	2.3439
	% control	-	104	105	107	-	108	119	98
Day 734 - 737	Mean	16.5051	↑17.9773	↑17.6834	↑18.6139 *	14.9135	↑15.2995	↓14.3320	↓14.9291
	Std. Dev.	2.3151	1.9917	1.9964	2.4702	3.2161	3.0758	3.3668	4.5552
	% control	-	109	107	113	-	103	96	100
	Relative to bw [% control]	-	93	102	103	-	92	88	106
Testes									
Day 370 - 374	Mean	6.1248	↑6.5223	↑6.1930	↓6.1014	-	-	-	-
	Std. Dev.	0.8161	0.6688	1.2419	0.6868	-	-	-	-
	% control	-	106	101	98	-	-	-	-
Day 734 - 737	Mean	5.6191	↓5.4769	↑5.9488	↓5.1414	-	-	-	-
	Std. Dev.	0.9345	0.8481	2.0428	1.1316	-	-	-	-
	% control	-	97	106	91	-	-	-	-

*/** Significantly different from the control at 5 % and 1 % level of probability, respectively.

Histopathology

Non-neoplastic lesions

Apart from the eye findings mentioned above histopathological examination showed only one other lesion that reached statistical significance. This was an increased incidence of inflammation of the stomach squamous mucosa in females fed 8000 ppm glyphosate (see table below).

Table 5.5-86 Chronic study of glyphosate administered in feed to Albino rats ([redacted] 1990): Incidence of inflammation and hyperplasia of the stomach squamous mucosa

		Dose group (ppm in diet)*			
		0	2000	8000	20000
Males	Inflammation	2/58	↑3/58	↑5/59	↑7/59
	Hyperplasia	3/58	3/58	↑4/59	↑7/59
Females	Inflammation	0/59	↑3/60	↑9/60**	↑6/59
	Hyperplasia	2/59	↑3/60	↑7/60	↑6/59

* Number of rats affected / number of rats examined
** Statistically significant at $p \leq 0.01$ (Fisher exact test with Bonferroni inequality)

Although the incidence of this lesion in mid-dose females (15 %) was slightly outside the historical control range (0 – 13.3 %) for the laboratory, there was no dose-related trend across all groups of females, and there was also no significance difference in male rats. Therefore, this finding is considered to be incidental and not related to treatment with glyphosate.

Neoplastic lesions
The only statistically significant difference in neoplastic lesions was an increased incidence of pancreatic islet cell adenomas observed in low-dose males (see **Table 5.5-87**). The incidence (14 %) in low-dose males was outside the historical control range (1.8 – 8.5 %) for this laboratory, but was in the historical control range ≥17 %) observed in reports from other laboratories. In addition, there was no dose-related trend for this finding in the male groups, as indicated by the lack of statistical significance in the Peto trend test. Due to the lack of a dose-related proliferative effect (hyperplasia) and or progression (carcinoma) of this lesion, and as such effects were not observed in females, this finding was not considered to be treatment-related.

Table 5.5-87 Chronic study of glyphosate administered in feed to Albino rats ([redacted] 1990): Incidence of pancreatic islet cell findings

Finding	Sex	Dose group (ppm in diet)*			
		0	2000	8000	20000
Hyperplasia	Males	2/58	↓0/57	↑4/60	2/59
	Females	4/60	↓1/60	↓1/60	↓0/59
Adenoma	Males	1/58	↑8/57**	↑5/60	↑7/59
	Females	5/60	↓1/60	↓4/60	↓0/59
Carcinoma	Males	1/58	↓0/57	↓0/60	↓0/59
	Females	0/60	↓0/60	↓0/60	↓0/59

* Number of rats affected / number of rats examined
** Statistically significant at $p \leq 0.01$ (Fisher exact test with Bonferroni inequality)

3. Assessment and conclusion

Assessment and conclusion by applicant: In conclusion, glyphosate was not carcinogenic in Sprague-Dawley rats following continuous dietary exposure of up to 20000 ppm for 24 months (corresponding to 940 mg/kg bw/day in males and 1183 mg/kg bw/day in females). The NOAEL for toxicity is 8000 ppm (corresponding to 362 mg/kg bw/day in males and 457 mg/kg bw/day in females), based on reduced body weights in females and cataract lens changes in males at 20000 ppm.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.5/011
Report author	[redacted]
Report year	1981
Report title	A Lifetime Feeding Study of Glyphosate (ROUNDUP® Technical) in Rats
Report No	77-2062
Document No	M-645914-01-1
Guidelines followed in study	None; in general accordance with OECD 451
Deviations from current test guideline (OECD 451, 2018)	Histopathological examinations of the cervix, coagulating glands, gall bladder, Harderian gland, lacrimal glands, rectum, skeletal muscle, upper respiratory tract, and vagina were not performed; Historical control attached for histopathological examinations. Dose levels selected were too low according to the recommendations given in OECD TG 451.
Previous evaluation	Not accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Invalid Dose levels too low, serious reporting deficiencies
Category study in AIR 5 dossier (L docs)	Category 3b

2. Full summary

This study, conducted for Monsanto Company, was designed to assess the toxicity of glyphosate (ROUNDUP® Technical) when administered orally *via* the diet to 300 Sprague-Dawley (CD) rats (50/sex/group).

During the first week of the study, the test substance was administered at dose levels of 30, 100 and 300 ppm. For the remainder of the study, dose levels of 3.05, 10.30 and 31.49 mg/kg bw/day for the males, and 3.37, 11.22 and 34.02 mg/kg bw/day for the females were maintained.

Clinical laboratory studies were performed at months 4, 8, 12, 18 and 24. Water consumption was measured over two 3-day periods at months 18 and 24. All male and female groups were terminated at 26 months, at which time survival had decreased to 30 % in one group/sex. Select organs were weighed and organ/body and organ/brain weight ratios were calculated. Histopathological evaluations were performed on all animals dying spontaneously, sacrificed in a moribund condition and sacrificed at terminal necropsy.

During most of the growth period, a slight but consistent trend toward reduced body weights in the treated males was evident. However, this difference decreased resulting in little difference in mean body weights between groups at termination. Because this effect was slight and not evident at termination, it is not considered to be toxicologically significant.

The treated females showed no statistically significant differences in mean body weights as compared to the controls through Month 19 of the study. However, for the following 2 months, the treated groups showed statistically significant reductions in group mean body weights, especially groups II and III, although not

in a dose-related fashion. Thereafter, the treated females gained weight relative to the control group, resulting in nearly identical group mean body weights at termination of the study.

Evaluations of mortality, food consumption and water consumption data, haematology, clinical chemistry, urinalysis and terminal organ and body weights, organ/body weight ratios and organ/brain weight ratios failed to reveal any effect attributable to the administration of glyphosate.

The incidence of interstitial cell tumours of the testes in the group IV males was elevated compared to the controls. Although an effect on the incidence of this tumour due to the administration of the test substance cannot be ruled out, the incidence in the group IV males is within the range observed in recent historical control data. In addition, the data suggest that the incidence in groups II through IV is within the normal biological variation observed for tumours at this site in this strain of rat. Where gross and microscopic changes occurred sporadically in the control and/or treated rats and were considered unrelated to the administration of the test substance.

I. MATERIAL AND MEDTHODS

A: Materials

1. Test material:

Identification:	Glyphosate (ROUNDUP® Technical)
Description:	Fine, white powder
Lot/Batch #:	XHJ-64
Purity:	98.7 %
Stability of test compound:	Not reported
2. Vehicle and/ or positive control:	Diet
3. Test animals:	
Species:	Rat
Strain:	Sprague-Dawley (CD®)
Source:	[redacted]
Age:	Approx. 6 weeks (at start of study)
Sex:	Males and females
Weight at dosing:	Males: approx. 124 g; females: approx. 102 g
Acclimation period:	12 days
Diet/Food:	Standard laboratory diet (Purina Lab Chow®), *ad libitum*
Water:	Automated water system (Elizabethtown Water Company), *ad libitum*
Housing:	Individually in elevated stainless steel cages
Environmental conditions:	Temperature: Not reported Humidity: Not reported Air changes: Not reported 12 hours light/dark cycle

B: Study design and methods

In life dates: 1978-07-12 to 1980-08-26 (males); 1978-07-12 to 190-09-04 (females)

Animal assignment and treatment:
In a lifetime feeding study groups of 50 Sprague-Dawley rats per sex received daily dietary doses of 0, 30, 100 and 300 ppm glyphosate (equivalent to mean achieved dose levels of 0, 3.05, 10.30 and 31.49 mg/kg bw/day for males and 0, 3.37, 11.22 and 34.02 mg/kg bw/day for females) for 26 months.

Appropriate amounts of test substance (adjusted by most recent weekly body weight and food consumption data) and standard laboratory diet were mixed weekly. Two 4 oz (about 120 g) samples of the control feed and each dietary level were taken at each feed preparation interval (Day 1). Extra feeder jars for each dietary level plus control were placed in the animal room and were sampled at the end of each feeding interval (Day 7). One set of samples for both Day 1 and Day 7 were shipped frozen to the sponsor for analyses. The other set of samples were stored frozen at the testing facility.

Clinical observations
All rats were examined for mortality and gross signs of toxicological or pharmacological effects twice daily. Detailed physical examinations for signs of local or systemic toxicity, pharmacologic effects and palpation for tissue masses were conducted weekly.

Body weight
Individual body weights were recorded twice prior to start of treatment, at weekly intervals from Week 1 to 14, biweekly thereafter and terminally (after fasting).

Food consumption and compound intake
Food consumption was recorded once prior to start of treatment, at weekly intervals for the first 14 weeks, and biweekly thereafter. The test substance intake was calculated from food consumption data. Based on nominal concentrations.

Water consumption
Water consumption was measured at month 18 and 24 over 2-three day periods.

H**aematology and clinical chemistry**
Blood was obtained *via* venepuncture of the orbital sinus (retrobulbar venous plexus) under light ether anaesthesia. Animals were selected randomly; the same animals were used at all intervals when feasible. Rats were fasted overnight prior to blood collections and were not dosed until after samples were collected. Blood was collected from 10 animals per sex and group at Months 4, 8, 12, 18 and 24. The following parameters were measured: haematocrit, haemoglobin, total erythrocyte count, platelet count, total leukocyte count, differential leukocyte count. The parameters for clinical chemistry were: serum glutamic oxaloacetic transaminase (AST), serum glutamic pyruvic transaminase (ALT), blood urea nitrogen, glucose, lactic acid dehydrogenase, total cholesterol, total bilirubin, direct bilirubin, total protein, albumin, globulin, inorganic phosphorus, calcium, potassium.

Urinalysis
Individual urine samples were collected from the same animals as those used for haematology analyses at Month 4, 12, 18 and 24. The following parameters were determined: appearance, specific gravity, pH, glucose, ketones, protein, bilirubin, occult blood and microscopic analysis.

Sacrifice and pathology
A gross necropsy was conducted on all surviving animals at scheduled sacrifice after 26 month and on all animals that died spontaneously or were killed in a moribund condition. The following organ weights were determined: Adrenals, brain, gonads, heart, kidneys, liver, pituitary, spleen and thyroid.

Tissue samples were taken from the following organs and subjected to a histopathological examination: adrenals, aorta, blood smear, bone & bone marrow, brain, caecum, colon, duodenum, eyes, heart, head, ileum, jejunum, kidneys, liver, lung, lymph nodes (mesenteric and mediastinal), mammary gland (right inguinal), oesophagus, ovaries, pancreas, pituitary, prostrate, sciatic nerve, seminal vesicles, skin (with

mammary gland), spinal cord (cervical, lumbar), spleen, stomach, mandibular salivary gland, testes with epididymides, thymus, thyroid/parathyroid, trachea, urinary bladder, uterus.

Statistics
Body weight, food consumption, haematology and clinical chemistry parameters, organ weights and organ/body weight ratios and organ/brain weight ration were analysed. Mean values of all dose groups were compared to control at each time interval.

Statistical evaluation of equality of means was made by the appropriate one way analysis of variance technique, followed by a multiple comparison procedure if needed. First, Bartlett's test was performed to determine if groups had equal variance. If the variances were equal, parametric procedures were used; if not, nonparametric procedures were used. The parametric procedures were the standard one way ANOVA using the F distribution to assess significance. If significant differences among the means were indicated, Dunnett's test was used to determine which means were significantly different from the control. If a nonparametric procedure for testing equality of means was needed, the Kruskal-Wallis test was used, and if differences were indicated a summed rank test (Dunn) was used to determine which treatments differed from control.

A statistical test for trend in the dose levels was also performed. In the parametric case (i.e. equal variance) standard regression techniques with a test for trend and lack of fit were used. In the nonparametric case Jonckheere's test for monotonic trend was used.

The test for equal variance (Bartlett's) was conducted at the 1 %, two-sided risk level. All other statistical tests were conducted at the 5 % and 1 %, two-sided risk level.

II. RESULTS

A. ANALYSIS OF DOSE FORMULATIONS
The stability analyses were in the responsibility of the sponsor.

B. MORTALITY
There was no significant difference between the control and treated groups of both sexes with regard to the survival rate during the course of this study. Survival was approximately 80-90 % through Month 20 of the study for all groups. Thereafter, significant reductions in the number of surviving animals occurred in all groups in roughly an equivalent fashion, culminating in the termination of the study at Month 26. At this time, survival had decreased to 30 % in the group I males and the group IV females, requiring that the study be terminated to ensure a sufficient number of animals at the terminal necropsy. At 24 months, survival levels equalled or exceeded 50 %, which is comparable to historical control data for rats of this strain.

Table 5.5-88 A Lifetime Feeding Study of Glyphosate (ROUNDUP® Technical) in Rats ([redacted] 1981): Percentage survival at termination after 26-month dietary exposure to glyphosate

Sex	Dose group (ppm)			
	0	30	100	300
Male	35/50	24/50	34/50	24/50
Female	32/50	27/50	20/50	35/50

Number of dead animals/total number of animals

C. CLINICAL OBSERVATIONS
Physical observations noted during the course of this study included alopecia, excessive lacrimation, nasal discharge and rales. These findings were present in animals in all groups, both male and female, in approximately the same incidence and are common observations in the laboratory rat. Time of appearance and duration was approximately the same in all groups, including the controls. Therefore, it is concluded

that the administration of the test substance did not significantly affect the physical condition of the animals on test in this study.

D. BODY WEIGHT

No statistically significant differences were noted among the mean body weights of the treated males as compared to the group I controls during the course of this study. However, during a portion of the growth period, a slight but consistent trend toward reduced body weights in the treated males was evident. The maximum decrease was approximately 6 % in the group IV males, occurring during Month 16. Thereafter, this difference decreased resulting in little difference in mean body weights between groups at termination. Because this effect was slight and not evident at termination of the study and did not affect survival, it is not considered to be toxicologically relevant.

No statistically significant differences in mean body weights were found among the treated females as compared to the controls through Month 19 of the study. However, for the following 2 months, the treated groups showed statistically significant reductions in mean body weights as compared to the control, although not in a dose-related fashion. The magnitude of the reduction ranged between 10-15 % with the greatest difference evident in groups II and III. Thereafter, the treated females gained weight relative to the control group resulting in nearly identical group mean body weights at termination of the study. This pattern is similar to that evident in the males.

The pattern of increasing body weights for the first 16 months, followed by a plateau and then a slight decline, observed in the males and females in group I is typical for the laboratory rat. In the treated animals, particularly in the group IV males and groups II through IV females, a slight delay in reaching the plateau phase was observed. This effect was not dose-related in the treated females and may be due to biological variation.

Table 5.5-89 A Lifetime Feeding Study of Glyphosate (ROUNDUP® Technical) in Rats ([redacted] 1981): Body weight data

ppm		Males				Females			
		0	30	100	300	0	30	100	300
Number of animals		50	50	50	50	50	50	50	50
Week 62	Mean body weight (g)	694.7 (N=47)	↓671.3 (N=48)	↓670.9 (N=46)	↓659.1	-	-	-	-
	Std. Dev.	83.7	85.4	66.7	66.3	-	-	-	-
	Std. Err.	12.2	12.3	9.8	9.4	-	-	-	-
Week 64	Mean body weight (g)	701.5	↓684.4	↓673	↓664.2	-	-	-	-
	Std. Dev.	84.0	80.4	74.1	66.6	-	-	-	-
	Std. Err.	Not readable in original report				-	-	-	-
Week 66	Mean body weight (g)	709.7 (N=47)	↓690.6 (N=47)	↓684.6 (N=45)	↓671.5	-	-	-	-
	Std. Dev.	85.4	80.0	71.9	69.0	-	-	-	-
	Std. Err.	12.5	11.7	10.7	9.9	-	-	-	-
Week 74	Mean body weight (g)	-	-	-	-	426.6 (N=48)	↓398.2 (N=46)	↓398.1 (N=47)	↓410.7 (N=47)
	Std. Dev.	-	-	-	-	88.0	65.3	61.0	75.8
	Std. Err.	-	-	-	-	12.7	9.6	8.9	11.1
Week 76	Mean body weight (g)	-	-	-	-	436.7 (N=48)	↓410.7 (N=46)	↓404.1 (N=47)	↓409.8 (N=45)
	Std. Dev.	-	-	-	-	93.7	67.8	63.8	82.4
	Std. Err.	-	-	-	-	13.5	10.0	9.3	12.3
Week 78	Mean body weight (g)	-	-	-	-	427.3 (N=47)	↓404.4 (N=36)	↓405.5 (N=37)	↓419.9 (N=33)
	Std. Dev.	-	-	-	-	86.9	71.4	65.3	87.3

Table 5.5-89 A Lifetime Feeding Study of Glyphosate (ROUNDUP® Technical) in Rats ([redacted] 1981): Body weight data

ppm		Males				Females			
		0	30	100	300	0	30	100	300
Number of animals		50	50	50	50	50	50	50	50
	Std. Err.	-	-	-	-	14.3	11.9	10.7	15.2

The number in brackets represents the animal number of the respective dose/timepoint.

E. FOOD CONSUMPTION AND COMPOUND INTAKE

Food consumption values relative to body weight were highest during the early stage of the study, gradually declining through Month 18. Thereafter these values remain essentially unchanged for the duration of the study. This pattern was observed in all groups of both sexes and is typical for long-term rat studies. Occasional statistically significant differences were noted in the treated animals of both sexes relative to their respective controls, but these differences in mean food consumption were slight and occurred sporadically unrelated to dose level. Therefore, it is concluded that the dietary administration of Glyphosate at the doses utilised in this study did not significantly affect food consumption values in either sex.

Test substance intake

During the first week of the study, the test substance was administered at the following dose levels for both sexes: 0, 30, 100 and 300 ppm for groups I through IV, respectively. Based on group mean food consumption, body weight values and nominal dietary concentrations for Week 1, test substance intake was calculated on a mg/kg/day basis. Thereafter, weekly through 14 weeks and biweekly for the duration of the study, the dietary levels of the test substance were adjusted to maintain the test substance intake attained for Week 1. These values were as follows: 0, 3.05, 10.30 and 31.49 mg/kg bw/day for groups I through IV males, respectively and 0, 3.37, 11.22 and 34.02 mg/kg bw/day for groups I through IV females, respectively. These values were maintained within a narrow range throughout the study.

Water consumption

At 18 and 24 months of the study, water consumption was measured for 10 animals/sex/group for two 3-day intervals over a 2-week period. Mean values for both the treated male and female groups were not significantly different from their respective control groups at the intervals studied. As is usual, the females consumed more on a body weight basis as compared to the males. Thee data indicate that the administration of the test substance did not significantly affect water consumption in either sex.

G. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

Analysis of the group mean haematology data for both sexes indicates no toxicologically significant differences in any of the parameters evaluated. All mean data were within the normal physiological range for the laboratory rat. The few statistically significant differences noted appear to be due to random variation as no consistent treatment-related pattern is evident. On the basis of this data it is concluded that the administration of the test substance did not affect the haematology parameters evaluated.

Clinical Chemistry

Mean clinical chemistry data for all groups of both males and females fall within the normal physiological range for the laboratory rat. Occasional statistically significant differences were noted, but these appear due to random fluctuation, as no treatment-related pattern emerged. Thus, on the basis of this data, the administration of the test substance did not significantly affect any of the clinical biochemistry parameters evaluated during the course of this study.

H. URINALYSIS

No significant differences were noted in the urinalysis data when the control groups were compared to the

treated groups for both sexes. Occasional values outside the normal range were found; however, these values occurred sporadically exhibiting no consistent pattern.

I. NECROPSY

Gross pathology

Gross observations noted at necropsy were similar in incidence between control and treated animals of both sexes. Lesions noted were those commonly found in chronic studies conducted in this laboratory on the same strain of rats.

Organ weights

No statistically significant differences were noted in the terminal organ weights, organ/body weight ratios and organ/brain weight ratios of the treated groups as compared to their respective controls. Slight differences in these parameters were observed in a group by group comparison; however, no consistent pattern related to the administration of the test substance was evident.

Histopathology

Non-neoplastic lesions

In general, a good correlation was found between gross lesions noted at necropsy and microscopic findings of those tissue sections. The incidence and severity of the microscopic findings were similar in the control and treated animals of both sexes. Among the more common findings were changes found in the kidneys and lungs, lesions frequently found in chronic rat studies. Therefore, these findings are considered unrelated to the administration of the test substance.

Neoplastic lesions

A variety of neoplasms were found in both the control and treated animals of both sexes. The most common tumours were found in the pituitary in both sexes. In the females, mammary gland tumours were the next most common neoplasm found. In general, the incidence of all neoplasms observed occurred in the treated and control animals to a similar degree, or occurred infrequently such that a treatment-related association could not be made. The only exception to the above was the incidence of interstitial cell tumours of the testes in male rats. The incidence of this neoplasm in both the scheduled terminal sacrifice animals as well as the total number of animals on test is presented along with recent historical control data for comparison in the table below:

Table 5.5-90 A Lifetime Feeding Study of Glyphosate (ROUNDUP® Technical) in Rats ([redacted] 1981): Neoplastic findings compared to historical control data

	Interstitial cell tumour of the testes			
[ppm]	0	30	100	300
Terminal sacrifice	0/15 (0)	2/26 (8)	1/16 (6)	4/26 (15)
All animals	0/50 (0)	3/50 (6)	1/50 (2)	6/50 (12)

	Recent historical control data					
Study number	I	II	III	IV	V	All Studies
Terminal sacrifice	4/65 (6)	3/11 (27)	3/26 (12)	3/24 (13)	3/40 (8)	16/166 (9.6)
All animals	4/116 (3)	5/75 (7)	4/113 (4)	6/113 (5)	5/118 (4)	24/535 (4.5)

(): incidence [%]

Based on the results in the present study, the data suggest a treatment-related response with regard to the incidence of testicular interstitial cell tumours in male rats. This tumour, as is frequently the case, increases in frequency among older animals as can be seen from comparing the incidence in the animals surviving

until termination to the total incidence. A review of recent control data from animals sacrificed at 12 months suggests an incidence near zero at this age. However, in animals ranging in age from 24-29 months at necropsy, the incidence increases to about 10 %. Therefore, the studies selected for comparison were all terminated within this time interval, as compared to 26 months for the present study.

A comparison of the incidence of interstitial cell tumours in the group IV males to the control data from recent chronic studies suggests that the two incidence are similar. At terminal sacrifice, the incidence in the present study was 15.4 % (4/26), while the range in control animals from 5 concurrent studies was 6.2 % (4/65) to 27.3 % (3/11) with an overall mean value of 9.6 % (16/166). When all animals on test are included, the incidence for the group IV males was 12 % (6/50) compared to a range of 3.4 % (4/116) to 6.7 % (5/75) with a mean of 4.5 % (24/535). Therefore, this comparison suggests an incidence of this tumour in the group I males which is slightly lower (0 %), and an incidence in the group IV males which is slightly higher than recent historical control data. Although an effect on the incidence of this tumour due to the administration of the test substance cannot be ruled out, the data suggest that the incidence in groups II through IV is within the normal biological variation observed for tumours at this site in this strain of rat.

3. Assessment and conclusion

Assessment and conclusion by applicant: In conclusion, glyphosate (ROUNDUP® Technical) was not carcinogenic in Sprague-Dawley rats following continuous dietary exposure of up to 300 ppm for 26 months (corresponding to 31.49 mg/kg bw/day in males and 34.02 mg/kg bw/day in females). The NOAEL for toxicity is 300 ppm (corresponding to 31.49 mg/kg bw/day in males and 34.02 mg/kg bw/day in females), based on the absence of findings in body weight changes, haematology, clinical chemistry, organ weight data or histopathological examinations. Based on the reporting deficiencies and the use of too low dose groups the outcome of the study is not considered reliable for the hazard and risk assessment of glyphosate.

Assessment and conclusion by RMS:

Carcinogenicity studies in the mouse

The most recent 80-Week dietary mouse study was conducted by [redacted] 2009 (CA 5.5/012-015). There were no adverse treatment related effects at the highest dose tested. The NOAEL for this study was 810/1081 mg/kg bw/day in males and females respectively.

A combined toxicity and carcinogenicity study in mice ([redacted] 2001, 5.5/016) demonstrated a slightly higher mortality in the high dose group. Mortality was within the upper end of the historical control range. However, treatment with glyphosate might slightly have affected the mortality at the highest dose of 10000 ppm, above the limit dose of 1000 mg/kg bw/day, and because a relationship to treatment was unclear, a conservative NOAEL for toxicity at the mid dose of 1000 ppm (150.5 mg/kg bw/day for combined sexes) was set for this study. The number of malignant lymphoma, the most common tumour in the mouse, was slightly higher in the high dose group compared to control, but this was considered as incidental variation based on historical control data and was not considered to be related to treatment. A re-evaluation of the statistical analysis of histopathology findings was performed in 2017 (CA 5.5/017). When appropriate statistics are applied to the tumour incidence data, (and the incidence data of malignant lymphoma in particular), no statistically significant increase in tumour incidence is found in this analysis. Moreover, the study was compromised by the presence of non-identified ecto- and endoparasites in a large number of animals and non-identified micro-organisms in single animals. In the absence of any detailed information it must be considered that some of the findings in this study (e.g. dermal lesions) are induced by infectious agents. All this taken together, it can be concluded that Glyphosate Technical (glyphosate acid) is not

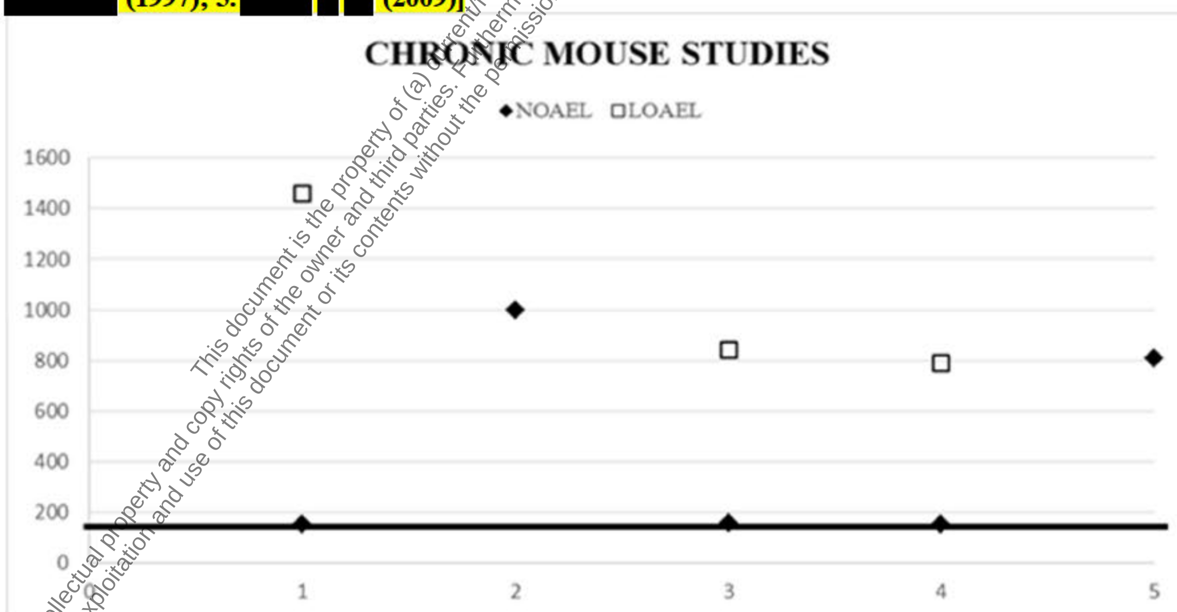
carcinogenic in Swiss albino mice when exposed to dietary concentrations of up to 10000 ppm for a duration of 18 months.

In the study by [redacted] 1997 (CA 5.5/018-018) the low effect level was 8000 ppm (equivalent to 787 mg/kg bw/day) in females only based on a reduction in body weight gain. At the top dose of 40000 ppm (equivalent to 4348/4116 mg/kg bw/day in males and females, respectively) signs of toxicity included loose stools, reduced body weight gain, food consumption and food utilisation, caecum distention and increased absolute and relative caecum weight (without corollary histopathological findings), increased incidence of anal prolapse consistent with histopathological erosion/ulceration of the anus.

A further 2-year oral diet study in mice was performed by [redacted] (1989, CA 5.5/021). In this study glyphosate was found to be not carcinogenic in male and female CD-1 mice following continuous dietary exposure of up to 1000 mg/kg bw/day (the limit dose for this type of study) for 104 weeks. Based on the study results and the lack of toxicological relevance of both the thymus weight findings and increased mineral deposit in the brain observed at 1000 mg/kg bw/day, the NOAEL in mice after chronic exposure to glyphosate technical for 104 weeks is considered to be 1000 mg/kg bw/day.

Two more long-term studies in mice (18-month) were considered not acceptable for reliable assessment of carcinogenicity ([redacted], 1988, CA 5.5/022; [redacted] 1982/1992, CA 5.5/024). Thus, they were not taken into consideration for this re-evaluation. A further study ([redacted], 1983, CA 5.5/023) also concluded that glyphosate technical was not carcinogenic in male and female CD-1 mice following continuous dietary exposure of up to 30000 ppm (equivalent to 5342.4mg/kg bw/day for males and 7045.5 mg/kg bw/day for females) for 104 weeks. Based on the findings of non-neoplastic bladder epithelial effects in male mice at 5000 ppm in diet (891.8 mg/kg bw/day), the NOAEL after chronic exposure to glyphosate for 104 weeks is considered to be 1000 ppm (equivalent to 157 mg/kg bw/day.

Figure 5.5.2-1: Valid chronic mouse NOAEL and LOAEL values, showing an overall NOAEL of 150 mg/kg bw/day [1. [redacted] (2001); 2. [redacted] (1993); 3. [redacted] (1983); 4. [redacted] (1997); 5. [redacted] (2009)]



The overall chronic NOAEL in mice is 150 mg/kg bw/day. No test substance related carcinogenic potential in mice was concluded in all studies.

Table 5.5-91: Summary of long-term toxicity and carcinogenicity studies in mice

Annex Point	Study	Study type	Substance(s)	Reference list-related category§	Result NOAEL (NOAEL)* (mg/kg bw/day)
CA 5.5/012-015	[redacted], 2009	18-month, oral diet Mouse, CD-1	Glyphosate technical (Batch: H05H016A, Purity: 95,7 %)	Valid, Category 2a	810/1081 (♂/♀) 946 (♂+♀) (810/1081 (♂/♀)
CA 5.5/016	[redacted], 2001	18-month, oral diet Mouse, Swiss albino	Glyphosate technical (Batch: 01/06/97, Purity: >95.14 %)	Valid, Category 2a	149.7/151.2 (♂/♀) 150.5 (♂+♀) (1460)
CA 5.5/017	[redacted], 2017	Statistical evaluation of pre-neoplastic and neoplastic lesions	Glyphosate technical (Batch: 01/06/97, Purity: >95.14 %)	Category 1	(1460)
CA 5.5/018-019	[redacted] 1997	18-month, oral diet Mouse, ICR	Glyphosate technical (Batch: T-941209, T-950308, Purity: 97.56 %, 94.61 %	Valid, Category 2a	838/153 (♂/♀) (4348/4116 (♂/♀))
CA 5.5/020-021	[redacted], 1993	2-year, oral diet Mouse, CD-1	Glyphosate technical (Batch: 206-JaK-25-1, Purity: 97.5 %, 100 %)	Valid, Category 2a	1000 (♂/♀) (1000 (♂/♀))
CA 5.5/022	[redacted] 1988	18-month, oral diet, Mouse, Balb/c	Glyphosate technical (Batch, Purity: not reported)	Invalid, Category 3b	5.87/5.42 (♂/♀) (5.87 / 5.42 (♂/♀))
CA 5.5/023	[redacted], 1983	2-year, oral diet Mouse, CD-1	Glyphosate technical (Batch: NB 1782608/3 and NB 1782610/7, Purity: 99.7 %)	Valid, Category 2a	157/190 (4841/5874)
CA 5.5/024	[redacted], 1982	18-month, oral diet, Mouse, CFLP/LATI	Glyphosate (Batch: 14/980-03090380, Purity: not reported	Invalid, Category 3b	37.7 /44.5 (37.7 /44.5)

* NOAEL for carcinogenicity
§ The category describes the acceptability of the study within the AIR 5 submission

1. Information on the study

Data point:	CA 5.5/012 CA 5.5/013 CA 5.5/014 CA 5.5/015

Report author	[redacted].
Report year	2009
Report title	Glyphosate Technical: Dietary Carcinogenicity Study in the Mouse
Report No	2060-0011
Document No	NA
Guidelines followed in study	OECD 451 (1981), JMAFF guideline 2-1-15 (2005), US-EPA OPPTS 870.4200 (1996)
Deviations from current test guideline (OECD 451, 2018)	Yes, histopathological examinations of the cervix, coagulating glands and the upper respiratory tract were not performed.
Previous evaluation	Yes, accepted in the RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The carcinogenic potential of Glyphosate technical was assessed in an 18-month feeding study in male and female CD-1 mice. Groups of 51 mice per sex received daily dietary doses of 0, 500, 1500 and 5000 ppm Glyphosate technical (equivalent to an average intake of 84.7, 266.8 and 945.6 mg/kg bw/day). Observations covered clinical signs, body weight, food and water consumption, palpation of masses, organ weights, necropsy and histopathological examination. The latter involved examination of all sampled organ tissues for all control and high dosage group animals killed at termination. In addition, differential white blood cell counts were performed for animals that were killed or died in extremis and for selected animals at twelve and eighteen month of treatment. The dose-levels were chosen based on available toxicity data.

There were no treatment-related deaths or clinical signs in any of the dose-groups. In the carcinogenicity study, survival after 78 weeks of treatment was 76, 80, 76 and 69 % in males and 73, 75, 75 and 78 % in females in the control through high dosage groups, respectively.

There were no treatment-related effects on body weight gain or food and water consumption noted. No significant treatment-related effects were noted on differential white blood cell counts in both sexes. There were no treatment-related trends in the proportion of masses observed, number of mice affected or time to appearance of palpable masses. Gross pathology, organ weight data and histopathological examination revealed no treatment-related effects.

I. MATERIALS AND METHODS

A: Materials

1. Test material: Glyphosate technical

Identification: Glyphosate

Description: White crystalline solid

Lot/Batch #: H05H016A

Purity: 95.7 %

Stability of test compound:	Expiry: 2008-03-25

2. Vehicle and/ or positive control: Diet

3. Test animals:

Species:	Mouse
Strain:	CD-1, Crl:CD-1 (ICR) BR
Source:	[redacted]
Age:	Approx. 5 – 6 weeks
Sex:	Males and females
Weight at dosing:	Males: 22 – 32 g, females: 18 – 28 g
Acclimation period:	At least ten days
Diet/Food:	Rat and Mouse SQC Ground diet No. 1, Special Diet Services Limited, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Initially in groups of three per sex in polypropylene solid-floor cages.
Environmental conditions:	Temperature: 21 ± 2 °C Humidity: 55 ± 15 % Air changes: at least 15/hour 12 hours light/dark cycle

B: Study design and methods

In life dates: 2005-10-10 to 2007-11-19

Animal assignment and treatment:
In a carcinogenicity feeding study groups of 51 CD-1 mice per sex received daily dietary doses of 0, 500, 1500 and 5000 ppm (equivalent to mean achieved dose levels of 0, 84.7, 266.8 and 945.6 mg/kg bw/day) Glyphosate technical in diet. Additional 12 mice per sex, designated for veterinary controls, were housed and maintained alongside treated animals. Ten animals per sex from each group were set aside for an interim kill (toxicity assessment), which was carried out on the survivors after 39 weeks of dosing. The remaining 50 mice per sex and dose-level were dosed for a maximum of 79 weeks (carcinogenicity assessment).

Test diets were prepared prior to start of treatment and then weekly by mixing a known amount of the test substance with a small amount of basal diet and blending for 19 minutes. This pre-mix was then added to larger amount of basal diet and blended for further 30 minutes.

The stability and homogeneity of the test material in diet were determined. Samples of each dietary admixture were analysed for achieved concentration monthly for the first six months and then every three months thereafter.

Clinical observations
A check for clinical signs of toxicity, ill health and behavioural changes was made once daily on all mice and recorded weekly. Observations for morbidity, and mortality were made twice daily. Additional unscheduled examinations were performed on animals that showed ill-health.
All surviving animals were palped weekly for size, position and appearance of new or existing masses.

Body weight
Individual body weights were recorded on Day 1 (prior to treatment) and at weekly intervals until the end

of week 13 and every 4 weeks thereafter until termination. Body weights were also determined before sacrifice. Bodyweight data were reported only until Week 77.

Food consumption and compound intake
Food consumption was recorded once weekly for each cage group from Week 1 to Week 13 and subsequently over one week in every 4 weeks until termination. Food consumption data were reported only until Week 77. Food efficiency and compound intake was calculated from the recorded food consumption data.

Water consumption
Water intake was observed daily, for each cage group, by visual inspection of the water bottles for any overt changes.

Haematology
Blood smear samples were collected after 12 months and at termination from all animals, and from mice that were killed in extremis. Differential white cell counts were performed on all control and high-dose animals and on the animals killed in extremis.

Sacrifice and pathology
All animals that died or were killed in extremis during the conduct of the study, and all animals sacrificed t scheduled termination were subjected to a gross pathological examination. Any macroscopic findings were recorded.
The following organ weights were determined from 10 mice per sex per group: adrenals, brain, epididymides, heart, kidneys, liver, lungs, ovaries, spleen and testes.
Tissue samples were taken from the following organs and preserved in buffered formalin: adrenals, aortic (thoracic), bone & bone marrow (sternum and femur (incl. stifle joint)), brain (incl. cerebrum, cerebellum pons), caecum, colon, duodenum, epididymides, eyes (with optic nerve), gross lesions incl. palpable masses, head (incl. pharynx, nasopharynx and paranasal sinuses), heart, Harderian and lacrimal glands, ileum, jejunum, kidneys, larynx, liver and gall bladder, lungs (with bronchi), mammary gland, lymph nodes (cervical and mesenteric), muscle (skeletal), oesophagus, ovaries, pancreas, pituitary, preputial gland, prostrate, rectum, salivary glands, sciatic nerve, seminal vesicles, skin (hind limb), spinal cord (cervical, mid-thoracic and lumbar), spleen, stomach, testes, thymus, thyroid/parathyroid, tongue, trachea, urinary bladder, uterus and vagina.

A detailed histopathological examination was performed on all sampled tissues of the control and high-dose animals, and on animals that died or were killed in extremis. In addition, tissues of the liver, lungs and kidneys, as well as gross macroscopic lesions and palpable masses from low and intermediate dose groups at termination were examined microscopically.

Statistics
All data were summarised in tabular form and analysed by computerised analysis using ProvantisTM Tables and Statistics Module. For each variable the of variance incorporating Student´s t-test and F-test. For each variable the most suitable transformation of data was found, the use of possible covariates checked and the homogeneity of means assessed using ANOVA or ANOVA and Bartlett´s test. The lowest treatment-related significant effects were determined using the Williams Test for parametric data or the Shirley Test for non-parametric data. If no response is found, but the data showed non-homogeneity of means, data were further analysed by a stepwise Dunnet (parametric) or Steel (non-parametric) test to determine significant differences from control. If required, pair-wise tests are performed using Students t-test (parametric) or the Mann-Whitney U test (non-parametric).

The levels of probability chosen as significant were $p < 0.01$** and $p < 0.05$*.

Histopathology data were analysed using Chi squared analysis (differences in the incidence of lesions occurring with an overall frequency of 1 or greater) and the Kruskal-Wallis one-way non-parametric

analysis of variance (comparison of severity grades).

The levels of probability chosen as significant were $p < 0.001$, $p < 0.01$, $p < 0.05$, and $p < 0.1$.

Results

A. ANALYSIS OF DOSE FORMULATIONS

Analyses for homogeneity and stability indicated that the dose preparations were homogeneous and stable for at least six weeks. Analyses for achieved concentration demonstrated that the mean prepared dietary admixture concentrations were within ± 5 % of the nominal concentration for all but 1 sample (500 ppm – level), which was + 10 % of the nominal concentration.

The group mean achieved doses are summarised below.

Table 5.5-92: Glyphosate technical: Dietary Carcinogenicity Study in the Mouse ([redacted] 2009b): Group mean achieved dose levels

Dose group	Dietary concentration (ppm)	Achieved dose level (mg/kg bw/day)*				
		Males		Females		Overall mean
		Mean	Range	Mean	Range	
1 (control)	0					
2 (low)	500	71.4	33 – 104	97.9	55 – 155	84.7
3 (mid)	1500	234.2	101 – 365	299.5	176 – 466	266.8
4 (high)	5000	810	461 - 1143	1081.2	610 - 1728	945.6

* based on actual food intake and body weight data

The results show a higher test material intake for females when compared to males for each dose level. Highest intakes were achieved within the first few treatment weeks, with subsequent decline thereafter. The mean intake for each dose group is therefore 84.7, 266.8 and 945.6 mg/kg bw/day for 500, 1500 and 5000 ppm, respectively.

B. MORTALITY

No treatment-related effects on the deaths occurred during the study, as well as no treatment-related effects on the time of death. From three male mice that were killed in extremis, examination results suggest that the morbidity of these animals was due to fighting between cage mates.

Table 5.5-93: Glyphosate technical: Dietary Carcinogenicity Study in the Mouse ([redacted] 2009b): Cumulated mortalities after 78-week dietary exposure to Glyphosate technical

Sex	Dose group (ppm)			
	0	500	1500	5000
Male	12 (6)	10 (8)	12 (6)	16 (6)
Female	14 (10)	13 (7)	13 (10)	11 (8)

() number of animals killed in extremis

The percentage of survival in each of the dose groups are summarised below.

Table 5.5-94: Glyphosate technical: Dietary Carcinogenicity Study in the Mouse ([redacted] 2009b): Percentage survival at termination after 78-week dietary exposure to Glyphosate technical

Sex	Dose group (ppm)			
	0	500	1500	5000
Male	76	80	76	69
Female	73	75	75	78

C. CLINICAL OBSERVATIONS

There were no significant treatment-related clinical signs of toxicity observed.

There were no trends in the proportion of palpable masses observed during the study period. A significant proportion observed showed evidence for regression before the animal reached the point of death or termination. Based on the results (see **Table 5.5-95**) no treatment-related effect on the development of palpable masses is seen for either sex. The slight increase in the mean number of masses per animal for high-dose females and mid-dose males was considered a coincidence. The median time to appearance of palpable masses was comparable for all dose groups of either sex.

Table 5.5-95: Glyphosate technical: Dietary Carcinogenicity Study in the Mouse ([redacted] 2009b): Group summary of palpable masses

Dose	Total number of animals in group		Number of animals with palpable masses		Total number of masses per group		Mean number of masses per animal		Median time (weeks) to appearance of masses	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
0	51	51	28	23	45	38	0.88	0.75	42.00	45.75
500	51	51	↑32	↑28	↑49	↑49	↑0.96	↑0.96	42.00	↑46.08
1500	51	51	↑39	23	↑60	38	↑1.20	0.75	↑42.43	↓44.83
5000	51	51	↓25	23	↑49	↑51	↑0.96	↑1.00	↓41.67	↓42.50

D. BODY WEIGHT

There were no treatment-related effects on male and female overall body weight gain during the conduct of study.

E. FOOD CONSUMPTION AND COMPOUND INTAKE

There were no treatment-related effects on food consumption for either sex noted during the study.

F. WATER CONSUMPTION

There were no treatment-related effects on water consumption for either sex noted during the study.

G. HAEMATOLOGY AND CLINICAL CHEMISTRY

There were no significant treatment related effects on haematology parameters that were consistently observed for animals of both sexes that persisted through the course of the study. Similarly, clinical chemistry did not reveal any consistent changes except ALP activities that were elevated in both sexes and at various time points at the high dose.

At 5000 ppm there was an increase in alkaline phosphatase activity for satellite group males and females compared with controls at 6 and 12 months ($p<0.01$). Main group males were also affected at 18 months ($p < 0.05$). The magnitude of the effect does not appear to increase with age. Furthermore, the lack of a consistent effect for females indicates that this effect is of limited toxicological importance.

Table 5.5-96: Glyphosate technical: Dietary Carcinogenicity Study in the Mouse ([redacted] 2009b): Clinical chemistry findings (group mean values)

ALP [IU/L]	Dose group (mg/kg bw/day)							
	0		500		1500		5000	
	♂	♀	♂	♀	♂	♀	♂	♀
Month 6	88 ± 26	50 ± 18	95 ± 24	63 ± 22	103 ± 31	62 ± 21	129 ± 33**	92 ± 28**

Month 12	88 ± 24	46 ± 18	97 ± 22	60 ± 16	116 ± 45	58 ± 10	140 ± 47**	91 ± 24**
Month 18	93 ± 37	66 ± 31	111 ± 15	56 ± 16	111 ± 30	71 ± 41	125 ± 31*	93 ± 47
Month 24	107 ± 33	66 ± 25	99 ± 19	59 ± 21	101 ± 55	82 ± 34	112 ± 35	87 ± 42

* $p < 0.05$
** $p < 0.01$

H. NECROPSY

Gross pathology

There were no treatment-related macroscopic findings observed for any mice sacrificed at termination or mice that died or were killed in extremis during the study period.

Organ weights

There were no treatment-related findings observed in organ weights or relative organ weights.

Histopathology

There were no treatment-related histopathological findings observed in any dose group of either sex.

3. Assessment and conclusion

Assessment and conclusion by applicant: In conclusion, Glyphosate technical was not carcinogenic in the CD-1 mouse following continuous dietary exposure of up to 945.6 mg/kg bw/day (average for both sexes) for 18 months. The NO(A)EL for toxicity was 810 mg/kg bw/day for male mice and 1081 mg/kg bw/day for female mice, the highest dosage tested.
No non-neoplastic or neoplastic treatment-related findings were observed.
No increases in malignant lymphoma or salivary gland histopathological findings were observed.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.5/016
Report author	[redacted]
Report year	2001
Report title	Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice
Report No	Toxi: 1559.CARCI-M
Document No	NA
Guidelines followed in study	OECD 451 (1981)
Deviations from current test guideline (OECD 451, 2018)	Yes, animals were observed for mortality only once per day; food consumption was not measured monthly after the first 13 weeks; histopathological examination of the cervix, Harderian gland, ileum, lacrimal gland, mammary glands, upper respiratory tract and vagina were not performed. The statistical evaluation of incidences of non-neoplastic and neoplastic lesions were evaluated using the Z-test, which is inappropriate for the analysis of tumour incidence data (the Z-test assumes a normal distribution). Peto's incidental tumour analysis was performed without assigning a Petocode to the neoplasms, which is inappropriate for the

	application of this test. The Cochran-Armitage test was only applied to the tumour incidences in the high dose group and the control group, which is inappropriate for a trend test, where all dose levels should be considered. To address these issues, it was decided to re-evaluate the statistical significance of all tumour incidence data, starting from the raw data tables of this report and applying appropriate statistical methods (see CA 5.5-017)
Previous evaluation	Yes, accepted in the RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The carcinogenic potential of glyphosate technical was assessed in an 18-month feeding study in male and female Swiss albino mice. Groups of 50 mice per sex received daily dietary doses of 0, 100, 1000 and 10000 ppm glyphosate technical (equivalent to an average intake of 14.7, 150.5 and 1460.3 mg/kg bw/day). Observations covered survival, clinical signs, neurological changes, body weight, food- and water consumption, ophthalmological examinations, masses formation, blood smears with differential count analysis, organ weights, necropsy and histopathological examination. The latter involved examination of all sampled organ tissues and lesions for all control and high dosage group animals died, sacrificed moribund or killed at termination.

The survival after 18-month of treatment was 56, 60, 56 and 46 % in males and 68, 68, 60 and 60 % in females in the control through high dosage groups, respectively. The mortality (combined for both sexes) was slightly increased at the high dose level with 38, 36, 42 and 47 % for the control, low, mid- and high-dose group, respectively. Despite being in the upper range of the historical control data for mortality, the mortality in the high dose is not considered to be mediated by the administration of the test substance. There were no treatment-related effects on clinical signs, behaviour, eyes, body weight, body weight gain, food consumption, and differential white blood cell counts in both sexes. Gross pathology, organ weight data and histopathological examination demonstrated no treatment-related effects. The number of malignant lymphoma, the most common tumour in the mouse, was slightly elevated in the high dose group compared to control, but this was considered as incidental background variation based on historical control data and in agreement with the study director.

I. MATERIALS AND METHODS

A: Materials

1. Test material: Glyphosate technical

Identification: Glyphosate

Description: Solid white, odourless crystals

Lot/Batch #: 01/06/97

Purity: > 95.14 % (w/w)

Stability of test compound:	Expiry: December 1999
2. Vehicle and/ or positive control:	Diet
3. Test animals:	
Species:	Mouse
Strain:	Swiss albino, HsdOla: MF1
Source:	[redacted]
Age:	6 weeks
Sex:	Males and females
Weight at dosing:	Males: 25 – 47 g, females: 21 – 26 g
Acclimation period:	5 days
Diet/Food:	Ssniff rat/mouse powder food maintenance meal – low in germs (M/s Ssniff Spezialdiäten, D-59494 Soest, Germany), *ad libitum*
Water:	Well water passed through activated charcoal filter and exposed to UV rays, *ad libitum*
Housing:	In groups of five per sex in polypropylene mouse cages with stainless steel top grill and steam sterilised clean paddy husk bedding.
Environmental conditions:	Temperature: 19 - 25 °C
	Humidity: 30 - 70 %
	Air changes: 12 - 15/hour
	12 hours light/dark cycle

B: Study design and methods

In life dates: 1997-12-23 to 1999-06-29

Animal assignment and treatment:
In a carcinogenicity feeding study groups of 50 Swiss albino mice per sex received daily dietary doses of 0, 100, 1000 and 10000 ppm (equivalent to mean achieved dose levels of 0, 14.5, 149.7 and 1453 mg/kg bw/day for males, and 0, 15.0, 151.2 and 1466.8 mg/kg bw/day for females) glyphosate technical in diet for 18 month. The dose levels were chosen based on results of a 50-day pre-study in mice. Test diets were prepared prior to start of treatment and then in intervals ranging from 10 to 23 days. Diets were prepared in quantities of 10, 12 or 15 kg. For preparation of 12 kg diet mixtures 1.2 g, 12 g and 120 g for the low-, mid- and high-dose group, respectively, of the test substance was mixed with approximately with 0.5 kg basal diet and blended for 3 minutes. This pre-mix was then mixed manually with approximately 0.5 kg food and then added in portions to the remaining bulk amount of food (approximately 11.0 kg) and blended in a stainless steel ribbon mixer for 20 minutes.

The homogeneity of the test material in diet was determined at beginning of treatment, and at 12 and 18 month. Analyses for achieved concentration were done at three and six month of the study. The stability of glyphosate technical in the diet was determined prior to start of the study for the 100 and 10000 ppm dose levels.

Clinical observations
A detailed veterinary examination of all mice was done before and after grouping and monthly thereafter. A check for clinical signs of toxicity, appearance, behaviour, and neurological changes and mortality was made once daily on all mice. For mice with observed tumours a separate record was maintained with details of the tumour development.

Body weight
Individual body weights were recorded on Day 1 (prior to treatment) and at weekly intervals until the end of week 13 and every 4 weeks thereafter until termination.

Food consumption and compound intake
Food consumption was recorded once weekly for each cage group from week 1 to week 13 and subsequently at weeks 26, 39, 52, 65 and 78. Food efficiency and compound intake was calculated from the recorded food consumption data.

Haematology
Blood smear samples were collected at 9 month and at termination (18 month) from all surviving animals, and from mice that were killed in extremis. Differential white cell counts were performed on all blood smear samples.

Ophthalmological examination
Ophthalmological examinations were performed on all mice prior to start of treatment at 6, 12 and 18 month of the study. Mydriasis was induced before examination by adding 1 % Tropicamide solution into the eyes. All other grossly visible eye findings were recorded also at the daily observations.

Sacrifice and pathology
All animals that died or were killed in extremis during the conduct of the study, were necropsied immediately or preserved in 10 % buffered neutral formalin until necropsy.

All surviving mice were sacrificed at scheduled termination. A gross pathological examination was performed on all mice. Any macroscopic findings were recorded.

The following organ weights were determined from 10 mice per sex per group: adrenals, kidneys, liver and gall bladder, ovaries and testes.

Tissue samples were taken from each mice from the following organs and preserved in 10 % buffered neutral formalin: adrenals, bone & bone marrow (sternum and femur (incl. joint)), brain (incl. cerebrum, cerebellum pons), caecum, colon, duodenum, epididymides, eyes (with optic nerve), heart, jejunum, kidneys, larynx, liver and gall bladder, lungs, lymph nodes (mandibular, mesenteric, and superficial inguinal), muscle (femoral), oesophagus, ovaries, pancreas, pituitary, prostrate, rectum, salivary glands, sciatic nerve, seminal vesicles and coagulating glands, skin, spinal cord (cervical, mid-thoracic and lumbar), spleen, stomach, testes, thymus, thyroid/parathyroid, trachea, urinary bladder, uterus and all lesions and tumours/masses.
A detailed histopathological examination was performed on all sampled tissues of the control and high-dose animals, and on animals that died or were killed in extremis. In addition, tissues of gross lesions and masses from all mice were examined microscopically.

Statistics
Body weight, body weight gain, food consumption and differential leukocyte counts of different groups were compared by Bartlett´s test for homogeneity of intra group variances. Heterogeneous data were transformed using log transformation. Data with homogeneous intra group variances were subjected to one-way analysis of variance using ANOVA. When "F" values were significant, Dunnetts pair wise comparison of means of treated groups with control means was done individually.

Incidence of gross lesions and non-neoplastic histopathological changes and incidences of benign and malignant neoplasms in the test substance groups were statistically compared with control group by Z-test where necessary. The incidence of neoplasms was analysed by Cochran-Armitage linear trend test, Life table analysis for fatal tumour incidence and Peto´s incidental tumour analysis. When a significant difference over the control group was observed in any of the treatment groups, the dose correlation co-

efficient was estimated and subjected to t-test. All analyses and comparisons were evaluated at the 5 % level and statistically significant differences ($p \leq 0.05$) were indicated

II. RESULTS

A. ANALYSIS OF DOSE FORMULATIONS

Stability analyses indicated that the dose preparations were stable for up to 30 days with a loss 8.37 % at the 100 ppm level and 6.99 % at the 10000 ppm level, when stored at room temperature in PE bags inside stainless steel drums.

Analyses for homogeneity at the start and at 12 and 18 month of treatment indicated that the dose preparations were homogeneous. Analyses for achieved concentration demonstrated that the mean prepared dietary admixture concentrations were within ± 10 % of the nominal concentration for all diet samples. The overall mean achieved concentrations were 94.0 ± 1.66, 949.5 ± 15.84 and 950.7 ± 142.28 as compared to the nominal concentrations of 100, 1000 and 10000 ppm, respectively.

B. MORTALITY

The cumulated pre-terminal deaths (including moribund sacrifice) are summarised in the table below.

Table 5.5-97: Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice ([redacted], 2001): Cumulated mortalities after 78-week dietary exposure to glyphosate technical

Sex	Historical control#		Dose group (ppm)**			
	min- max*	Mean ± SD	0	100	1000	10000
Male	11/50 – 27/50	18 ± 5	22 (6)	20 (6)	22 (8)	27 (8)
Female	12/50 – 20/50	16 ± 3	16 (7)	16 (7)	20 (2)	20 (3)
Combined sex	12/100 – 47/100	17 ± 4	38 (13)	36 (13)	42 (10)	47 (11)

\# Derived from the control groups of 9 studies performed in the timeframe embracing the study summarised here
* Number of dead animals / total number of animals
** Total number of animals per group = 50
() number of animals killed in extremis

The percentage of survival in each of the dose groups are summarised below.

Table 5.5-98: Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice ([redacted], 2001): Percentage survival at termination after 18-month dietary exposure to glyphosate technical

Sex	Dose group (ppm)			
	0	100	1000	10000
Male	56	60	56	46
Female	68	68	60	60
Combined	62	64	58	53

The survival percentage was slightly decreased at the high dose level, but the decrease did not attain statistical significance.

As can be seen from the historical control data, the mortality in the high-dose group is, at the upper end, but within the historical control range. The number of animals that were killed in extremis were comparable or higher in the controls compared to the treated groups.

C. CLINICAL OBSERVATIONS

There were no significant treatment-related clinical signs of toxicity observed.

D. BODY WEIGHT

There were no significant treatment-related effects on male and female body weight and overall body weight gain during the conduct of study.

In males incidences of slightly decreased body weights in week 10 at 100 ppm and in months 7 and 8 at 1000 ppm were considered incidental, since no effects on body weights were observed in the high-dose group.

In females receiving 100 ppm, decreased net body weight gain (85 % of control) was observed in month 18 only. At 1000 and 10000 ppm the body weight gain was 89 % and 99 % of the control. Therefore, this finding was also considered as incidental.

E. FOOD CONSUMPTION AND COMPOUND INTAKE

There were no treatment-related effects on food consumption for either sex noted during the study.

The observed slightly lower food consumption observed in males in week 1 at 100 ppm and in weeks 1 and 7 at 10000 ppm was considered incidental, since the changes were minimal and the effects was not consistent during the remaining parts of the study period.

In females lower food consumptions were observed in week 2 for all dose levels, in week 26 at 10000 ppm. Higher food consumption occurred in week 11 at 100 ppm and in weeks 3 and 4 at 10000 ppm. These findings were also considered incidental, since the changes were minimal and food consumption during the remaining parts of the study was comparable with the control group.

The calculated mean daily test substance intake is summarised in the **Table 5.5-99** below.

Table 5.5-99: Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice ([redacted] 2001): Group mean compound intake levels

Dose group	Dietary concentration (ppm)	Mean daily test substance intake (mg/kg bw/day)*		
		Males	Females	Combined
1 (control)	0	0.0	0.0	0.0
2 (low)	100	14.5	15.0	14.7
3 (mid)	1000	149.7	151.2	150.5
4 (high)	10000	1453.8	1466.8	1460.3

* based on actual food intake and body weight data

F. HAEMATOLOGY

Differential leukocyte counts at 9 and 18 month

There were no significance treatment-related changes in the white blood cell counts for either sex at both 9 and 18 month. Slightly higher neutrophil counts and slightly lower lymphocyte counts in high dose males at 9 month were within the historical control ranges. The slightly higher eosinophil counts, higher neutrophil and monocyte counts, as well as slightly lower lymphocyte counts observed in high dose females at 18 month were comparable with historical control values and therefore considered incidental.

Differential leukocyte counts of moribund sacrificed mice

Although the differential leukocyte count data were not statistically analysed, they appeared to be within the range of biological variation.

G. OPHTHALMOLOGICAL EXAMINATION

There were no treatment-related findings observed at the ophthalmological examinations performed at 6,

12 and 18 month of treatment.

H. NECROPSY

Gross pathology

There were no treatment-related macroscopic findings observed for any mice sacrificed at termination or mice that died or were killed in extremis during the study period.

In animals found dead or sacrificed moribund across control and all dose levels the incidence of enlargement of superficial inguinal lymph nodes and thymus in mid dose females and in the high dose for combined sexes was statistically significant increased. These enlargements were associated with neoplasms of the hemolymphoreticular system. Other changes included enlargement of various lymph nodes, and thymus, both associated with neoplasms of the hemolymphoreticular system, enlargement of the spleen, associated with neoplasia and amyloidosis and increased extramedullary haematopoiesis. The low incidence of observed liver enlargements was associated with neoplasia and amyloidosis. However, none of these findings were dose-dependent.

In mice sacrificed at termination the following changes were observed: Kidney surface rough/uneven in high dose males, discoloration / enlargement of mesenteric lymph nodes in high dose females and discoloration in high dose combined sex, and enlargement of spleens in both sexes combined at the high dose were significantly higher than in control mice. Since none of these changes showed a dose-dependency, and the corresponding histopathological changes were not significantly higher in these groups, the findings were considered incidental.

Organ weights

There were no treatment-related findings observed in organ weights or relative organ weights.

Histopathology

There were no treatment-related histopathological findings observed in any dose group of either sex.

In mice found dead or sacrificed moribund during the study period the following significant histopathological changes were seen. Cystic glands of the stomach were significantly increased in high dose mice of both sexes combined.

There was a more frequent occurrence of cystic glands of the stomach in male mice. The effect becomes more evident when the incidences in animals found dead or moribund and those sacrificed at scheduled termination are combined. The difference between the treated groups is not large and, taking into account the large dose spacing, a clear dose response may be doubted but, according to the study author, the incidence was higher than the historical control data. Unfortunately, this historical control data was notpresented in the report. In contrast, no increase became apparent in females.

Table 5.5-100: Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice ([redacted] 2001): Summary of non-neoplastic histopathological findings (cystic gland), total incidence

	Dietary concentration of glyphosate (ppm)											
	Males				Females				Combined sex			
Finding	**0**	**100**	**1000**	**10000**	**0**	**100**	**1000**	**10000**	**0**	**100**	**1000**	**10000**
Number examined	50	50	50	50	50	16	20	50	100	66	70	100
Stomach Cystic glands (n)	17	27	31	33	23	4	5	25	40	31	36	58
Cystic glands, incidence [%]	34	54	62	66	46	25	25	50	40	47	51	58
Cystic glands, HCD [%], range (determined	0 - 45				0 - 35				0 - 39			

Table 5.5-100: Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice ([redacted] 2001): Summary of non-neoplastic histopathological findings (cystic gland), total incidence

	Dietary concentration of glyphosate (ppm)											
	Males				Females				Combined sex			
Finding	**0**	**100**	**1000**	**10000**	**0**	**100**	**1000**	**10000**	**0**	**100**	**1000**	**10000**
from 5 studies)												

Based on the incidences and the statistical significance mentioned for male animals, there was no NOEL in this study because it cannot be excluded that this finding was due to treatment. The clinical relevance of cystic glands of the stomach is not clear. In any case, there was no increase in severity (always minimal to mild) and, more important, the cysts formation did not progress to any other pathological lesion, even at a dose level that was 100 times higher than the lowest. Thus, this finding should not be taken into account when the NOAEL for this study is set.

As can be seen in the tables below, an increase in malignant lymphoma was noted in both the male and female groups receiving the highest dose. The incidence was statistically significantly elevated as compared to the actual control groups in this study, was above the mean values of the (relatively small) historical control and, for males, outside the historical control range. Even though malignant lymphoma is a common tumour in mice (accounting for 54.6 % of all tumours in this study), it cannot be completely excluded that the higher incidence in the top dose groups were somehow related to treatment. The RMS conclusion is that there was limited evidence of a carcinogenic potential of glyphosate in this mouse strain at the very high dose level of 10000 ppm (about 1460 mg/kg bw/day for sexes combined) in this study, with male animals being more affected. The NOAEL should be set at the mid dose level of ca 150 mg/kg bw/day.

Increased haematopoiesis was seen in the bone (femur) of high dose males, mid- and high-dose animals combined sex. Cell debris in tubules of epididymides was increased in mid dose males and the incidence of sub-capsular cell hyperplasia was increased in adrenals of low dose males. In addition, the incidence of kidney nephropathy in mid-dose females, as well as the incidence of lymphocyte infiltration of epididymides in mid dose males was significant decreased. All these findings were also observed at lower doses and/or were not dose dependent. Thus, these findings were also considered incidental.

In mice sacrificed at termination the following more frequent observed changes were observed: Cystic glands of the stomach were significantly increased in low-, mid- and high-dose males. However, this finding was not dose-dependent. Furthermore, high incidences were observed in females of the control group when compared to the HCD data.

Degenerative heart changes were higher in high-dose males and females, and significant higher in combined sex. Since the incidences were similar or slightly higher than historical controls, and no dose-dependency was observed this finding is considered incidental. The number of malignant lymphoma was slightly elevated in the high dose group compared to control. This tumour of the hemolymphoreticular system is one of the most common tumours of mice accounting for the highest percentage of spontaneous tumours in this species. Therefore, the observed tumour incident is considered incidental and not treatment-related. In addition, there was no increase with dose, and the incidences of this tumour varied with sex and fate (i.e. pre-terminal and terminal deaths). In mandibular lymph nodes lymphoid hyperplasia was significantly increase in low and mid-dose males and combined sex, whereas the incidence was significantly lower in high dose females. In addition, extramedullary haematopoiesis was significantly increased in these lymph nodes at the mid-dose level in combined sex. In spleen extramedullary haematopoiesis was significantly increased in females and combined sex at the low dose level. In the absence of any dose-relation these findings, as well as several not statistically significant changes considered incidental (see tables below).

Table 5.5-101: Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice ([redacted], 2001): Summary of non-neoplastic histopathological findings for dead and moribund animals

Finding	Dietary concentration of glyphosate (ppm)											
	Males				Females				Combined sex			
	0	100	1000	10000	0	100	1000	10000	0	100	1000	10000
Number examined	22	20	22	27	16	16	20	20	38	36	42	47
Stomach Cystic glands (n)	8	8	9	16	1	4	5	6	9	12	14	↑22+
Cystic glands, incidence [%]	36	40	41	59	6	25	25	30	24	33	33	47
Cystic glands, HCD [%], range (determined from 5 studies)	0 - 45				0 - 35				0 - 39			
Kidney Nephropathy (n)	9	7	10	12	5	1	1	3	14	8	11	15
Bone (femur) Increased haematopoises (n)	1	1	↑8+	5	0	1	2	3	1	2	↑10+	↑8+
Epididymes Cell debris in tubules (n)	0	1	4	0	--	--	--	--	--	--	--	--
Epididymes Lymphocyte infiltration (n)	4	1	0	3	--	--	--	--	--	--	--	--
Heart Degenerative changes (n)	11	14	13	16	4	2	4	1	15	16	17	17
Adrenals sub-capsular cell hyperplasia (n)	3	↑8+	7	10	12	11	13	15	15	19	20	25
Mandibular LN extramedullary haematopoiesis (n)	3	2	5	3	1	1	1	2	4	3	6	5

n Number of animals affected
LN Lymph Node
+ significantly increased;
-- not examined/determined

Table 5.5-102: Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice ([redacted], 2001): Summary of non-neoplastic and neoplastic histopathological findings at termination

Finding	Dietary concentration of glyphosate (ppm)											
	Males				Females				Combined sex			
	0	100	1000	10000	0	100	1000	10000	0	100	1000	10000
Stomach(N)	28	30	28	23	34	-	-	30	62	64	58	53
Cystic glands (n)	9	↑19+	↑22+	↑17+	22	-	-	19	31	-	-	36
Cystic glands, incidence [%]	32	63	79	74	65	-	-	63	50			68
Cystic glands, HCD [%], range (determined from 5 studies)	0-38				0-24				0-32			
Kidney(N)	28	6	4	23	34	2	1	30	62	64	58	53

Table 5.5-102: Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice ([redacted] 2001): Summary of non-neoplastic and neoplastic histopathological findings at termination

Finding	Dietary concentration of glyphosate (ppm)											
	Males				Females				Combined sex			
	0	100	1000	10000	0	100	1000	10000	0	100	1000	10000
Nephropathy (n)	7	4	3	6	5	2	0	2	12	6	3	8
Bone (femur) (N)	28	-	-	23	34	2	1	30	62	--	--	53
Increased haematopoises (n)	1	-	--	0	1	0	0	2				
Epididymes (N)	28	1	-	23	--	--	--	--	--	--	--	--
Lymphocyte infiltration (n)	0	0	--	1	--	--	--	--	--	--	--	--
Mandibular LN (N)	28	30	28	23	34	33	28	30	62	64	58	53
extramedullary haematopoiesis (n)	5	7	9	9	3	9	7	4	8	16	16+	13
Heart (N)	28	2	--	23	34	--	-	30	62	--	--	53
Degenerative changes (n)	14	1	--	17	2	--	-	6	16	--	--	23+
Adrenals (N)	28	--	--	23	34	-	-	29	62	--	--	53
sub-capsular cell hyperplasia (n)	15	--	--	13	27	--	--	22	42	--	--	35
Hemolymphoreticular system (N)	28	30	28	23	34	34	30	30	62	64	58	53
malignant lymphoma (n)	1	3	3	6	9	10	6	13	10	13	9	19

N Number examined
n Number of animals affected
LN Lymph Node
+ significantly increased
-- not examined/determined

Table 5.5-103: Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice ([redacted] 2001): Incidences of malignant lymphoma and comparison with historical control data

			Dietary concentration of glyphosate (ppm)							
			Males				Females			
	♂	♀	0	100	1000	10000	0	100	1000	10000
Dead & moribund										
Number examined	75	77	22	20	22	27	16	16	20	20
Number affected	20	49	9	12	13	13	9	10	13	12
Percentage affected	26.7	63.6	41.0	↑60.0+	↑59.0+	48.0	56.0	63.0	65.0	60.0
Mean %	26	61.8	--	--	--	--	--	--	--	--
Range %	0-44	0-100	--	--	--	--	--	--	--	--
			--	--	--	--	--	--	--	--
Terminal sacrifice										
Number examined	175	175	28	3028	23	34	34	30	30	28
Number affected	26	50	1	3	3	↑6+	9	10	6	13
Percentage affected	14.9	28.9	3.6	10.0	10.7	↑26.1+	26.5	29.4	20.0	↑43.3+
Mean %	14.8	28.8	--	--	--	--	--	--	--	--
Range %	8-24	20-43	--	--	--	--	--	--	--	--
			--	--	--	--	--	--	--	--
All fates										
Number examined	250	250	50	50	50	50	50	50	50	50

Table 5.5-103: Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice ([redacted] 2001): Incidences of malignant lymphoma and comparison with historical control data

			Dietary concentration of glyphosate (ppm)							
			Males				Females			
	♂	♀	0	100	1000	10000	0	100	1000	10000
Number affected	46	99	10	15	16	↑19+	18	20	19	25
Percentage affected	18.4	39.6	20.0	30.0	32.0	↑38.0+	36.0	40.0	38.0	↑50.0+
Mean %	18.4	41.6	--	--	--	--	--	--	--	--
Range %	6-30	14-58	--	--	--	--	--	--	--	--

+ significantly increased
-- not examined/determined

3. Assessment and conclusion

Assessment and conclusion by applicant:
In conclusion, glyphosate technical was not carcinogenic in Swiss albino mice following continuous dietary exposure of up to 1460.3 mg/kg bw/day (average for both sexes) for 18 months. Based on, a more frequent occurrence of cystic glands of the stomach in male mice and an increase in malignant lymphoma was noted in both the male and female groups receiving the highest dose mortality at the upper limit of the historical control range, the NOAEL in mice after chronic exposure to Glyphosate technical for 18 month is conservatively set at 1000 ppm, corresponding to 149.7 mg/kg bw/day for males, 151.2 mg/kg bw/day for females and 150.5 mg/kg bw/day for both sexes combined.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.5.017
Report author	[redacted]
Report year	2017
Report title	Statistical Evaluation of Pre-Neoplastic and Neoplastic Lesions from Study: Study No. TOXI: 1559.CARCI-M; Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice
Report No	11921
Document No	90017583
Guidelines followed in study	No guideline followed
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

This is a re-evaluation of the statistical analysis of histopathology findings recorded in the [redacted] Study No. TOXI: 1559.CARCI-M, CD-1, [redacted], 2001 (see CA 5.5/016), in which an increased incidence of malignant lymphoma was reported.

Groups of 50 male and 50 female Swiss albino HsdOla:MF1 mice were exposed to glyphosate technical (glyphosate acid) in the diet at 0 (control), 100, 1000 or 10000 ppm for 18 months. The incidences of non-neoplastic and neoplastic lesions were statistically analysed using the Z-test (pair-wise comparisons). The Cochran-Armitage linear trend test, Peto's incidental tumour analysis and life table analysis for fatal tumours were used for the statistical analysis of the neoplastic lesions.

The statistical evaluation performed within the report of [redacted] (2001) has to be considered inadequate and generally lacks clarity.

Non-neoplastic and neoplastic lesions were evaluated using the Z-test, which is inappropriate for the analysis of tumour incidence data (the Z-test assumes a normal distribution). Peto's incidental tumour analysis was performed without assigning a Peto code to the neoplasms, which is inappropriate for the application of this test. The Cochran-Armitage test was only applied to the tumour incidences in the high dose group and the control group, which is inappropriate for a trend test, where all dose levels should be considered.

To address these issues, the statistical significance of all tumour incidence data was re-assessed, starting from the raw data tables of the original report and applying appropriate statistical methods.

When appropriate statistics are applied to the tumour incidence data, (and the incidence data of malignant lymphoma in particular), no statistically significant increase in tumour incidence is found in this analysis. Moreover, the study was compromised by the presence of non-identified ecto- and endoparasites in a large number of animals and non-identified micro-organisms in single animals. In the absence of any detailed information it must be considered that some of the findings in this study (e.g. dermal lesions) are induced by infectious agents.

All this taken together, it can be concluded that Glyphosate Technical (glyphosate acid) is not carcinogenic in Swiss albino mice when exposed to dietary concentrations of up to 10000 ppm for a duration of 18 months.

I. MATERIAL AND METHODS

Data entry and compilation

The animal heading data (animal number, sex, group, first and last day under test) were entered into the Pathdata System Version 6.2e2 (PDS Systems, Switzerland) at AnaPath GmbH, Switzerland, including:

- Groups
- Animal numbers and sex
- The first treatment day (according to study report, December 23, 1997) was entered for all animals.
- The last treatment day was entered according to the study report. In the study report, a list of decedents was provided. A list of interim decedent animals with the respective study day of necropsy is the basis for decedents (Table 10, study report pages 126-127). In this table, the study day of death was specified. The study day was calculated for the correct date. This date was entered as last day under study and as necropsy day for each decedent.
- For terminal sacrifices, no exact data were available. Therefore, the final day under study was calculated for these animals by the last day under treatment (June 29, 1999) (page 14 of study report) + a 14 days necropsy period (July 14, 1999).

- The mode of death (found death, sacrificed moribund, terminal sacrifice) was entered.

Thereafter the following data were entered:

- all neoplastic data
- all pre-neoplastic data insofar they are not related to inflammatory or infectious lesions
- all findings were entered as unilateral findings in bilateral organs
- All findings were entered as unilateral findings in bilateral organs.
- All neoplastic lesions were entered in the PathData system for statistical analysis. Systemic neoplasms (malignant lymphoma, myeloid leukemia and histiocytic sarcoma) were entered under 'Systemic Neoplasms'. In the original report, they were entered under 'Hemolymphoreticular System'. Systemic neoplasms were entered using pathology codes, i.e. ML for malignant lymphoma, MY for myeloid leukemia, HS for histiocytic sarcoma, along with a severity degree (scale 1 to 5). Non-inflammatory tissue infiltrates were given the code "M" for metastasis. A severity grade was not given for metastases because this was not available in the original study report.
- Systemic neoplasms that were originally described under 'bone (femur and joint)' or 'sternum with bone marrow' were separately entered for both organs in this report. Therefore, a 'metastasis' in these organs appears twice, for example for animal no. 1129, the original report states: 'sternum with bone marrow: malignant lymphoma'. Under the re-evaluation, malignant lymphoma is entered as metastasis under sternum and bone marrow.
- Tumours were counted only once per animal, without any consideration of metastases.

Since no Peto codes were assigned to the primary neoplasms in the original study, the following Peto codes were assigned for statistical calculation:

- N0 (malignant neoplasm, no Peto code) in survivors
- N1 (incidental malignant neoplasm) was not applied
- N2 (probably incidental malignant neoplasm) in cases of systemic neoplasms in animals that survived and only one organ was affected
- N3 (probably fatal malignancy) in cases of systemic neoplasms or large squamous cell carcinomas in animals that were sacrificed moribund or survived the course of the study.
- N4 (fatal malignancy) in cases of systemic neoplasms or large squamous cell carcinomas or alveolar-bronchiolar carcinoma in animals that were found dead during the course of the study.
- B0 (benign neoplasm, no PETO code) in survivors
- B1 (incidental benign neoplasm) in Leydig cell tumours,
- B2 (probably incidental benign neoplasm) in cases of small hepatocellular adenoma, renal cell adenoma, adenoma in cecum, haemangioma in tail, and osteoma,
- B3 (probably fatal benign neoplasm) and B4 (fatal benign neoplasm) were not assigned.

The correctness of the transfer of the data from the original report to the PathData system was quality-checked. 100 % of the data have been quality checked of which 20 % under GLP by the local QAU. The microscopic diagnoses were entered directly into the PathData system.

An explanation of Codes and Symbols can be found at the beginning of the Tables section.

Following microscopic data are presented in the Tables:

- NUMBER OF ANIMALS WITH MICROSCOPIC LESIONS BY ORGAN/GROUP/SEX (incidences of non-neoplastic (pre-neoplastic) lesions without gradings)
- OVERALL CHRONOLOGICAL LISTING OF NEOPLASMS BY WEEKLY INTERVALS
- AOFT (Animal Organ Finding Table = table of individual microscopic findings)
- TEXT OF GROSS AND MICROSCOPIC FINDINGS (complete narrative of both the macroscopic and microscopic findings, including Animal Heading Data for each dose group).

Gross lesions were not entered since they are not relevant for the statistical evaluation of microscopical lesions.

Statistical re-evaluation

Neoplastic lesions
Statistical evaluation of all neoplastic lesions was carried out using the Fisher's Exact test and the test for positive trend with respect to dose rates according to Peto *et al*. (1980) with assignment of Peto codes to all neoplasms. For the Fisher's Exact test the one-tailed p-values are given for each pair-wise comparison and for the Peto test the trend and one-tailed p-values are given in the tables in annex to this report. When a neoplasm is marked with a "#", it is considered to be a common tumour.

Non-neoplastic lesions
Statistical evaluation of all non-neoplastic (pre-neoplastic) findings was carried out with the Fisher's Exact test and the trend test over all dose groups according to Armitage (1955), the Cochrane-Armitage linear trend test.

For the Fisher's Exact test the one-tailed p-values are given for each pair-wise comparison and for the Cochran Armitage test the trend and one-tailed p-values are given in the tables in annex to this report.

Assessment of statistical significance
For tumour incidences to be considered as statistically significant according to the "Guidance for Industry: Statistical Aspects of the Design, Analysis, and Interpretation of Chronic Rodent Carcinogenicity Studies of Pharmaceuticals" (CDER 2001) one-tailed p values should be less than 0.025 for rare neoplasms and less than 0.005 for common neoplasms (Haseman 1983, Lin and Rahman 1998). A neoplasm is regarded as rare, if in an assay involving one or two hundred animals there may be no such neoplasm, or at most one or two such neoplasms in animals of one sex and strain. Based on this definition of rare tumors, if only one or two animals have a particular type of neoplasm in a standard assay, a statistically significant result is not relevant. This holds true even if one or two such neoplasms occur in the top dose group and there are none elsewhere in the study. A neoplasm is regarded as common, "if it occurs spontaneously in five or ten or more animals in most experiments performed with animals of one strain" (Peto *et al*. 1980).

II. RESULTS

A: NEOPLASTIC LESIONS

No statistically significant trend was found for systemic neoplasms (including malignant lymphoma) in the Peto test. When the same tumours were analysed using the Fisher's Exact test, no statistically significantly increases were found in pair-wise comparisons of all dose groups with the control group.

Table 5.5-104: Statistical Evaluation of Pre-Neoplastic and Neoplastic Lesions from Study: Study No. TOXI: 1559.CARCI-M; Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice ([redacted], 2017): Selected neoplastic lesions (malignant neoplasms)

Dietary concentration of glyphosate (ppm)		Males				Females			
		0	100	1000	10000	0	100	1000	10000
Number of animals		50	50	50	50	50	50	50	50
Cerebrum	Malignant lymphoma	2	-	2	1	-	2	4	2
Cerebellum	Malignant lymphoma	-	-	-	-	-	-	3	-
Medulla	Malignant	-	-	-	-	-	-	2	-

Table 5.5-104: Statistical Evaluation of Pre-Neoplastic and Neoplastic Lesions from Study: Study No. TOXI: 1559.CARCI-M; Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice ([redacted], 2017): Selected neoplastic lesions (malignant neoplasms)

Dietary concentration of glyphosate (ppm)		Males				Females			
		0	100	1000	10000	0	100	1000	10000
oblangata	lymphoma								
Spinal chord	Malignant lymphoma	-	-	2	-	1	-	1	1
Sciatic nerve	Malignant lymphoma	-	-	1	1	-	2	1	-
Heart	Malignant lymphoma	4	-	3	1	5	4	4	4
Trachea	Malignant lymphoma	-	-	-	-	1	1	1	1
Lung	Malignant lymphoma	6	7	10	9	10	5	6	12
Systemic neoplasms	Malignant lymphoma	12	16	18	19	19	20	19	25
Oesophagus	Malignant lymphoma	-	-	1	-	-	-	-	-
Stomach	Malignant lymphoma	7	6	4	4	3	4	3	4
Duodenum	Malignant lymphoma	1	4	4	5	-	3	1	1
Jejunum	Malignant lymphoma	-	1	2	3	-	1	-	-
Ileum	Malignant lymphoma	4	3	4	8	2	7	2	5
Caecum	Malignant lymphoma	5	5	6	2	1	1	3	1
Colon	Malignant lymphoma	3	2	3	5	-	3	-	1
Rectum	Malignant lymphoma	4	2	2	5	2	4	3	3
Liver	Malignant lymphoma	7	9	10	14	9	6	10	10
Gallbladder	Malignant lymphoma	2	1	3	6	-	2	2	1
Pancreas	Malignant lymphoma	3	5	2	7	4	6	6	8
Kidneys	Malignant lymphoma	11	10	14	15	10	8	9	14
Urinary bladder	Malignant lymphoma	5	4	5	6	5	8	9	10
Ovaries	Malignant lymphoma	-	-	-	-	4	9	8	6
Uterus	Malignant lymphoma	-	-	-	-	1	8	7	7
Testes	Malignant lymphoma	-	1	-	-	-	-	-	-
Epididymides	Malignant lymphoma	5	5	6	4	-	-	-	-
Prostate gland	Malignant lymphoma	3	4	-	4	-	-	-	-

Table 5.5-104: Statistical Evaluation of Pre-Neoplastic and Neoplastic Lesions from Study: Study No. TOXI: 1559.CARCI-M; Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice ([redacted], 2017): Selected neoplastic lesions (malignant neoplasms)

Dietary concentration of glyphosate (ppm)		Males				Females			
		0	100	1000	10000	0	100	1000	10000
Coagulating glands	Malignant lymphoma	1	3	2	4	-	-	-	-
Seminal vesicles	Malignant lymphoma	1	5	2	3	-	-	-	-
Pituitary gland	Malignant lymphoma	1	1	1	-	1	3	1	-
Thyroid gland	Malignant lymphoma	-	1	1	-	1	-	2	1
Parathyroid glands	Malignant lymphoma	-	-	-	-	-	1	1	1
Adrenal glands	Malignant lymphoma	2	4	3	4	4	6	4	5
Spleen	Malignant lymphoma	10	13	10	14	17	15	13	12
Bone marrow (sternum)	Malignant lymphoma	4	10	11	11	5	7	6	6
Bone marrow (smear)	Malignant lymphoma	2	6	5	5	1	3	5	2
Thymus	Malignant lymphoma	9	5	7	10	7	6	8	13
Inguinal lymph node	Malignant lymphoma	12	13	14	13	7	11	12	11
Other lymph node	Malignant lymphoma	1	5	5	3	8	8	6	4
Mesenteric lymph node	Malignant lymphoma	10	13	15	15	14	15	15	20
Mandibular lymph node	Malignant lymphoma	11	10	17	12	14	13	16	13
Salivary glands	Malignant lymphoma	7	5	9	6	9	5	6	10
Mammary glands	Malignant lymphoma	-	-	-	-	3	6	7	2
Skin/subcutis	Malignant lymphoma	-	2	2	2	1	-	3	-
Skeletal muscle	Malignant lymphoma	1	1	2	-	-	1	-	-
Mesentery	Malignant lymphoma	1	1	1	-	3	3	2	4
Joints	Malignant lymphoma	5	11	9	11	6	8	7	6
Eyes	Malignant lymphoma	2	2	4	4	2	1	2	2
Optic nerve	Malignant lymphoma	2	2	4	4	2	1	2	2
Femur	Malignant lymphoma	6	11	9	11	6	7	7	6
Sternum	Malignant lymphoma	4	10	11	11	5	7	6	6
Body cavities	Malignant	-	-	-	-	-	-	-	1

Table 5.5-104: Statistical Evaluation of Pre-Neoplastic and Neoplastic Lesions from Study: Study No. TOXI: 1559.CARCI-M; Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice ([redacted], 2017): Selected neoplastic lesions (malignant neoplasms)

Dietary concentration of glyphosate (ppm)		Males				Females			
		0	100	1000	10000	0	100	1000	10000
	lymphoma								

B: NON-NEOPLASTIC LESIONS

Non-neoplastic lesions (only pre-neoplastic lesions), including lymphoid hyperplasia's of lymphoid organs were analysed to determine possible early stages of systemic neoplasms such as malignant lymphoma. The only exceptions were the hyperplastic lesions in the skin. In most cases they were diagnosed as being associated with dermal inflammation or the presence of ectoparasites, which were not further specified. No statistically significant trend was found for any of the non-neoplastic lesions when the Armitage trend test was applied. Also, no statistically significant increases were evident in pair-wise comparisons with the control group using the Fisher's Exact test. The only exception was a statistically significant ($p < 0.05$) increase in the incidence of lymphoid hyperplasia in the inguinal lymph node ($p = 0.0269$) in high dose males which was not related to any neoplastic change.

Table 5.5-105: Statistical Evaluation of Pre-Neoplastic and Neoplastic Lesions from Study: Study No. TOXI: 1559.CARCI-M; Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice ([redacted], 2017): Selected non-neoplastic lesions

Dietary concentration of glyphosate (ppm)		Males				Females			
		0	100	1000	10000	0	100	1000	10000
Number of animals		50	50	50	50	50	50	50	50
Inguinal lymph node	Lymphoid hyperplasia	4	8	1	4	1	1	**12***	8

* Statically significant from control (Fisher's exact test, $p \leq 0.05$)

3. Assessment and conclusion

Assessment and conclusion by applicant:

It can be concluded that Glyphosate Technical (glyphosate acid) is not carcinogenic in Swiss albino mice when exposed to dietary concentrations of up to 10000 ppm (equivalent to 1460.3 mg/kg bw/day) for a duration of 18 months.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.5/018 CA 5.5/019
Report author	[redacted]
Report year	1997

Report title	HR-001: 18-Month Oral Oncogenicity Study in Mice
Report No	[redacted] 94-0151
Document No	NA
Guidelines followed in study	Japan MAFF Guidelines 59 NohSan No.4200, 1985 U.S. EPA FIFRA Guidelines Subdivision F, 1984 OECD 451 (1981).
Deviations from current test guideline (OECD 451, 2018)	Yes, mortality was observed only once per day; histopathological examination of the cervix, lacrimal glands and the upper respiratory tract were not performed. No historical control data [Additional Historical control data was submitted from the performing laboratory ([redacted] 2013)].
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

In order to evaluate the oncogenic potential of glyphosate technical in mice, the test substance was administered to SPF ICR mice (Crj:CD-1) by incorporating it into a basal diet at a concentration of 0, 1600, 8000 or 40000 ppm (equivalent to 0, 165.0, 838.1 and 4348 mg/kg bw/day for males and 0, 153.2, 786.8 and 4116 mg/kg bw/day for females) for a period of 18 months (78 weeks). During the treatment period, all animals were observed for clinical signs and measured body weights as well as food consumption. At week 21, urinalysis was carried out on 20 males from all groups. Differential leukocytes counts were determined on the blood smears from 10 males and 10 females of all groups at week 52 and after 78 weeks of treatment, organ weight analysis was conducted on 10 males and 10 females which were served to the determination of differential leukocytes counts. All animals of both sexes were subjected to necropsy and histopathological examinations.

- 40000 ppm group: In clinical observations, the incidence of pale-coloured skin was increased in males. In addition, loose faeces were observed in all cages beginning at week 21 in males and at week 20 in females. Retarded growth was persistently observed during treatment period showing statistically significant differences in weight from week 16 to 36 in males and from week 6 to end of treatment in females. These changes were associated with depressed food consumption and food efficiency. At necropsy, the increased incidences of distention of caecum were noted in males and females at terminal kill and in all animals examined, which were consistent to increase in absolute and relative weights of the caecum. However, no abnormalities were recorded in the caecum histopathologically. In males a significant increase was noted for the overall incidence of anal prolapsed which was correspondent to erosion/ulcer of the anus in histopathology.

- 8000 ppm group: Retarded growth was observed in females with statistically significant decreases in weight at week 6 and weeks 9-24. No treatment related changes were seen in males.

- 1600 ppm group: There were no treatment related changes in either sex in any parameters.

Histopathological examinations failed to show increases of any types of neoplastic lesions in all treatment groups of both sexes.

I. MATERIAL AND METHODS

A: Materials

1. Test material: Glyphosate technical

Identification:	HR-001	
Description:	Solid crystals	
Lot/Batch #:	T-941209	T-950308
Purity:	97.56 %	94.61 %
Stability of test compound:	Not mentioned in the report	

2. Vehicle and/or positive control: Diet

3. Test animals:

Species:	Mouse
Strain:	SPF ICR (Crj:CD-1)
Source:	[redacted]
Age:	5 weeks
Sex:	Males and females
Weight at dosing:	Males: 15 – 25 g, females: 14 – 23 g
Acclimation period:	9 days in males, 7 days in females
Diet/Food:	Certified diet MF Mash (Oriental Yeast Co., Ltd.), *ad libitum*
Water:	Filtered and sterilised water, *ad libitum*
Housing:	In groups of four per sex in aluminium cages with wire mesh floors
Environmental conditions:	Temperature: 24 ± 2 °C
	Humidity: 55 ± 15 %
	Air changes: 15/hour
	12 hours light/dark cycle

B: Study design and methods

In life dates: 1995-02-21 to 1996-09-06

Animal assignment and treatment:
Groups of 50 males and 50 females Specific-Pathogen-Free (SPF) ICR (Crj:CD-1) mice received the test material by incorporating it into the basal diet at a level of 0, 1600, 8000 or 40000 ppm for a period of 18 months.

Clinical observations
All animals were conducted a cage-side observation daily for clinical signs and their deaths during the study. In addition, a detailed examination including palpation of the body was performed at least once a week. Moribund animals showing marked debility were euthanised by exsanguinations under deep ether anaesthesia and necropsied when an unfavourable prognosis was predicted. Dead animals were taken from the cage as soon as possible after discovery in order to minimise the loss of tissues by cannibalism and necropsied. Mortality was expressed as ratios of cumulative number of animals found dead or killed *in extremis* to effective number of animal group.

Body weight
Individual body weights were recorded weekly from week 1 to 13 and every 4 weeks from week 16 to 76. Body weights were also measured at week 78, at the end of treatment, and used for calculation of relative organ weights. Group mean body weights were calculated at each measurement.

Food consumption and compound intake
Food consumption by each cage was recorded for a period of 3 or 4 consecutive days weekly during the first 13 weeks and every 4 weeks from week 16 to 76. Food efficiency and compound intake was calculated from the recorded food consumption data.

Haematology
Blood smear samples were collected at week 52 and at termination (18 month) from all surviving animals, and from mice that were killed *in extremis*. Differential white cell counts were performed on all blood smear samples.

Sacrifice and pathology
All animals that died or were killed *in extremis* during the conduct of the study were necropsied immediately.
All surviving mice were sacrificed at scheduled termination. A gross pathological examination was performed on all mice. Any macroscopic findings were recorded.

The following organ weights were determined from 10 mice per sex per group: brain, adrenals, kidneys, spleen, liver and gall bladder, ovaries, and testes.

Tissue samples were taken from each mice from the following organs and preserved in 10 % buffered neutral formalin: brain, spinal cord, sciatic nerve, pituitary, thymus, thyroids with parathyroids, adrenals, spleen, bone with marrow, tibio-femoral joint, lymph nodes, heart, aorta, salivary glands, oesophagus, stomach, liver with gallblader, pancreas, duodenum, jejunum, ileum, cecum, colon, rectum, head, pharynx, larynx, trachea, lung, kidneys, urinary bladder, testes, prostate, seminal vesicles, epididymides, coagulating glands, ovaries, uterus, vagina, Harderian glands, eyes, skeletal muscle, skin, mammary gland, all gross lesions.

A detailed histopathological examination was performed on all sampled tissues of the control and high-dose animals, and on animals that died or were killed *in extremis*. In addition, tissues of gross lesions and masses from all mice were examined microscopically. The following tissues were examined: brain, spinal cord, sciatic nerve, pituitary, thymus, thyroids with parathyroids, adrenals, spleen, bone with marrow, tibio-femoral joint, lymph nodes, heart, aorta, salivary glands, oesophagus, stomach, liver with gallblader, pancreas, duodenum, jejunum, ileum, cecum, colon, rectum, trachea, lung, kidneys, urinary bladder, testes, prostate, seminal vesicles, epididymides, coagulating glands, ovaries, uterus, vagina, Harderian glands, eyes, skeletal muscle, skin, mammary gland, all gross lesions.

Statistics
Body weight, food consumption and organ weights were evaluated by Bartlett´s test for homogeneity of intra group variances. When group variances were homogenous, a parametric analysis of variance of a one way layout type was conducted to determine if any statistical differences exist among groups. When the analysis of variance was significant, Dunnett's or Scheffe's multiple comparison test was applied. When the group variance were heterogeneous, the data were evaluated by Kruskal-Wallis non-parametric analysis of variance. When significant Dunnett type mean rank test or Scheffe's type mean rank test was applied.

Mortality was assessed by a life table analysis.

Urinalysis were analysed by Mann-Whitney's U test to compare data between the treatment groups and the controls.

Mann-Whitney's U test was used to analyse difference of the differential leukocyte counts between the high dose groups and the controls. For comparison of the data from all groups, Dunnett's and Scheffe's multiple comparison test was applied. The data from males killed *in extremis* during the treatment were examined by Mann-Whitney's U test.

Fisher's exact probability test was used to analyse the data of clinical signs and incidences of gross lesions at necropsy and histopathological lesions.

Results

A. ANALYSIS OF DOSE FORMULATIONS

Stability analyses indicated that the dose preparations were stable for up to 30 days with a loss of 8.37 %. Homogeneity of the test substance in diet was analysed on the samples taken from the top, middle, and bottom portion of the mixer. The coefficient of variation for each test diet was within 5.2 % or less. The results indicated that homogeneity of the test substance in diet was satisfactory in each test diet.

In order to verify concentration of the test substance in test diets, every batch of test diet was analysed during the treatment period. Mean concentration of the test substance in test diet at a nominal level of 1600, 8000 or 40000 ppm was 1561 ± 86.7, 7790 ± 394.4 or 38783 ± 1655.0 ppm (mean ± standard deviation), respectively. The values were within 97-98 % of the target concentrations and satisfied the acceptable limit of concentration for test substance.

B. MORTALITY

No significant differences were noted for mortality between the treated groups and the respective control of either sex. Cumulative mortality of each group of either sex is shown in the following table:

Table 5.5-106: HR-001: 18-Month Oral Oncogenicity Study in Mice ([redacted], 1997): Final mortality at termination of treatment (%)

Dose group (ppm)	Male	Female
0	24/50 (48)	18/50 (36)
1600	16/50 (32)	14/50 (28)
8000	23/50 (46)	10/50 (20)
40000	21/50 (42)	15/50 (30)

C. CLINICAL OBSERVATIONS

Statistically significant changes in clinical signs observed in the treated groups of either sex are shown in the following table:

Table 5.5-107: HR-001: 18-Month Oral Oncogenicity Study in Mice ([redacted], 1997): Statistically significant changes in clinical signs

	Male				Female			
	Dose group (ppm)							
	0	1600	8000	40000	0	1600	8000	40000
Number of animals examined	50	50	50	50	50	50	50	50
Perinasal region: tactile hair loss	0	↑3	↑3	↑6*	5	↑13*	↑9	↑8
Anus: mass(es)	0	0	0	↑8**	0	0	0	0
Integument :								
wound	22	↓16	↓20	↓6*	3	↓0	↓0	↓0
Erosion/Ulcer	9	↓5	↑12	↓8	16	↓4**	↓1**	↓2**
Swelling	16	↓6*	↑13	↓9	6	↓2	↓0	↓1
Mass(es)	15	↓13	↓13	↓10	13	↓11	↓9	↓4*
Pale-coloured skin	2	↑3	↑6	↑10*	4	↓2	↑6	↑6

Table 5.5-107: HR-001: 18-Month Oral Oncogenicity Study in Mice ([redacted], 1997): Statistically significant changes in clinical signs

	Male				Female			
	Dose group (ppm)							
	0	1600	8000	40000	0	1600	8000	40000
Hair loss	11	↑12	↑21*	↑12	22	↑23	↓18	↓14
Wetted fur	11	↓9	↓7	↓4*	1	1	1	1

* $p < 0.05$
** $p<0.01$ (Fisher's exact probability test).

In the 40000 ppm group, males showed increased incidences of tactile hair loss, pale-colored skin, and mass(es) of anus and decreases of wound and wetted fur. In females of this group decreased incidences were observed in ulcer/erosion and mass(es) of skin. Although, in addition to these signs, loose stool was observed in the cages of both sexes beginning at week 21 in males and 20 in females, the group housing failed to identify which animal excreted the loose stool.

In the 8000 ppm group, males showed an increased incidence in hair loss of the skin and females represented decreases in ulcer/erosion and swelling of the skin.

In the 1600 ppm group, males showed a decrease in swelling of the skin and females represented an increase in tactile hair loss as well as a decrease in ulcer/erosion of the skin.

None of the observed effects seems to be dose-related. Whatever the dose tested, females were more sensitive to erosion/ulcer of the integument than males.

D. BODY WEIGHT

In the 40000 ppm group, males and females showed retarded growth during the treatment manifesting significantly lowered weights at weeks 16 to 36 in males and at weeks 6 and thereafter in females compared to the respective control. At the end of treatment, mean average weights were 93 % and 86 % of the respective control in males and females, respectively.

In the 8000 ppm group, females showed significantly decreased weights at week 6 and weeks 9 to 24 compared to the control and the final mean average weight was 92 % of the control at the end of the treatment, while growth rate in males was comparable to the control.
In the 1600 ppm group, males and females showed similar growth curves to the controls during the treatment period.

Effects on the body weight were more important in females than in males. These effects were durable in the 40000 ppm female group whereas they were stopped at week 36 in the male group of the same treatment dose. Sporadic effects were observed in the 8000 ppm female group. No significant effects were seen in the 1600 ppm male and female groups.

Table 5.5-108: HR-001: 18-Month Oral Oncogenicity Study in Mice ([redacted], 1997): Body weights at week 1, 24, 52 and 78

Timepoint	Sex	Males				Females			
	Dose (ppm)	0	1600	8000	40000	0	1600	8000	40000
		Body weight [g], mean ± SD							
Week 1		31.9 ± 1.8	32.0 ± 1.9	32.3 ± 2.0	31.4 ± 1.7	25.8 ± 1.4	25.3 ± 1.6	25.5 ± 1.5	25.7 ± 1.6
Week 24		47.6 ±	48.2 ±	48.5 ±	**44.6 ±**	47.4 ±	44.6 ±	**42.8 ±**	**38.6 ±**

Table 5.5-108: HR-001: 18-Month Oral Oncogenicity Study in Mice ([redacted], 1997): Body weights at week 1, 24, 52 and 78

Timepoint	Sex / Dose (ppm)	Males 0	Males 1600	Males 8000	Males 40000	Females 0	Females 1600	Females 8000	Females 40000
		5.7	5.9	5.3	**4.3***	7.1	7.2	**7.4***	**4.2****
Week 52		49.7 ± 7.0	51.4 ± 7.2	50.3 ± 6.3	46.3 ± 5.7	51.8 ± 10.9	51.7 ± 9.0	50.8 ± 9.3	**45.4 ± 5.9****
Week 78		50.7 ± 6.4	52.8 ± 6.9	50.1 ± 5.7	47.3 ± 5.8	55.8 ± 7.8	53.2 ± 8.2	51.5 ± 9.1	**47.8 ± 6.4****
Body weight [%], mean									
Week 1		100	100	101	98	100	98	99	100
Week 24		100	101	102	94	100	94	90	81
Week 52		100	103	101	93	100	100	98	88
Week 78		100	104	99	93	100	95	92	86

E. FOOD CONSUMPTION AND COMPOUND INTAKE

In the 40000 ppm group, males showed significant depressions in food consumption at weeks 1 and 68, revealing an overall group mean food consumption at 94 % of the control during the treatment period. Females in this group also showed significantly decreased food consumption at weeks 1, 4, 8, 12, 20, 28, 40, 48 and 68, revealing an overall group mean food consumption at 93 % of the control during the treatment period.

In the 8000 ppm group, females showed significantly lowered food consumption at weeks 28, 40, and 68 compared to the control manifesting an overall group mean food consumption at 96 % of the control. Whereas, food consumption in males was comparable to the control during the treatment period.

No statistically significant effects was observed in the 1600 ppm group either in males or females.

The food consumption depressions were more important in female than in males. They were not time-related.

Overall average chemical intake in each treated group of either sex was calculated from food consumption and nominal concentration as shown in the following table:

Table 5.5-109: HR-001: 18-Month Oral Oncogenicity Study in Mice ([redacted], 1997): Calculated test substance intake in mg/kg bw/day

Dose level (ppm)	Dose level (mg/kg bw/day) Male	Female
1600	165.0	153.2
8000	838.1	786.8
40000	4348	4116

F. HAEMATOLOGY

Statistically significant changes in differential leucocyte counts observed in the treated group of either sex are shown in the following table.

Table 5.5-110: HR-001: 18-Month Oral Oncogenicity Study in Mice ([redacted] 1997): Statistically significant changes in haematology parameters

Parameter	Sex	Fate of animals[a]	Dose group (ppm)		
			1600	8000	40000
Lymphocytes	Males	ke	ND[b]	↑139	↑172
	Females	tk	↓ 92	↑131	↑[c]163
Neutrophil (segmented)	Males	ke	ND	↓ 91	↓ 81

Numbers in the above table show values in the treated groups when the corresponding value in the control group is 100.
a: ke, killed *in extremis*; tk, terminal kill; b: ND, not determined; c: Dunnett's or Scheffe's multiple comparison test
↓↑ Mann-Whitney's U test

In the 40000 ppm group, males killed *in extremis* during the treatment period showed an increase of lymphocytes in differential leukocyte counts and a decrease of neutrophil (segmented form). In females of this group, differential count of lymphocytes was significantly increased at week 78.

There were no significant differences in differential leukocyte counts at other intervals of examination in the 40000 ppm group of both sexes, males killed *in extremis* in the 8000 ppm group, and females at week 78 in the 8000 and 1600 ppm groups compared to the controls. No significant treatment-related effects were conceived in morphology of the leukocytes.

G. NECROPSY

Gross pathology

Statistically significant changes in incidence of macroscopic lesions observed in the treated groups of either sex are shown in the following table.

Table 5.5-111: HR-001: 18-Month Oral Oncogenicity Study in Mice ([redacted], 1997): Statistically significant changes in macroscopic lesions

Sex	Male				Female			
Dose group (ppm)	0	1600	8000	40000	0	1600	8000	40000
78tk (N=)	(26)	(34)	(27)	(29)	(32)	(36)	(40)	(35)
External appearance: Loss of tactile hair	0	0	↑1	↑5*	4	↑8	↑8	↓0*
Soiled fur on external genital region	9	↓7	↓2*	↓6	0	0	0	0
Spleen: Swelling	5	↓1*	↓4	↓2	7	↓2	↓3	↓3
Lung: Mass(es)	4	↑12	↑11*	↑9	8	↓6	↑18	8
Cecum: Distention	0	0	0	↑11**	0	0	0	↑16**
Kidney: Cyst(s)	4	4	↓2	0*	2	↓0	↑4	↓1
Uterus: Cyst(s)	-	-	-	-	6	↓2	↓2	↓0*
Skin: Loss of hair	1	↑4	↑7*	↑6	8	↑11	↑16	↓5
Ke/fd (N=)	(24)	(16)	(23)	(21)	(18)	(14)	(10)	(15)
Lymph nodes (mesenteric): Swelling	0	↑2	0	↑5*	1	↑2	1	↑4
Lymph nodes (others): Swelling	5	↓2	↓4	↑9	0	↑3	↑4*	↑4*
Kidney: Coarse surface	4	↓2	↓1	↓1	6	↓3	↓0*	↓4
Skin: Loss of hair	5	↓4	↑7	↓4	11	↓5	↓2*	↓4
Wound	6	↓2	↓3	↓0*	0	0	0	0
Ulcer/Erosion	6	↓3	↓4	6	5	↓3	↓0	↓0
All (N=)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
External appearance: Loss of tactile hair	0	0	↑1	↑6*	5	↑11	↑9	↓3
Lymph nodes (cervical): Swelling	5	↓3	↑6	↑9	12	↓6	↓4*	↓7

Table 5.5-111: HR-001: 18-Month Oral Oncogenicity Study in Mice ([redacted], 1997): Statistically significant changes in macroscopic lesions

Sex	Male				Female			
Dose group (ppm)	**0**	**1600**	**8000**	**40000**	**0**	**1600**	**8000**	**40000**
Lymph nodes (mesenteric): Swelling	0	↑2	0	↑6*	3	↓2	↓1	↑5
Spleen: Swelling	16	↓4**	↓12	↓14	17	↓8*	↓8*	↓10
Lung: Mass(es)	9	↑14	↑17	↑15	10	↓8	↑20*	↑11
Cecum: Distention	0	0	0	↑14**	0	0	0	↑18**
Anus: Anal prolapse	0	0	0	↑5*	0	0	0	0
Kidney: Pale in color	6	↓2	↓4	↓2	7	↓4	↓1*	↓4
Coarse surface	6	↓2	↓2	↓1	7	↓4	↓0**	↓5
Testis: Atrophy	5	↓2	5	↓0*	-	-	-	-
Uterus: Cyst(s)	-	-	-	-	6	↓2	↓2	↓0*
Eye: Opacity	1	1	↑5	↑2	5	↓1	↓0*	↓0*
Auricle: Partial amputation	6	↓2	↓1	0*	4	↓2	↓0	↓1
Skin: Loss of hair	6	↑8	↑14*	↑10	19	↓16	↓18	↓9*
Wound	9	↓3	↓3	↓1*	0	0	0	0
Ulcer/Erosion	7	↓4	↑9	↓6	8	↓3	↓1*	↓0**
Swelling	7	↓1*	↓3	↓1*	3	↓0	↓0	↓0

Tk: Terminal kill; Ke/fd: Killed *in extremis* or found dead; All: All animals examined;
(N=) Number of animals examined
* $p < 0.05$ (Fisher's exact probability test);
** $p<0.01$

In the 40000 ppm group, males and females showed significant increases in incidence of distention of the cecum at terminal kill after 78 weeks of treatment. Significant increases in incidence of the lesion were also noted in all animals examined recording 28 % (14/50) in males and 36 % (18/50) in females. Distended cecum was filled with loose stool-like materials. In addition, males showed an increase in loss of tactile hair and a decrease of cyct(s) in the kidney in those necropsied at terminal kill, and an increase of swelling in the lymph nodes (mesenteric) and a decrease of wound in the skin in those killed *in extremis* or found dead during the treatment period when compared to the controls. Among these, significant differences in incidence were also noted in all animals examined for increases in loss of tactile hair and swelling of the lymph nodes (mesenteric) and a decrease in wound in the skin. Moreover, significant differences in incidence were also noted in all animals examined for an increase in anal prolapse of the anus and decreases in atrophy of the testis, partial amputation of the auricle, and swelling of the skin. Females showed decreases in loss of tactile hair and cyst(s) of the uterus in those necropsied at terminal kill, and an increase in swelling of the lymph nodes (others) and a decrease in ulcer/erosion of the skin in those killed *in extremis* or found dead during the treatment period. Among these, significant differences in incidence were noted in all animals examined for decreases in cyst(s) of the uterus and ulcer/erosion of the skin. Moreover, significant differences in incidence were also noted in all animals examined for decreases in opacity of the eye and loss of hair of the skin.

In the 8000 ppm group, males showed increases in mass(es) of the lung and loss of hair of skin and a decrease in soiled fur on external genital region in those necropsied at terminal kill when compared to the control. An increased incidence was also noted in all animals examined for loss of hair of the skin. Females killed *in extremis* or found dead during the treatment period in this group showed an increase in swelling of the lymph nodes (others) and decreases in coarse surface of the kidney and loss of hair of the skin. Moreover, significant differences in incidence were noted in all animals for an increase in mass(es) of the lung and decreases in swelling of the lymph nodes (cervical) and spleen, pale in colour and coarse surface of the kidney, opacity of the eye, and ulcer/erosion of the skin.

In the 1600 ppm group, males showed decreased incidences in swelling of the spleen in those necropsied at terminal kill and in all animals examined and in swelling of the skin in all animals examined, while

females disclosed a decreased incidence in swelling of the spleen in all animals examined.

Organ weights
In the 40000 ppm group, males and females showed significant increases in absolute and relative weights of the cecum. The percentages of the values to those of the respective control were 173 % in males and 187 % in females for absolute weight, respectively, and 174 % and 212 % for relative weight, respectively. In females, relative weight of the kidney was also increased significantly at a level of 111 % of the control.

Histopathology
Neoplastic lesions
The table below shows neoplastic lesions in the treated groups of either sex with statistically significant differences in incidence from those of the controls.

Table 5.5-112: HR-001: 18-Month Oral Oncogenicity Study in Mice ([redacted], 1997): Statistically significant changes in histopathology findings: Incidence of malignant lymphoma at terminal sacrifice and over all animals

Sex	Male				Female			
Dose group (ppm)	0	1600	8000	40000	0	1600	8000	40000
Hematopoietic & Lymphatic system: General: Malignant lymphoma								
All animals (N=)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
All animals: Incidence	2	2	0	6	6	4	8	7
All animals: Incidence [%]	4	4	0	12	12	8	16	14
Tk (N=)	(26)	(34)	(27)	(29)	(32)	(36)	(40)	(35)
Tk: Incidence	0	0	0	2	4	0*	5	3
Tk: Incidence [%]	0	0	0	7	12.5	0	12.5	8.5
HCD (12 studies between 1993 and 1998; 458/459 m/f mice examined in the 12 studies) [mean (range)] (see CA 5.5/019)	6.33 % (3.85-19.23)				15.03 % (7.84-26.92)			

Tk Terminal kill;
(N=) Number of animals examined;
* $p < 0.05$ (Fisher's exact probability test)

During the last evaluation historical control data for malignant lymphoma from the performing laboratory ([redacted], 2013; ASB2014-9146) were submitted. A total of 9 long-term studies (no information on actual duration provided) in the same mouse strain was covered that had been performed or at least terminated (perhaps commenced before) between 1993 and 1998, i.e. exactly the time in which the study under review was conducted. In male mice, the total incidence of malignant lymphoma in control groups varied considerably, ranging from ca 4 (actually 3.58) to ca 19 % (19.23). In fact, 8 of 9 studies had a control incidence below 12 % (6 % or lower) as observed now at the top dose level but, in principle, this incidence fell into the historical control range. Thus, the conclusion is that the higher incidence at the exaggerated dose level of 40000 ppm as compared to the control group is a chance findings and cannot be used to support the assumption of a carcinogenic effects of glyphosate in mice that is based on the results of the study by [redacted] (2001, ASB2012-11491).

In female control groups, malignant lymphoma incidence was between 8 and 27 % and, thus, the actual incidences in the control and treated groups were well covered.

As to neoplastic lesions, the incidence of malignant lymphoma was significantly decreased in females of the 1600 ppm group necropsied at terminal kill compared to the control. Neither increases in incidence nor nearly occurrences compared to the controls were noted for neoplastic lesions in the treated groups of both sexes.

Non-neoplastic lesions
Statistically significant changes in incidence of non-neoplastic lesions observed in the treated groups of either sex are shown in the following table.

Statistically significant changes in incidence of non-neoplastic lesions:

Table 5.5-113: HR-001: 18-Month Oral Oncogenicity Study in Mice ([redacted], 1997): Statistically significant changes in non-neoplastic lesions

Sex	Male				Female			
Dose group (ppm)	0	1600	8000	40000	0	1600	8000	40000
78tk (N=)	(26)	(34)	(27)	(29)	(32)	(36)	(40)	(35)
Spleen: Increased extramedullary haematopoiesis	5	2	4	3	6	5	1*	4
Liver: Micro-granuloma	1	5	5	4	15	16	14	7*
Kidney: Cortical cyst(s)	9	6	9	0*	2	1	5	0
Tibio-femoral joint: Proliferation of cartilageous tissue	14	17	11	15	18	14	11*	15
Ke/fd (N=)	(24)	(16)	(23)	(21)	(18)	(14)	(10)	(15)
Bone marrow (femur): Increased haematopoiesis	6	3	7	6	7	1*	1	2
Lymph nodes (cervical): Plasma cell hyperplasia	6	1	5	4	5	3	0	0*
Spleen: Amyloid deposition	2	3	2	0	8	3	0*	1*
Small intestine: Amyloid deposition	1	1	1	0	5	0*	0	2
Liver: Amyloid deposition	3	3	2	0	10	3	0**	1**
Thyroid: Amyloid deposition	2	2	2	0	8	1*	0*	2
Parathyroid: Amyloid deposition	1	1	2	0	7	1	0*	2
Skin: Wound	9	5	9	4	9	5	1*	3
All (N=)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Bone marrow (femur): Increased haematopoiesis	9	3	10	10	9	2*	2*	2*
Bone marrow (sternum): Increased haematopoiesis	9	3	9	10	9	3	2*	2*
Bone marrow (Vertebra): Increased haematopoiesis	9	3	10	10	9	3	2*	2*
Lymph nodes (cervical): Plasma cell hyperplasia	6	2	8	5	8	3	1*	0*
Lymph nodes (mesenteric): Myeloid cell aggregation	5	0*	3	2	4	1	1	1
Spleen: Increased extramedullary haematopoiesis	20	7*	14	14	13	10	5*	9
Amyloid deposition	3	3	4	0	10	3*	0**	1**
Lung: Alveolar epithelial cell hyperplasia	0	5*	1	1	3	4	5	5
Small intestine: Amyloid deposition	2	1	1	0	8	0**	0**	3
Liver: Micro-granuloma	1	6	5	5	16	16	14	7*
Amyloid deposition	5	3	4	0*	12	3*	0**	1**
Kidney: Cortical cyst(s)	10	8	13	2*	5	1	5	0*
Glomerular amyloidosis	1	1	2	0	7	2	0**	2
Uterus: Amyloid deposition	-	-	-	-	6	0*	0*	1
Thyroid: Amyloid deposition	3	2	4	0	11	1**	0**	2**
Parathyroid[c]: Amyloid deposition	2	1	4	0	10	1**	0**	2*
Eye: Cataract	4	5	5	5	5	2	0*	2
Skin: Skin subcutaneous abscess	3	1	2	5	5	1	0*	1

Tk: Terminal kill; Ke/fd: Killed *in extremis* or found dead; All: All animals examined
(N=): Number of animals examined
* $p < 0.05$;
** $p<0.01$ (Fisher's exact probability test)
c: The number animals examined in the control, 1600, 8000 or 40000 ppm groups were 46, 48, 48 or 46 in males and 48, 48, 50 or 49 in females, respectively.

In the 40000 ppm group, males showed significant decreases in incidence of amyloid deposition in the liver in all animals examined and cyst(s) in the kidney in those necropsied at terminal kill and in all animals examined, when compared to the control. In these males, erosion/ulcer in the anus was observed in a total

of 8 animals including 6 cases killed *in extremis* or found dead during the treatment period and 2 cases necropsied at terminal kill. There was even a large abscess in one case. Among these, regressive hyperplasia of mucous epithelium of the large intestine was seen in 2 cases with severe lesions in the anus. However, as the histopathological examinations were carried out only on the anus which were observed macroscopic lesions, the incidence of erosion/ulcer in the anus was not assessed by a statistical method. In females of this group, statistical significant decreases in incidence were noted in all animals examined as follows; increase haematopoiesis in bone marrow (femur, sternum and vertebra), plasma cell hyperplasia in the lymph nodes (cervical), cyst(s) in the kidney, micro-granuloma in the liver, and amyloid deposition in the spleen, liver, thyroid, and parathyroid. Among these, significant decreases in incidence were also noted for micro-granuloma in the liver in those necropsied at terminal kill and plasma cell hyperplasia in the lymph nodes (cervical) and amyloid deposition in the spleen and liver in those killed *in extremis* or found dead during the treatment period.

In the 8000 ppm group, although males did not show any non-neoplastic lesions with statistically significant differences in incidence from the control, females disclosed significant decreases in incidence of proliferation of cartilageous tissue in the tibio-femoral joint in those necropsied at terminal kill, wound in the skin in those killed *in extremis* or found dead during the treatment period, and subcutaneous abscess in the skin in all animals examined. In addition, significant decreases in incidence, when compared to the control, were observed in all animals examined as follows; increase haematopoiesis in bone marrow (femur, sternum and vertebra), plasma cell hyperplasia in the lymph nodes (cervical), extramedullary haematopoiesis in the spleen, amyloid deposition in the spleen, small intestine, liver, kidney (glomerular amyloidosis), uterus, thyroid, and parathyroid, and cataract in the eye. Among these, the incidences of extramedullary haematopoiesis in the spleen in those necropsied at terminal kill and amyloid deposition in the spleen, liver, thyroid, and parathyroid in those killed *in extremis* or found dead during the treatment period were also decreased significantly.

In the 1600 ppm group, males in all animals examined showed a significant increase in incidence of alveolar epithelial cell hyperplasia in the lung and decreases in incidence of myeloid cell aggregation in the lymph nodes (mesentery) and extramedullary haematopoiesis in the spleen. In females of this group, the incidences in all animals examined were decreased significantly in increased haematopoiesis in bone marrow (femur) and amyloid deposition in the spleen, small intestine, liver, uterus, thyroid, and parathyroid. Among these, significantly decreased incidences were also noted for increased haematopoiesis in bone marrow (femur) and amyloid deposition in the small intestine and thyroid in those killed *in extremis* or found dead during the treatment period.

Study conclusion:

	No-observable effect level	Based on
Males	8000 ppm (838.1 mg/kg/day)	- Increased incidences of tactile hair loss, pale-colored skin and mass(es) of anus at 40000 ppm - Decreased body weight gain at 40000 ppm - Decrease in food consumption at 40000 ppm - Decrease in urinary pH at 8000 and 40000 ppm - Increase of lymphocytes and decrease in neutrophil (segmented form) at 40000 ppm - Increase in mass(es) of the lung and loss of hair of skin at 1600 ppm but not observed at 40000 ppm. - Increase in distension and absolute and relative weight of the cecum at 40000 ppm - Increase in incidence of alveolar epithelial cell hyperplasia in the lung at 1600 ppm but not observed at higher doses.
Females	1600 ppm (153.2 mg/kg/day)	- Decreased body weight gain at 40000 ppm and 8000 ppm - Increase in tactile hair loss at 1600 ppm but not observed in

		higher dose groups - Decrease in food efficiency at 8000 ppm - Increase in swelling of the lymph nodes and in mass(es) of the lung

3. Assessment and conclusion

Assessment and conclusion by applicant: Based on the effects in female mice on food consumption and body weight gain at the mid dose level of 8000 ppm, the lowest dose of 1600 ppm (ca. 153 mg/kg bw/day) is considered the NOAEL in this study. In contrast, the masses in lung mentioned in the dossier were not dose-related and there was no convincing evidence of lymph node swelling. Male mice appeared less vulnerable.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.5/020 CA 5.5/021
Report author	[redacted]
Report year	1989
Report title	Glyphosate: 104-week dietary carcinogenicity study in mice
Report No	7793
Document No	154-GLY
Guidelines followed in study	US-EPA Pesticide Assessment Guidelines Subdivision F, 83-2 (1982); in general compliance with OECD 451
Deviations from current test guideline (OECD 451, 2018)	Yes, histopathological examinations were performed without Harderian gland, cervix, eyes, coagulating glands, gross lesions, lacrimal glands, upper respiratory tract, seminal vesicles and vagina. No historical control data.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The carcinogenic potential of glyphosate technical was assessed in a 104-week feeding study in male and female CD-1 mice. Groups of 50 mice per sex received daily dietary doses of 0, 100, 300 or 1000 mg/kg

bw/day glyphosate technical for 24 months. Observations covered clinical signs, body weight, food and water consumption, differential blood count, as well as organ weights, necropsy and histopathological examination.

Achieved doses throughout the study period were generally close to nominal. There were no treatment-related deaths or clinical signs in any of the dose-groups. A slight decrease in absolute body weight at Week 40 was evident in the low and high groups of both sexes but this finding was not considered to be treatment-related. Food and water consumption did not differ significantly from the controls. Moreover, there were no treatment-related changes in differential blood count.

At necropsy the incidence of lung masses was slightly higher in the 1000 mg/kg bw/day group. Organ weight data showed increased thymus weights in males at 300 and 1000 mg/kg bw/day after 104 weeks, but not in females. Histopathological examination noted increased mineral deposit in the brain of high dose males. These changes were not considered treatment related. No treatment-related neoplastic lesions were observed at termination.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate technical
Description:	White powder
Lot/Batch #:	206-JaK-25-1
Purity:	97.5 % and 100 %
Stability of test compound:	At least two years at ambient temperature in the dark

2. Vehicle and/or positive control: Diet

3. Test animals:

Species:	Mouse
Strain:	CD-1
Source:	[redacted]
Age:	Approx. 4 weeks upon arrival at testing facility
Sex:	Males and females
Weight at dosing:	Males: 30.9 ± 0.5 g, females: 23.5 ± 0.3 g
Acclimation period:	21 days
Diet/Food:	SQC Expanded (Fine Ground) Rat and Mouse Maintenance Diet No. 1 (Special Diet Services Limited, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Either one male or one female in suspended polypropylene cages with stainless steel wire grid tops and bottoms
Environmental conditions:	Temperature: 20 ± 2 °C Humidity: 55 ± 10 % Air changes: 15 – 20 / hour 12 hours light/dark cycle

B: Study design and methods

In life dates: 1989-11-30 to 1991-12-23

Animal assignment and treatment:
In a carcinogenicity study groups of 50 CD-1 mice per sex received daily dietary doses of 0, 100, 300 or 1000 mg/kg bw/day glyphosate technical for 104 weeks. The dose levels were selected based on the results of a 13-week dietary toxicity study in mice.

Test diets were prepared once per week for the first 13 weeks and at least once every two weeks thereafter by direct admixture of the test substance to the plain diet and mixing for 20 minutes.

Analyses for achieved concentrations of the test substance in the diet were conducted from formulated diets or Weeks 1, 4, 8, 12, 15, 23, 30, 38, 46, 54, 62, 76, 83 and 93 of dosing. The stability and homogeneity of the test substance in the diet was determined prior to the start of the study.

Clinical observations
A check for mortality was made twice daily on all animals throughout the study. In addition, all animals were examined for clinical signs during each day. A detailed clinical examination and check for palpable masses were done once each week on every animal.

Body weight
Individual body weights were recorded for each animal before dosing, at weekly intervals until the end of week 13 and approximately every 4 weeks thereafter until termination.

Food and water consumption and compound intake
Food consumption was recorded once weekly for each cage group starting one week before treatment until Week 13 and subsequently every 4 weeks until termination. Water consumption was monitored by visual inspection throughout the study period.
Achieved dosages were calculated from nominal dietary concentration, taking into account actual food consumption and body weight data.

Haematology
During Weeks 52, 77 and 102 of dosing, a blood sample was taken from all surviving animals *via* tail snip without anaesthesia. Differential blood smears were prepared, fixed and stained from all animals. A differential blood count was performed on smears from all surviving Control and High dose animals at each time point.

The following parameters were measured: Differential leukocyte count (neutrophils, lymphocytes, monocytes and eosinophils). Absolute indices were calculated.

Sacrifice and pathology
All surviving animals were sacrificed and necropsied. Method of killing was by carbon dioxide asphyxiation followed by exsanguination. The gross dissections and necropsies were performed under the supervision of a pathologist, with the organs/tissues listed below being weighed and/or fixed.

The following organs were weighed: adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, prostrate, salivary glands (parotid, sublingual and submaxillary), spleen, testes including epididymides, thymus and uterus.

The following organs were examined histopathologically: adrenals, aortic arch, any abnormal tissue, bladder, bone and bone marrow (sternum and rib), brain, intestine (duodenum, jejunum, ileum, caecum, colon, rectum), heart, kidneys, liver with gall bladder, lungs, mammary gland, lymph nodes (mesenteric), muscle (thigh), oesophagus, ovaries, pancreas, parotid salivary glands, pituitary, prostrate, sciatic nerve,

skin, spinal cord (cervical, thoracic and lumbar), spleen, stomach (glandular and non-glandular), sublingual salivary glands, submaxillary salivary glands, testes with epididymis, thymus, thyroid/parathyroid, trachea and uterus.

Statistics
Body weight and organ weight data were statistically analysed for homogeneity of variance using the 'F-max' test. If the group variances appeared homogeneous, a parametric ANOVA was used and pairwise comparison made *via* Students's t-test using Fishers's F protected LSD. If the variances were heterogeneous, log or square root transformations were used in an attempt to stabilise the variances. If the variances remained heterogeneous, then a nonparametric test such as Kruskal-Wallis ANOVA was used.

Organ weights were also analysed conditional on body weight (i.e. analysis of covariance). Differences in survival data between Control and groups receiving Glyphosate were assessed graphically using Kaplan-Meier plots. Histology and tumour data were analysed using Fisher's Exact Probability test.

II. RESULTS

A. ANALYSIS OF DOSE FORMULATIONS
Periodic analyses for achieved concentrations showed that the diet preparations of all dose groups were within an acceptable degree of accuracy (± 10 %), with the exception of 2 instances where deviation of the mean from the theoretical concentration exceeded ± 10 % (Group 2♀ - Week 62: +248 % and Group 3♀ - Week 93: -10.1 %).

B. MORTALITY
There were 208 pre-terminal deaths throughout the study. There was no evidence to suggest that any of these deaths were treatment-related.

The numbers of deaths are summarised in the table below.

Table 5.5-114: Glyphosate – 104 week dietary carcinogenicity study in mice ([redacted] 1989): Cumulated mortalities after 104-week dietary exposure to glyphosate technical

Sex	Dose group (mg/kg bw/day)*			
	0	100	300	1000
Male	24/50	25/50	21/50	25/50
Female	29/50	34/50	24/50	26/50

* Number of dead / total number

C. CLINICAL OBSERVATIONS
There were no notable intergroup differences in either sex. The clinical signs seen were distributed equally throughout all groups and included emaciation, a hunched posture, subdued behaviour and exophthalmic eyes. These are considered to be typical for mice of this age and strain in a study of this type and duration.

There were no notable intergroup differences in the incidences of externally palpable masses.

D. BODY WEIGHT
A slight decrease in absolute body weight at Week 40 was evident in all groups of both sexes except control and intermediate dose females. This transient effect is not considered to be attributable to administration of glyphosate. Generally, slight (incidental) increases of body weights were observed in substance treated animals of all dose groups until week 52. The effect was most often observed in high dose males being constantly about 10 % and less often observed in high dose females. Between week 52 and 104 the body weights were comparable within all groups. All groups receiving glyphosate showed a comparable weight gain to that of their respective controls. The mean body weight gain data are summarised in the table below.

Table 5.5-115: Glyphosate – 104 week dietary carcinogenicity study in mice ([redacted] 1989): Body weight development (mean values) after 104-week dietary exposure to glyphosate technical

	Dose group (mg/kg bw/day)							
	0		100		300		1000	
	♂	♀	♂	♀	♂	♀	♂	♀
Weight gain (g) 0-104 weeks	12.8	14.9	↑13.1	↓14.1	↑14.4	↓14.7	↑13.0	↑15.2
% of control	--	--	102	95	113	99	102	102

E. FOOD AND WATER CONSUMPTION AND COMPOUND INTAKE

There were no treatment-related effects on food and water consumption for either sex noted during the study.
The overall group mean achieved doses are summarised in table below.

Table 5.5-116: Glyphosate – 104 week dietary carcinogenicity study in mice ([redacted] 1989): Group mean achieved dose levels – oncogenicity study

Dose group	Nominal dose (mg/kg bw/day)	Mean achieved dose level (mg/kg bw/day)		Mean achieved dose level (% of nominal)	
		Males	Females	Males	Females
1 (control)	0	--	--	--	--
2 (low)	100	98 ± 6	102 ± 11	98	102
3 (mid)	300	297 ± 17	298 ± 30	99	99
5 (high)	1000	988 ± 56	1000 ± 113	99	100

Over the entire study duration the mean achieved dosages in all groups were close to the nominal.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology – Differential Blood Counts

There were no notable intergroup differences in either sex at any time point.

H. NECROPSY

Gross pathology

The incidence of lung masses was slightly higher in the male high dose group (18/50) compared to the male control (10/50). There were no other findings in males or females noted at necropsy that could be related to treatment with Glyphosate.

Organ weights

Absolute thymus weight was increased in the male intermediate and high dose groups ($p < 0.01$ and $p < 0.05$, respectively) compared to control. Thymus weight was also increased in the male intermediate and high dose groups ($p < 0.05$ and $p < 0.01$, respectively) after covariance analysis.

There were no other notable intergroup differences in males or females.

Table 5.5-117: Glyphosate – 104 week dietary carcinogenicity study in mice ([redacted] 1989): Absolute thymus weights

Dose group	Nominal dose	absolute Thymus weight (g)

	(mg/kg bw/day)	males	females
control	0	0.02 ± 0.01	0.02 ± 0.01
2 (low)	100	0.02 ± 0.01	0.05 ± 0.05
3 (mid)	300	0.03 ± 0.02**	0.04 ± 0.03
5 (high)	1000	0.03 ± 0.03*	0.06 ± 0.08

* Significantly different from control, $p < 0.05$;
** Significantly different from control, $p < 0.01$

Histopathology
Non-neoplastic changes
The incidence of increased mineral deposits in the brain was higher in the male high dose group (13/50, $p < 0.05$) compared to control (4/49).

All other statistically significant findings in males and females were considered to be typical background changes commonly seen in mice of this age and strain.

Neoplastic findings
There were no statistically significant increases in incidence of any tumour.
The number of animals with tumours, both benign and malignant, was similar between the control and high dose groups of both sexes. However, the number of animals with multiple tumour types was slightly increased in the high dose group of both sexes (males: 16/50 and females: 11/50) compared to control (males: 11/50 and females: 6/50). This led to a slight increase in the total number of tumours in the high dose group of both sexes (males: 60 and females: 43) compared to control (males: 49 and females: 36).

Hemangiosarcoma was evident in 4/50 high dose males, 2/50 low dose females and 1/50 high dose females (not significant) compared to the respective controls (both 0/50).

Histiocytic sarcoma in the lymphoreticular/haemopoietic tissue was evident in 2/50 low and 2/50 high dose males and 3/50 low, 3/50 intermediate and 1/50 high dose females (not significant) compared to the respective Controls (both 0/50).

Other tumours seen were considered to be typical for mice of this age and strain due to the very low incidence of occurrence and were not considered to be due to administration of Glyphosate.

3. Assessment and conclusion

Assessment and conclusion by applicant: In conclusion, glyphosate technical was not carcinogenic in male and female CD-1 mice following continuous dietary exposure of up to 1000 mg/kg bw/day (the limit dose for this type of study) for 104 weeks. Based on the study results and the lack of toxicological relevance of the thymus weight findings, as well as an increase of mineral deposit in the brain observed at 1000 mg/kg bw/day, the NOAEL in rats after chronic exposure to glyphosate technical for 104 weeks is considered to be 1000 mg/kg bw/day.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.5/022
Report author	[redacted]

Report year	1988
Report title	Carcinogenicity and Chronic Toxicity Study of Glyphosate (Technical) of Excel Industries LTD., Bombay
Report No	Not reported
Document No	Not reported
Guidelines followed in study	No guideline followed; similar to OECD 453 (1981)
Deviations from current test guideline (OECD 453, 2018)	Yes, less than 50 (25) animals/sex/dose were used for the main test and less than 10 (5) animals/sex/dose were used as a satellite group; no ophthalmology performed; haematology was performed without determining mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, prothrombin time, activated partial thromboplastin time; clinical chemistry was performed without determining inorganic phosphorous, calcium, chloride, sodium, potassium, cholesterol, AST, any hepatobiliary evaluation, albumin, creatinine, T3, T4, TSH; organ weights of brain, epididymides, spleen, (para)thyroids and uterus were not determined; histopathology was performed without determining bone/bone marrow, caecum, harderian gland, cervix, coagulating gland, epididymides, jejunum, lacrimal gland, upper respiratory tract, rectum, mammary gland, peripheral nerve, prostate, skeletal muscle, spinal cord, trachea, vagina. No historical control data.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Not reported
Acceptability/Reliability:	Invalid Due to restrictions regarding dose levels, number of animals used and insufficient investigations the study is not considered reliable for hazard and risk assessment. In view of the discussion ongoing on the endpoint carcinogenicity a full summary is provided in order to allow a complete re-evaluation. The test substance (batch reference or purity) was also not clearly defined in the report.
Category study in AIR 5 dossier (L docs)	Category 3b

2. Full summary

Glyphosate (Technical) manufactured by Excel Industries Limited, Bombay was administered *via* the diet in concentrations of 75, 150 or 300 ppm (equivalent to mean achieved dose levels of 0, 1.63, 3.35 or 5.87 mg/kg bw/day (males) and 0, 1.65, 3.35 or 5.42 mg/kg bw/day (females)) to 25 Balb/c mice per sex and dose for 80 weeks. In addition 5 animals/sex/dose were used as a satellite group and were treated for 40 month.

Animals were observed for any abnormal toxicity, body weight, food consumption, haematology, clinical chemistry, organ weights, histopathology and occurrence of tumours.

No toxic symptoms were noted during the study. The body weight and the food intake of the high dose animals of both sex was reduced. Haematology and clinical chemistry values of all treated groups were comparable to the control. No significant differences were found in organ weights or histopathological examination and no abnormal rise in tumour occurrence was found in the animals fed with the test material. Glyphosate (Technical) supplied by Excel Industries Ltd., Bombay, did not show any significant increase in tumour formation or toxicological effects when Balb/c mice were fed at the dose of 300 ppm (mixed

with food) for 80 weeks.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate technical
Description:	No data given in the report
Lot/Batch #:	No data given in the report
Purity:	No data given in the report
Stability of test compound:	No data given in the report

2. Vehicle and/or positive control: Diet

3. Test animals:

Species:	Albino mouse
Strain:	Balb/c inbred
Source:	No data given in the report
Age:	5-8 weeks
Sex:	Males and females
Weight at dosing:	Males: 11.64 – 12.24 g, females: 11.30 – 12.04 g
Acclimation period:	No data given in the report
Diet/Food:	Pelleted food (Lipton India Ltd, India), mixed with the test material
Water:	Water, *ad libitum*
Housing:	Initially in groups of five in polypropylene cages
Environmental conditions:	Temperature: 19 - 25 °C
	Humidity: 30 - 70 %
	Air changes: No data given in the report
	12 hours light/dark cycle

B: Study design and methods

In life dates: Not reported

Animal assignment and treatment:
In a combined chronic toxicity and carcinogenicity study groups of 25 Balb/c mice per sex received daily dietary doses of 0, 75, 150 and 300 ppm (equivalent to mean achieved dose levels of 0, 1.63, 3.35 and 5.87 mg/kg bw/day (males) and 0, 1.65, 3.35 and 5.42 mg/kg bw/day (females)) Glyphosate technical for 80 weeks. In addition, for the control and each dose group 5 rats per sex were included for interim sacrifice in month 9 to study blood chemistry and haematology (chronic toxicity study).

Clinical observations
General observations were performed once a day to start with and twice daily later on to prevent death of animals at night. Animals were palpated for occurrence and size of the tumour.

Body weight

Individual body weights were recorded on Day 0, once a week until the end of Week 13 and every 4 weeks thereafter until termination.

Food consumption and compound intake
Food consumption was recorded once weekly for each group from Week 1 to Week 13 and then every 4 weeks.

Haematology and clinical chemistry
The satellite group of 10 animals was sacrificed at the end of 9 months, blood was collected and processed for the following haematological and biochemical studies. All the surviving animals were sacrificed at the end of 18 months, their blood was collected and processed for haematological and biochemical studies.

Haematology
The following parameters were measured: Haemoglobin, total red blood cell count, packed cell volume (PCV), platelets, total white blood cell count and differential white blood cell count.

Blood chemistry
The following parameters were measured: Total serum proteins, alanine transaminase (ALT), blood urea nitrogen and blood glucose.

Sacrifice and pathology
Necropsy was performed on all animals that died during the observation period and all animals at scheduled termination.

The following absolute and relative (percent of body weight) organ weights were determined: adrenals, heart, gonads, kidneys, liver and spleen.

Tissue samples for histopathology were taken from the following organs of all animals: adrenals, aorta, brain, colon, duodenum, eyes, gall bladder, heart, ileum, kidneys, liver, lungs, lymph nodes, oesophagus, ovaries, pancreas, pituitary, salivary glands, seminal vesicles, skin, spleen, stomach, testes, thymus, thyroid, urinary bladder, uterus and tumour.

Statistics
Not reported.

II. RESULTS

A. ANALYSIS OF DOSE FORMULATIONS
Analysis of the dose formulation was not performed.

B. MORTALITY
No treatment-related deaths were observed in this study.

The numbers of pre-terminal deaths in the main group are displayed in the below **Table 5.5-118**:

Table 5.5-118: Carcinogenicity and Chronic Toxicity Study of Glyphosate (Technical) of Excel Industries LTD., Bombay ([redacted], 1988): Cumulated mortalities after 80-week dietary exposure to Glyphosate technical

Sex	Dose group (ppm)			
	0	75	150	300
Male	4/25	7/25	5/25	7/25
Female	4/25	5/25	3/25	7/25

C. CLINICAL OBSERVATIONS

None of the animals under treatment exhibited any toxic symptoms during the course of the study.

D. BODY WEIGHT

The gain in body weight of animals treated with Glyphosate (Technical) at two dose levels 75 ppm and 150 ppm was found to be comparable with that of control animals, while that of animals in the higher dose group (i.e. fed with 300 ppm) was found to be reduced.

Table 5.5-119: Carcinogenicity and Chronic Toxicity Study of Glyphosate (Technical) of Excel Industries LTD., Bombay ([redacted], 1988): Group mean body weights

Timepoint	Sex	Males				Females			
	Dose (ppm)	0	75	150	300	0	75	150	300
	No. of rats	25	25	25	25	25	25	25	25
Week 0		12.00 ± 0.32	↑12.24 ± 0.30	↓11.72 ± 0.27	↓11.64 ± 0.23	11.80 ± 0.29	↑12.04 ± 0.29	↓11.30 ± 0.21	↑11.90 ± 0.30
Week 1		13.88 ± 0.33	↑14.16 ± 0.36	↓13.12 ± 0.35	↓13.28 ± 0.24	14.00 ± 0.37	↑14.10 ± 0.35	↓13.10 ± 0.32	↓13.20 ± 0.39
Week 2		15.48 ± 0.28	↑15.80 ± 0.37	↓13.24 ± 0.28	↓15.00 ± 0.28	16.00 ± 0.33	↑16.20 ± 0.31	↓15.00 ± 0.22	↓15.00 ± 0.40
Week 3		16.80 ± 0.25	↑17.37 ± 0.39	↓16.60 ± 0.31	↓16.52 ± 0.28	17.70 ± 0.32	↑18.30 ± 0.30	↓16.10 ± 0.26	↓16.60 ± 0.41
Week 13		26.24 ± 0.32	↑26.48 ± 0.27	↓25.72 ± 0.45	↓25.72 ± 0.42	27.00 ± 0.31	↑27.60 ± 0.60	↓25.60 ± 0.23	↓25.60 ± 0.54
Week 53		34.79 ± 0.72	↑34.96 ± 0.71	↓34.10 ± 0.48	↓33.10 ± 0.34	33.69 ± 0.67	↑35.12 ± 0.70	↑34.46 ± 0.47	↓33.40 ± 0.52
Week 80		41.70 ± 1.20	↑42.44 ± 1.18	↓40.60 ± 0.79	↓37.83 ± 0.70	39.62 ± 1.01	↑45.25 ± 1.04	↑41.36 ± 0.73	↓36.50 ± 0.56

E. FOOD CONSUMPTION AND COMPOUND INTAKE

Glyphosate (Technical) up to the dose of 150 ppm did not cause any effect on food consumption while the animals in the high group (300 ppm) showed reduction in food intake. The mean intake for each dose group is 1.63, 3.35 and 5.87 mg/kg bw/day (males) and 1.65, 3.35 and 5.42 mg/kg bw/day (females) for 75, 150 and 300 ppm, respectively.

The group mean achieved doses are summarised below.

Table 5.5-120: Carcinogenicity and Chronic Toxicity Study of Glyphosate (Technical) of Excel Industries LTD., Bombay ([redacted], 1988): Group mean achieved dose levels

Dose group	Dietary concentration (ppm)	Mean achieved dose level (mg/kg bw/day)	
		Males	Females
low	75	1.63	1.65
mid	150	3.35	3.35
high	300	5.87	5.42

Table 5.5-121: Carcinogenicity and Chronic Toxicity Study of Glyphosate (Technical) of Excel Industries LTD., Bombay (1988): Group mean food consumption

Timepoint	Sex	Males				Females			
	Dose (ppm)	0	75	150	300	0	75	150	300
	No. of rats	25	25	25	25	25	25	25	25
Week 1		2.20	↓2.16	↓2.16	2.20	2.16	↑2.24	2.16	↑2.28
Week 2		2.36	↑2.40	↓2.24	2.36	2.44	↑2.52	↓2.32	↓2.20
Week 3		2.64	↓2.60	↓2.44	↓2.56	2.68	2.68	↓2.48	↓2.44
Week 4		2.92	↓2.80	↓2.76	↓2.84	3.04	↓2.96	↓2.76	↓2.64
Week 5		3.20	↓3.00	↓3.08	↓3.16	3.36	↓3.08	↓2.88	↓2.84
Week 6		3.60	↓3.36	↓3.48	↓3.48	3.76	↓3.32	↓3.20	↓2.72
Week 7		3.96	↓3.68	↓3.88	↓3.72	4.12	↓3.80	↓3.56	↓2.80
Week 8		4.28	↓4.04	↓4.12	↓3.96	4.40	↓3.92	↓3.84	↓2.84
Week 9		4.64	↓4.52	↑4.72	↓4.00	4.72	↓4.28	↓4.32	↓3.04
Week 10		4.80	↓4.76	↑4.92	↓4.00	4.92	↓4.64	↓4.44	↓3.12
Week 11		4.92	↓4.88	↓4.76	↓3.92	4.72	↓4.68	↓4.60	↓3.40
Week 12		4.80	↑4.88	↓4.60	↓4.16	4.68	↑4.76	↓4.64	↓3.64
Week 13		4.80	4.80	↓4.60	↓4.08	4.60	↑4.68	↓4.52	↓3.80
Week 17		4.60	4.60	↑4.80	↓4.04	4.76	↓4.68	↓4.64	↓3.92
Week 21		4.72	↓4.68	↑4.78	↓4.08	4.84	↓4.56	↓4.52	↓3.60
Week 25		4.84	↑4.86	↑4.92	↓3.88	4.60	↑4.92	↓4.48	↓3.80
Week 29		4.80	↑4.92	↑4.92	↓3.88	4.68	↑4.76	↓4.64	↓3.48
Week 33		4.88	↓4.60	↓4.76	↓3.84	4.68	↑4.84	↓4.56	↓3.44
Week 37		4.84	↓4.76	↓4.72	↓3.72	4.84	↓4.68	↓4.52	↓3.48
Week 41		4.79	↑4.92	↓4.68	↓3.92	4.76	↑4.79	↓4.40	↓3.56
Week 45		4.83	↓4.72	↑4.95	↓3.68	4.84	↓4.71	↓4.56	↓3.60
Week 49		4.87	↓4.71	4.87	↓3.92	4.62	↓4.46	↑4.72	↓3.29
Week 53		4.87	↓4.66	↓4.48	↓3.83	4.52	↑4.58	↑4.54	↓3.16
Week 57		4.87	↓4.40	↓4.35	↓3.78	4.61	↓4.48	↑4.79	↓3.08
Week 61		4.65	↑4.86	↓4.61	↓3.61	4.34	↑4.64	↓4.30	↓3.26
Week 65		4.87	↓4.86	↓4.61	↓3.74	4.41	↑4.64	↓4.39	↓3.43
Week 69		4.74	↑4.76	↓4.55	↓3.67	4.32	↑4.77	↓4.26	↓3.54
Week 73		4.86	↑4.90	↓4.29	↓3.10	4.29	↑4.86	↓4.27	↓3.50
Week 77		4.76	↑5.00	↓4.43	↓3.00	4.19	↑4.80	↓3.82	↓2.90
Week 80		4.71	↑5.00	↓4.30	↓3.00	4.38	↑4.95	↓3.77	↓2.72

F. LABORATORY INVESTIGATION

Haematology

The values of haematological parameters in the animals of treated groups (Groups II, III and IV) were found to be comparable to the animals in the control group (Group I) at interim and final sacrifice. There was no significant difference in the values at interim sacrifice and those at final sacrifice.

Table 5.5-122: Carcinogenicity and Chronic Toxicity Study of Glyphosate (Technical) of Excel Industries LTD., Bombay (1988): Haematology

ppm	Males				Females			
	0	75	150	300	0	75	150	300
	HGB [g%]							
Month 9	14.04 ± 0.38	↑14.4 ± 0.38	↑14.5 ± 0.36	↑14.26 ± 0.29	14.34 ± 0.36	↓14.26 ± 0.47	↑14.44 ± 0.28	↑14.38 ± 0.26
Month 18	14.5 ± 0.16	↓14.4 ± 0.15	14.5 ± 0.19	↓14.4 ± 0.19	14.7 ± 0.16	↓14.5 ± 0.20	↓14.6 ± 0.21	14.7 ± 0.19

Table 5.5-122: Carcinogenicity and Chronic Toxicity Study of Glyphosate (Technical) of Excel Industries LTD., Bombay ([redacted], 1988): Haematology

ppm	Males				Females			
	0	75	150	300	0	75	150	300
HGB [g%]								
PCV [%]								
Month 9	43.8 ± 0.73	↑44.0 ± 1.22	↑45.0 ± 1.09	↓43.4 ± 1.21	44.4 ± 0.93	↓43.8 ± 1.24	↑44.8 ± 0.80	↓43.6 ± 1.08
Month 18	43.7 ± 0.43	↓43.0 ± 0.44	↓43.0 ± 0.44	↓43.2 ± 0.42	42.9 ± 0.42	42.9 ± 0.43	↑43.2 ± 0.21	↑43.4 ± 0.54
Platelet [10^5/mm^3]								
Month 9	2.86 ± 0.13	↓2.81 ± 0.16	↓2.78 ± 0.12	↓2.83 ± 0.16	2.80 ± 0.14	↓2.70 ± 0.07	↓2.63 ± 0.11	↓2.68 ± 0.09
Month 18	2.76 ± 0.08	↑2.89 ± 0.05	↓2.68 ± 0.04	↓2.75 ± 0.05	2.79 ± 0.05	↓2.73 ± 0.04	↑2.80 ± 0.05	↓2.78 ± 0.05
Red blood cell count [10^6/mm^3]								
Month 9	7.98 ± 0.24	↑8.02 ± 0.26	↑8.28 ± 0.28	↑8.2 ± 0.43	8.42 ± 0.27	↑8.46 ± 0.35	↓8.1 ± 0.33	↓8.26 ± 0.16
Month 18	8.4 ± 0.11	↓8.3 ± 0.10	8.4 ± 0.10	↓8.3 ± 0.24	8.0 ± 0.11	↑8.2 ± 0.07	↑8.3 ± 0.09	↑8.4 ± 0.11
Total white blood cell count [10^3/mm^3]								
Month 9	7.44 ± 0.33	↑8.32 ± 0.37	↑7.94 ± 0.45	↑8.1 ± 0.41	7.34 ± 0.35	↑7.62 ± 0.26	↓7.28 ± 0.40	↑7.7 ± 0.30
Month 18	7.7 ± 0.21	↑8.0 ± 0.20	↑8.1 ± 0.17	↑8.0 ± 0.79	8.0 ± 0.19	8.0 ± 0.20	↓7.8 ± 0.17	↓7.8 ± 0.23
Differential white blood cell count [%] - Polymorphs								
Month 9	19.2 ± 1.59	↑21.8 ± 1.82	↑21.4 ± 1.63	↑20.6 ± 1.86	20.4 ± 1.63	↑21.0 ± 1.64	↓19.6 ± 1.01	↑21.2 ± 1.49
Month 18	21.9 ± 0.81	↓21.2 ± 0.79	↓20.8 ± 0.68	↓19.9 ± 0.24	20.2 ± 0.63	↑20.8 ± 0.72	↓19.6 ± 0.56	↓19.8 ± 0.67
Differential white blood cell count [%] - Lymphocytes								
Month 9	80.8 ± 1.59	↓78.2 ± 1.82	↓78.6 ± 1.63	↓79.4 ± 1.86	79.6 ± 1.63	↓79.0 ± 1.64	↑80.4 ± 2.03	↓79.2 ± 1.35
Month 18	78.1 ± 0.81	↑78.8 ± 0.79	↑79.2 ± 0.68	↑80.1 ± 0.24	79.8 ± 0.63	↓79.2 ± 0.72	↑80.4 ± 0.56	↑80.2 ± 0.67

Clinical chemistry

The levels of ALT, blood urea nitrogen, total serum proteins and blood glucose were found to be comparable in all the animals at interim and final sacrifice. There was no significant difference in the values at final sacrifice as compared to those at interim sacrifice.

Table 5.5-123: Carcinogenicity and Chronic Toxicity Study of Glyphosate (Technical) of Excel Industries LTD., Bombay ([redacted] 1988): Clinical chemistry

ppm	Males				Females			
	0	75	150	300	0	75	150	300
Total Protein [g%]								
Month 9	6.2 ± 0.12	↑6.3 ± 0.18	↑6.4 ± 0.27	↑6.3 ± 0.16	6.3 ± 0.14	↓6.1 ± 0.11	↓6.0 ± 0.11	6.3 ± 0.12
Month 18	6.2 ± 0.04	6.2 ± 0.04	6.2 ± 0.05	6.2 ± 0.05	6.1 ± 0.05	↑6.2 ± 0.04	↑6.2 ± 0.04	↑6.3 ± 0.05
Blood urea nitrogen [mg%]								
Month 9	22.6 ± 1.29	↓22.2 ± 0.95	↓22.4 ± 0.66	↑23.2 ± 1.50	22.7 ± 1.43	↑23.1 ± 1.12	↑22.8 ± 1.11	↑23.1 ± 1.03

Table 5.5-123: Carcinogenicity and Chronic Toxicity Study of Glyphosate (Technical) of Excel Industries LTD., Bombay ([redacted], 1988): Clinical chemistry

	Males				Females			
ppm	**0**	**75**	**150**	**300**	**0**	**75**	**150**	**300**
Total Protein [g%]								
Month 18	22.8 ± 0.38	↑24.4 ± 0.44	↑23.7 ± 0.46	↑24.2 ± 0.51	24.4 ± 0.55	↓24.3 ± 0.48	↓24.0 ± 0.43	↓24.0 ± 0.46
ALT [1U/L]								
Month 9	37.2 ± 2.13	↑41.6 ± 3.12	↑40.2 ± 2.27	↑41.2 ± 3.44	43.0 ± 2.97	↓40.6 ± 2.02	↓39.4 ± 3.08	43.0 ± 4.00
Month 18	41.4 ± 1.04	↓40.8 ± 1.41	↑42.0 ± 1.40	↑41.9 ± 1.61	41.3 ± 1.18	↑43.3 ± 1.32	↑41.6 ± 1.37	↑46.3 ± 0.24
Blood glucose [mg%]								
Month 9	95.6 ± 4.49	↓94.0 ± 4.24	↓95.0 ± 3.10	↑98.0 ± 3.74	102.8 ± 4.47	↓98.4 ± 4.40	↓97.8 ± 3.37	↓99.0 ± 4.15
Month 18	95.3 ± 1.87	↓93.6 ± 2.10	↑95.7 ± 1.99	↑98.9 ± 1.91	95.1 ± 1.99	↑96.4 ± 2.01	↑95.8 ± 1.88	↑100.2 ± 1.85

G. NECROPSY

Organ weights

Relative values of organ weights of treated animals of (groups II, III and IV) were not significantly different from those of control animals of Group I.

Table 5.5-124: Carcinogenicity and Chronic Toxicity Study of Glyphosate (Technical) of Excel Industries LTD., Bombay ([redacted], 1988): Organ weights

mg/kg bw/d		Body Weight (g)	Liver (g)	Kidneys (g)	Heart (g)	Spleen (g)	Adrenals (g)	Testes (g)#	Ovaries (g)
Month 18 (males) absolute values	0	41.6 ± 1.21	2.41 ± 0.081	0.62 ± 0.019	0.25 ± 0.014	0.22 ± 0.016	0.008 ± 0.0006	0.19 ± 0.008	-
	75	43.4 ± 1.37	↓2.34 ± 0.048	0.62 ± 0.017	↑0.27 ± 0.011	↓0.19 ± 0.008	0.008 ± 0.0005	↑0.2 ± 0.0	-
	150	40.6 ± 0.81	↓2.23 ± 0.035	↓0.59 ± 0.009	↓0.20 ± 0.008	↓0.21 ± 0.007	↓0.007 ± 0.0001	↑0.20 ± 0.005	-
	300	37.8 ± 0.67	↓2.06 ± 0.027	↓0.55 ± 0.012	↓0.21 ± 0.009	↓0.19 ± 0.005	↓0.007 ± 0.0002	0.19 ± 0.008	-
Month 18 (males) relative values	0	41.6 ± 1.96	5.85 ± 0.26	1.48 ± 0.068	0.61 ± 0.040	0.53 ± 0.044	0.018 ± 0.0014	0.46 ± 0.023	-
	75	43.4 ± 1.37	↓5.46 ± 0.182	↓1.42 ± 0.038	↑0.63 ± 0.037	↓0.45 ± 0.026	↑0.019 ± 0.0008	↑0.47 ± 0.026	-
	150	40.6 ± 0.81	↓5.48 ± 0.060	↓1.45 ± 0.021	↓0.51 ± 0.011	↓0.51 ± 0.012	0.018 ± 0.0008	↑0.49 ± 0.005	-
	300	37.7 ± 0.67	↓5.45 ± 0.064	↓1.46 ± 0.019	↓0.53 ± 0.009	↓0.49 ± 0.007	↑0.019 ± 0.0004	↑0.50 ± 0.019	-
Month 18 (females) absolute values	0	39.6 ± 1.01	2.16 ± 0.070	0.50 ± 0.013	0.22 ± 0.015	0.23 ± 0.017	0.008 ± 0.0003	-	0.03 ± 0.002
	75	42.3 ± 1.04	↑2.44 ± 0.550	↑0.60 ± 0.018	↑0.26 ± 0.012	↓0.22 ± 0.010	↓0.007 ± 0.0004	-	0.030 ± 0.002
	150	41.4 ± 0.78	↑2.23 ± 0.039	↑0.61 ± 0.010	↑0.23 ± 0.009	0.23 ± 0.007	↓0.0077 ± 0.0001	-	0.03 ± 0.002
	300	36.5 ± 0.58	↓2.03 ± 0.022	↑0.51 ± 0.010	↓0.19 ± 0.005	↓0.18 ± 0.003	↓0.007 ± 0.002	-	0.03 ± 0.002
Month	0	39.6 ±	5.28 ±	1.26 ±	0.57 ±	0.58 ±	0.021 ±	-	0.080 ±

Table 5.5-124: Carcinogenicity and Chronic Toxicity Study of Glyphosate (Technical) of Excel Industries LTD., Bombay ([redacted], 1988): Organ weights

mg/kg bw/d		Body Weight (g)	Liver (g)	Kidneys (g)	Heart (g)	Spleen (g)	Adrenals (g)	Testes (g)#	Ovaries (g)
18 (females) relative values		1.01	0.298	0.026	0.033	0.049	0.0010		0.0073
	75	42.3 ± 1.04	↑5.78 ± 0.124	↑1.42 ± 0.030	↑0.60 ± 0.024	↓0.53 ± 0.032	↓0.016 ± 0.0013	-	↓0.072 ± 0.0053
	150	41.36 ± 0.6	↑5.42 ± 0.05	↑1.47 ± 0.010	↑0.58 ± 0.019	↓0.56 ± 0.015	↓0.018 ± 0.0003	-	↓0.076 ± 0.0040
	300	36.5 ± 0.55	↑5.57 ± 0.057	↑1.40 ± 0.013	↓0.51 ± 0.008	↓0.49 ± 0.006	↓0.018 ± 0.004	-	↓0.07 ± 0.004

Numbers of testes weight for male at 75 ppm were not readable properly in the report and may thus not be listed correctly.

Histopathology
Histopathological changes were found at all dose levels including control, hence it is concluded that these are no treatment-related effects.

Table 5.5-125: Carcinogenicity and Chronic Toxicity Study of Glyphosate (Technical) of Excel Industries LTD., Bombay ([redacted], 1988): List of histopathological findings

Organ	Finding
Liver	Degenerative changes, vacuolation of cytoplasm in a few hepatocytes
Lung	Emphysema, perivascular and peribronchiolar lymphoid aggregation
Spleen	Lymphoid hyperplasia, depletion of lymphoid tissue, necrosis
Kidney	Nephrotic changes
Thyroid	Less colloid
Adrenal	Stromal hyperplasia
Uterus	Cystic hyperplasia
Salivary gland	Lymphoid hyperplasia
Ileum	Catarrhal changes
Duodenum	Catarrhal changes

Neoplastic changes
The incidence of tumour finding was comparable in control animals and animals treated at various dose levels.

Table 5.5-126: Carcinogenicity and Chronic Toxicity Study of Glyphosate (Technical) of Excel Industries LTD., Bombay ([redacted], 1988): Neoplastic changes

ppm	Males				Females			
	0	75	150	300	0	75	150	300
Liver: Hepatocellular adenoma	1/25	1/24	0/24	1/24	1/25	0/25	1/25	1/25
Lung: Alveolar adenoma	1/25	1/24	1/24	2/24	1/25	1/25	0/25	1/25

3. Assessment and conclusion

Assessment and conclusion by applicant:
Balb/c mice did not show any significant increase in tumour formation or toxicological effects when given Glyphosate (technical) in the diet at up to 300 ppm (equivalent to 5.87 or 5.42 mg/kg/day in males and females respectively for 80 weeks.
Due to several limitations the study is not considered reliable for hazard and risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.5/023
Report author	[redacted]
Report year	1983
Report title	A chronic feeding study of glyphosate (Roundup® technical) in mice
Report No	77-2061
Document No	M-646425-01-1
Guidelines followed in study	No guideline specified, in general compliance with OECD 451
Deviations from current test guideline (OECD 451, 2018)	Yes, Histopathological examinations were performed without coagulating glands, lacrimal glands, upper respiratory tract, seminal vesicles and vagina. No historical control data.
Previous evaluation	Yes, accepted in the RAR (2015)
GLP/Officially recognised testing facilities	No (pre-GLP; no certificate)
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

This study was designed to assess the oncogenic potential and toxicity of glyphosate (Roundup® Technical) when administered orally, *via* dietary admixture to CD-1 mice (50/sex/group) at dose levels of 1000, 5000 or 30000 ppm (equivalent to 157, 814 and 4841 mg/kg bw/day for males and 190, 955 and 5874 mg/kg bw/day for females) for a period of twenty-four months. Control animals received basal diet.

Detailed physical observations for signs of toxic or pharmacologic effects and palpations for tissue masses were performed weekly throughout the study. Body weight and food consumption measurements were conducted on all animals. Water consumption was measured at month 12 on 10 animals/sex/group and at month 24 on 12 animals/sex/group. Haematology evaluations were conducted on 10 animals/sex/group at months 12 and 18 and 24 and on 12 males/group and all females surviving to the last day of sacrifice. After twenty-four months of treatment, all survivors were sacrificed, selected organs were weighed and organ/body and organ/brain weight ratios were calculated. Complete gross post-mortem examinations and histopathological evaluation of selected tissues were conducted on all animals.

Mean body weights for the high-dose males were generally lower than control; differences from control

were as great as -11 % (at Week 102) and were, for the most part, statistically significant. Mean body weights for the high-dose females and the males and females at the low- and mid-dose levels did not demonstrate a response to treatment.

Other parameters evaluated, i.e. general animal condition, body weight gain. food consumption, feed efficiency, water consumption and haematology revealed no consistent dose- or treatment-related response to administration of glyphosate.
At the terminal sacrifice, the mean absolute and relative (to body and brain weights) weight of the testes were elevated for the high-dose group. Other organ weight differences noted were attributed to differences in body weight or were sporadic and were not considered treatment-related.

Correlation of necropsy observations with microscopic findings revealed no treatment relationship.

Neoplastic findings were those commonly encountered in mice. Bronchioalveolar tumours of the lungs, hepatocellular neoplasms, and tumours of the lymphoreticular system accounted for the majority of those encountered. There were no suspected test substance-associated trends in the incidence of these tumours or in any of the other spontaneously occurring neoplasms. Lymphoreticular tumours tended to be more frequent in treated animals, particularly the females. The numbers were relatively small and differences from the control failed to provide supportive evidence that the neoplasms had a test substance relationship.

The other neoplasms that occurred with any frequency in treated mice only were renal tubule adenomas, which occurred in males. Three were present at the high-dose and one at the mid-dose level; however, the distribution of this benign tumour was considered spurious and unrelated to treatment. All other neoplasms occurred sporadically and were considered to have had no relationship to treatment.

Of the non-neoplastic findings, hepatic central lobular hypertrophy was noted with increased incidence in the high-dose group. This change may represent a hepatocellular adaptation to the metabolism of the test substance. A slight increase in the incidence of slight to mild epithelial hyperplasia was noted in the urinary bladder of the mid- and high-dose males; however, no treatment relationship was suspected.

All other tissue alterations occurred sporadically or were considered to have been spurious in distribution. Most occurred with approximately equal frequency and severity in control and treated mice and were judged to be unrelated to glyphosate administration.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate (Roundup® technical)
Description:	Fine, white clumped powder
Lot/Batch #:	NB 1782608/3 and NB 1782610/7
Purity:	99.7 %
Stability of test compound:	Not reported

2. Vehicle and/or positive control: Diet

3. Test animals:

Species:	Mouse
Strain:	CD-1, COBS (ICR derived)
Source:	[redacted]

Age:	at receipt: 29 days; at treatment: 40 days
Sex:	Males and females
Weight at dosing:	Males: 23 g (16 – 28 g); females: 20 g (15 – 24 g)
Acclimation period:	11 days
Diet/Food:	Purina® Rodent Laboratory Chow #5001, *ad libitum*
Water:	automated watering system (Elizabethtown Water Company), *ad libitum*
Housing:	Animals were doubly housed in elevated stainless steel wire mesh cages during the first week of the acclimation period and individually housed thereafter.
Environmental conditions:	Temperature: 18.3 – 23.9 °C
	Humidity: 30 – 80 %
	Air changes: not reported
	12 hours light/dark cycle

B: Study design and methods

In life dates: 1980-03-31 to 1982-03-14

Animal assignment and treatment:
In a carcinogenicity study groups of 50 CD-1 mice per sex received daily dietary doses of 0, 1000, 5000 or 30000 ppm (equivalent to 157, 814 and 4841 mg/kg bw/day for males and 190, 955 and 5874 mg/kg bw/day for females) glyphosate technical for 104 weeks.

Test diets were prepared once per week. Analyses for achieved concentrations of the test substance in the diet were conducted by the sponsor.

Clinical observations
A check for mortality and for gross signs of toxicology was made twice daily on all animals throughout the study. A detailed clinical examination and check were performed at the beginning of the study and once each week on every animal.

Body weight
Individual body weights were recorded for each animal twice before dosing, at weekly intervals until the end of week 14, biweekly thereafter until termination and at termination.

Food and water consumption and compound intake
Food consumption was recorded once weekly for each cage group starting one week before treatment until Week 14 and subsequently biweekly until termination.

Measured and recorded daily for 10 animals/sex/group at month 12 over one 3-day interval. A second 3-day interval, at month 12, was omitted due to technician error. Due to the high mortality across all groups during month 24, water consumption measurements were initiated on 12 animals/sex/group in an attempt to insure adequate sample size for statistical evaluation. Measurements at month 24 were performed over one 3-day and one 2-day interval.

Achieved dosages were calculated from nominal dietary concentration, taking into account actual food consumption and body weight data.

Haematology
During Month 12, 18 and 24 of dosing, blood was obtained *via* venipuncture of the orbital sinus (retrobulbar

venous plexus) under light ether anaesthesia. Animals were selected randomly; the same animals were used at all intervals when feasible. Mice were fasted overnight prior to blood collections and were not fed until after samples were collected.

Performed on 10 animals/sex/group, evaluations were conducted on 12 males/group (to insure adequate sample size for statistical evaluation) and on all females surviving to the last day of sacrifice (mortality occurring during the water consumption measurement period resulted in haematology evaluations on fewer than 10 animals/group in some groups).

The following parameters were measured: Haemoglobin, haematocrit, erythrocytes, platelets, total and differential leukocytes, erythrocyte morphology.

Sacrifice and pathology
Complete gross post-mortem examination was performed on all animals which died spontaneously, accidentally, or were killed moribund as well as at termination. External surface, all orifices, the cranial cavity, carcass, the external surfaces of the brain and spinal cord, nasal cavity and paranasal sinuses, the thoracic, abdominal and pelvic cavities and their viscera and the cervical tissues and organs were examined for all animals. Animals were fasted prior to the terminal sacrifice.

The following organs were weighed: adrenals, brain (with entire brain stem), heart, kidneys, liver, ovaries, spleen and testes.

The following organs were examined histopathologically: adrenals, abdominal aorta, bladder, bone and bone marrow (costochondral junction), bone marrow smear and blood smear, brain, epididymides, eyes (with optic nerve and contiguous Harderian glands), gross lesions, head, heart, intestine (duodenum, jejunum, ileum, caecum, colon, rectum), kidneys, liver with gall bladder, lungs, mammary gland, lymph nodes (mediastinal and mesenteric), muscle (biceps femoris), oesophagus, ovaries, pancreas, mandibular salivary glands, pituitary, prostrate, sciatic nerve, skin, spinal cord (cervical), spleen, stomach, testes, thymus, thyroid/parathyroid, tissue masses or suspect tumours and regional lymph nodes, trachea and uterus (with cervix).

Statistics
Body weight, body weight gain, food and water consumption, feed efficiency, haematology parameters, terminal organ and body weights, organ/body and organ/brain weight ratios were analysed. Mean values of all dose groups were compared to control at each time interval.

II. RESULTS

A. ANALYSIS OF DOSE FORMULATIONS
Analyses for achieved concentrations of the test substance in the diet were conducted by the sponsor.

B. MORTALITY
The incidence of mortality was low during the first 18 months of study, with survival rates greater than or equal to 62 % and 68 % for the males and females, respectively. The mortality rate increased after 18 months, as would be expected in aging mice. The incidence of mortality was considered to demonstrate no dose- or test substance-related adverse effect of glyphosate administration on survival, i.e. survival was greater than control in the high-dose males and mid- and high-dose females, with the lowest survival in the low-dose females.

The numbers of deaths are summarised in the table below.

Table 5.5-127: A chronic feeding study of glyphosate (Roundup® technical) in mice ([redacted], 1983): Cumulated mortalities after 104-week dietary exposure to glyphosate technical

Sex	Dose group (ppm)[1]			
	0	1000	5000	30000
Male	27/50 (3)	32/50 (2)	32/50 (1)	23/50 (1)
Female	27/50 (3)	38/50 (0)	22/50 (1)	23/50 (4)

[1] Number of dead / total number; accidental deaths are presented in parentheses

C. CLINICAL OBSERVATIONS

The physical observations noted throughout the study were considered common for CD-1 mice under laboratory conditions; these observations included yellow staining of the anogenital area, dermatological abnormalities (scabbing on the ears and alopecia), excessive lacrimation, displacement of the pupils and ocular opacities. These observations occurred with low incidence and did not occur in a pattern suggestive of a relationship to treatment.

D. BODY WEIGHT

Sporadic differences from control were noted in the mean body weight data for the high-dose females, and the males and females at the low- and mid-dose levels. However, these differences from control were slight and failed to demonstrate a consistent pattern with respect to treatment level over time, and were therefore, not considered to reflect a response to treatment.

Body weight gain data exhibited some variability, as is common with mouse studies. No treatment-related trends were apparent in these data for male and female animals.

Table 5.5-128: A chronic feeding study of glyphosate (Roundup® technical) in mice ([redacted] 1983): Summary of cumulative body weight changes

ppm		Males				Females			
		0	1000	5000	30000	0	2000	6000	20000
Week -1	Mean body weight (g)	21.5	21.5	↓21.4	↑21.6	19.4	↓19.3	19.4	↓19.3
	Std. Dev.	1.2	1.3	1.2	1.5	1.7	1.6	1.5	1.8
Week 0	Mean body weight (g)	22.6	↑22.8	↓22.5	↓22.5	20.3	↑20.5	↓19.9	↓19.6
	Std. Dev.	1.3	1.4	1.8	1.7	1.4	1.5	1.7	1.4
Week 1	Mean body weight (g)	25.8	↓25.5	↓25.0	↓**24.5****	22.0	↑22.6	↑22.4	↑22.4
	Std. Dev.	1.4	1.6	2.0	1.8	1.5	1.5	1.7	1.4
Week 2	Mean body weight (g)	29.1	↓**27.7****	↓**27.8****	↓**26.1****	22.5	↑**23.4***	↑23.2	↑**23.9****
	Std. Dev.	1.8	1.8	2.2	1.9	1.5	1.5	1.7	1.6
Week 3	Mean body weight (g)	28.7	↑28.8	↑29.2	↓**27.5***	23.7	↑**24.5***	↑24.4	↑**24.8****
	Std. Dev.	1.6	1.9	2.3	2.1	1.8	1.5	1.8	1.8
Week 13	Mean body weight (g)	34.8	↓**33.3***	↑35.1	↓**33.2****	28.9	↑29.5	↑29.3	↓28.8
	Std. Dev.	2.4	2.6	2.8	2.5	1.8	2.1	2.1	2.0
Week 24	Mean body weight (g)	35.6	↓34.7	↓35.3	↓35.5	31.0	↑31.3	↓30.7	↓30.9
	Std. Dev.	2.3	3.3	3.2	2.5	2.2	2.6	2.5	2.4
Week 52	Mean body weight (g)	36.4	↓35.0	↓36.1	↓**33.8****	32.3	↑33.1	↓**30.0****	↓32.1
	Std. Dev.	2.7	2.8	3.1	2.8	2.8	2.9	3.2	2.5
Week 78	Mean body weight (g)	38.0	↓37.6	↓37.4	↓**35.6***	33.1	↑**35.5**§	↑33.4	↓31.5

Table 5.5-128: A chronic feeding study of glyphosate (Roundup® technical) in mice ([redacted] 1983): Summary of cumulative body weight changes

ppm		Males				Females			
		0	1000	5000	30000	0	2000	6000	20000
	Std. Dev.	3.3	3.7	4.7	3.0	3.0	3.9	4.2	2.5
Week 100	Mean body weight (g)	36.4	↑**39.6***	↑**39.7****	↑36.6	35.1	↑37.6	↑35.7	↓33.6
	Std. Dev.	2.4	2.9	1.9	2.7	3.2	5.6	4.9	2.7
Week 102	Mean body weight (g)	37.7	↑37.9	↓35.7	↓**33.6****	-	-	-	-
	Std. Dev.	2.6	3.6	2.5	3.6	-	-	-	-

* Significantly different from control (Dunnett's test; $p \leq 0.05$)
** Significantly different from control (Dunnett's test; $p \leq 0.01$)
§ Significantly different from control (Dunn's Rank Sum; $p \leq 0.05$)
§§ Significantly different from control (Dunn's Rank Sum; $p \leq 0.01$)

Table 5.5-3129: A chronic feeding study of glyphosate (Roundup® technical) in mice ([redacted] 1983): Summary of cumulative body weight gains

ppm		Males				Females			
		0	1000	5000	30000	0	2000	6000	20000
Week 0	Mean body weight gain (g)	1.1	↑1.3	1.1	↓0.9	0.9	↑1.2	↓0.5	↓**0.2***
	Std. Dev.	1.1	1.8	1.2	1.2	1.2	1.3	1.3	1.6
Week 1	Mean body weight gain (g)	3.2	↓**2.7***	↓**2.5****	↓**2.0****	1.7	↑2.1	↑**2.5****	↑**2.8****
	Std. Dev.	0.9	0.6	1.0	0.9	0.9	1.0	1.1	1.1
Week 2	Mean body weight gain (g)	3.3	↓**2.2****	↓**2.8****	↓**1.6****	0.9	↓0.8	↓0.8	↑**1.5****
	Std. Dev.	0.6	0.6	0.7	0.6	0.9	0.9	1.0	0.8
Week 3	Mean body weight gain (g)	-0.5	↑**1.1****	↑**1.4****	↑**1.4****	1.1	1.1	↑1.2	↓0.9
	Std. Dev.	0.4	0.5	0.5	0.5	1.0	0.7	0.7	0.8
Week 13	Mean body weight gain (g)	0.2	↓-**1.1****	↓0.1	↓-**0.4****	1.4	↓1.1	↓-**0.2****	↓-**0.9****
	Std. Dev.	0.6	0.5	0.5	0.4	0.9	1.0	0.9	0.9
Week 24	Mean body weight gain (g)	0.0	↓-**1.0****	↓-0.2	↑0.2	0.3	↓0.2	↓0.2	↑0.7
	Std. Dev.	0.7	0.9	0.7	0.8	1.5	1.0	0.9	1.3
Week 52	Mean body weight gain (g)	-1.2	↑-1.1	↑-**0.3****	↓-1.5	-0.8	↑**0.1****	↓-**2.8****	↑**1.0****
	Std. Dev.	1.3	0.8	1.3	1.1	1.2	1.1	1.3	1.1
Week 78	Mean body weight gain (g)	-0.6	↑**0.5**§§	↓**-1.4**§§	↓**-2.1**§§	0.4	↑0.9	↑0.5	↓**-0.6**§§
	Std. Dev.	0.6	0.5	2.0	1.1	1.5	2.1	1.6	1.1
Week 100	Mean body weight gain (g)	-1.2	↑**0.4****	↑**2.2****	↑**1.7****	1.1	↑2.0	↓0.8	↑1.4
	Std. Dev.	0.8	0.8	0.8	0.5	1.4	1.8	1.1	1.2
Week 102	Mean body weight gain (g)	0.5	↓**-1.5***	↓-**3.1****	↓-**2.9****	-	-	-	-
	Std. Dev.	1.7	2.0	1.9	2.5	-	-	-	-

* Significantly different from control (Dunnett's test; $p \leq 0.05$)
** Significantly different from control (Dunnett's test; $p \leq 0.01$)
§ Significantly different from control (Dunn's Rank Sum; $p \leq 0.05$)
§§ Significantly different from control (Dunn's Rank Sum; $p \leq 0.01$)

E. FOOD AND WATER CONSUMPTION AND COMPOUND INTAKE

There were no treatment-related effects on food and water consumption for either sex noted during the study. Test substance intake was calculated from individual body weight and food consumption values and nominal concentrations of 1000, 5000 and 30000 ppm of glyphosate and expressed as milligrams of test substance per kilogram of body weight per day (mg/kg/day).

The overall group mean achieved doses are summarised in the table below.

Table 5.5-130: A chronic feeding study of glyphosate (Roundup® technical) in mice ([redacted] 1983): Summary of food consumption data

ppm		Males				Females			
		0	**1000**	**5000**	**30000**	**0**	**2000**	**6000**	**20000**
Week 0	Mean (g)	272.8	↑280.9	↑274.5	↓265.4	273.2	↓269.6	↓270.0	↑297.9
	Std. Dev.	36.6	40.4	42.6	54.0	60.5	64.6	50.3	80.1
Week 1	Mean (g)	250.7	↓249.9	↑252.8	↓**240.7**§§	287.3	↑287.8	↓**249.1**§§	↑328.6
	Std. Dev.	17.0	27.8	29.6	46.9	69.5	53.8	53.6	101.4
Week 2	Mean (g)	211.2	↑**230.6****	↑**232.8****	↑**234.7****	266.0	↑284.3	↓264.3	↑**298.7**§§
	Std. Dev.	20.4	16.8	19.0	21.4	32.0	52.0	30.9	53.2
Week 3	Mean (g)	202.0	↓197.7	↑208.7	↓197.1	246.4	↓244.2	↑260.8	↑289.8
	Std. Dev.	22.1	22.3	27.3	29.1	29.9	54.3	36.4	84.4
Week 13	Mean (g)	149.9	↑**163.7****	↑150.2	↑**161.1****	186.5	↑190.8	↑198.5	↑**215.6****
	Std. Dev.	12.3	17.7	12.2	12.7	27.4	46.8	19.0	48.6
Week 24	Mean (g)	162.4	↑164.8	↑**183.3**§§	↑170.2	171.1	↑181.9	↑**186.9***	↑**194.5****
	Std. Dev.	10.7	17.0	23.6	19.5	23.4	26.3	23.4	32.2
Week 52	Mean (g)	176.2	↓172.7	↓**164.3****	↓171.8	187.2	↑194.6	↑**226.5**§§	↓**149.5**§§
	Std. Dev.	12.8	15.3	16.3	13.9	18.9	42.3	24.4	29.9
Week 78	Mean (g)	146.5	↓137.3	↓143.9	↓**141.6****	165.7	↓153.7	↑172.7	↑173.2
	Std. Dev.	20.3	19.5	25.4	18.7	32.1	26.2	20.8	26.6
Week 98	Mean (g)	145.2	↑153.8	↑159.0	↑**178.5**§§	165.1	↑**187.6****	↑165.5	↑177.6
	Std. Dev.	20.6	46.9	46.8	62.1	28.9	25.3	20.7	22.1
Week 100	Mean (g)	158.5	↓**119.1****	↓**103.9****	↓**115.5****	164.7	↓**143.8****	↓**144.4****	↓**141.1****
	Std. Dev.	11.1	11.5	11.8	14.8	18.3	21.6	16.1	17.6
Week 102	Mean (g)	139.2	↑140.7	↑145.2	↑145.0	-	-	-	-
	Std. Dev.	20.1	20.5	27.6	17.4	-	-	-	-

* Significantly different from control (Dunnett's test; $p \leq 0.05$)
** Significantly different from control (Dunnett's test; $p \leq 0.01$)
§ Significantly different from control (Dunn's Rank Sum; $p \leq 0.05$)
§§ Significantly different from control (Dunn's Rank Sum; $p \leq 0.01$)

Table 5.5-4131: A chronic feeding study of glyphosate (Roundup® technical) in mice ([redacted] 1983): Group mean achieved dose levels – oncogenicity study

Dose group	Concentration (ppm)	Mean achieved dose level ranges (mg/kg bw/day)		Mean achieved dose level according to study report (mg/kg bw/day)	
		Males	Females	Males	Females
1 (control)	0	--	--	--	--
2 (low)	1000	110.9 – 249.9	128.9 – 287.8	157	190
3 (mid)	5000	519.3 – 1264.2	689.7 – 1321.5	814	955
4 (high)	30000	3465.0 – 7219.8	4232.4 – 9858.6	4841	5874

Mean water consumption values (expressed in millilitres of water consumed per kilogram of body weight per day) for the treated males at month 12 were lower than control values; however, differences from control were not statistically significant and at month 24, values for the treated males (particularly the high-dose group) were comparable to control values. Water consumption values for the low- and mid-dose females were somewhat elevated at month 12 relative to control, but a similar pattern was not evident at month 24. No consistent test substance-related effects on water consumption were apparent in these data.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Although a few statistically significant differences from control were noted for some of the haematology parameters evaluated, these differences occurred sporadically or did not occur in a dose-related pattern and were not consistent over time. Therefore, these slight differences were not considered to be of toxicological significance.

Evaluation of differential leukocytes and erythrocyte morphology revealed no evidence of an effect of glyphosate administration on these parameters.

Table 5.5-132: Chronic study of glyphosate administered in feed to Albino rats ([redacted] 1983): Summary of haematological data

ppm		Males				Females			
		0	1000	5000	30000	0	2000	6000	20000
HGB									
Month 12	Mean (g/dL)	14.5	↓13.7	↓14.1	14.5	14.7	↓14.2	↓14.6	↓14.3
	Std. Dev.	0.6	1.2	1.2	1.0	0.8	1.1	0.7	1.3
Month 18	Mean (g/dL)	14.2	↓13.7	↓13.5	↑14.4	13.8	↓13.3	↓13.7	↑14.4
	Std. Dev.	0.8	0.6	1.6	1.4	0.9	1.1	1.4	0.6
Month 24	Mean (g/dL)	14.5	↑15.3	↓14.4	↓13.6	13.8	↓12.9	13.8	↓13.7
	Std. Dev.	0.7	2.7	1.2	2.3	0.9	2.8	1.6	1.8
HCT									
Month 12	Mean (%)	43	↓41	↓42	43	44	↓43	44	↓43
	Std. Dev.	2	3	4	3	2	4	2	4
Month 18	Mean (%)	43	↓42	↓41	↑44	43	↓41	43	↑44
	Std. Dev.	3	2	5	4	2	3	4	2
Month 24	Mean (%)	43	↑46	43	↓41	42	↓39	↓40	↓41
	Std. Dev.	2	7	4	7	3	8	4	5
RBC									
Month 12	Mean ($10^6/mm^3$)	7.96	↓7.52	↓7.77	↑7.98	7.76	↓7.66	↑7.90	↓7.71
	Std. Dev.	0.53	0.74	0.68	0.54	0.50	0.69	0.65	0.75
Month 18	Mean ($10^6/mm^3$)	8.18	↓7.85	↓7.52	↑8.22	7.66	↓7.53	↑7.78	↑7.92
	Std. Dev.	0.65	0.33	0.92	0.83	0.56	0.66	0.78	0.39
Month 24	Mean ($10^6/mm^3$)	8.58	↑8.89	↑8.65	↓7.91	7.73	↓7.39	↑7.98	↑8.58
	Std. Dev.	0.66	1.36	0.90	1.43	0.58	1.59	0.79	3.21
Plat									
Month 12	Mean ($10^5/mm^3$)	16.05	↑**18.17***	↓14.75	↑17.48	14.00	↑15.84	↑16.17	↓13.43
	Std. Dev.	1.75	0.97	1.51	1.69	2.02	3.32	5.04	3.41
Month 18	Mean ($10^5/mm^3$)	16.42	↑19.86	↓13.86	↑16.70	11.12	↑12.15	↑11.32	↑11.17
	Std. Dev.	5.13	5.37	3.21	3.18	2.15	1.89	3.09	2.05
Month 24	Mean ($10^5/mm^3$)	16.97	↓16.90	↓14.41	↓15.42	9.27	↑12.42	↑10.51	↓8.58
	Std. Dev.	4.85	5.78	4.44	6.16	2.27	5.04	3.68	3.21
RBC									
Month 12	Mean ($10^3/mm^3$)	5.5	↑6.1	↑5.9	↓**3.3***	4.3	↑4.6	↑4.7	↓3.9
	Std. Dev.	1.8	2.6	2.0	1.4	2.8	2.8	2.8	2.1
Month 18	Mean ($10^3/mm^3$)	4.2	↑5.6	↑4.4	4.2	5.9	↓4.7	↓4.8	↓4.4
	Std. Dev.	1.5	2.6	2.8	2.1	2.3	1.8	2.9	2.0

Table 5.5-132: Chronic study of glyphosate administered in feed to Albino rats ([redacted] 1983): Summary of haematological data

ppm		Males				Females			
		0	1000	5000	30000	0	2000	6000	20000
HGB									
Month 24	Mean (10^3/mm^3)	4.5	↑5.5	↓3.9	↓3.9	4.2	↓4.1	↓3.9	↓3.4
	Std. Dev.	1.8	2.6	1.6	1.9	1.1	2.0	2.9	1.0

* Significantly different from control (Dunnett's test; $p \leq 0.05$)

H. NECROPSY

Gross pathology

Correlation of necropsy observations with microscopic findings revealed no trend indicating a test substance relationship.

Organ weights

Differences in mean absolute or relative (to body or brain weights) organ weights from control for this group were considered reflective of the difference in mean body weight and not of toxicological significance. The exception to this pattern was the increased mean absolute and relative weights of the testes for the high-dose group; although histopathologic evaluation did not reveal any morphologic abnormalities in this tissue. Sporadic organ weight differences were noted at lower dose levels; however, they were not considered toxicologically significant, as no dose response relationship was evident.

The mean absolute and relative (to body and brain weight) ovarian weights for the high-dose females were markedly elevated, although differences from control were not statistically significant. These differences were due to a single animal, whose ovarian weight of 0.213 g was approximately six times the mean ovarian weight for the group when this value was excluded ($\bar{x}$ = 0.033 grams). Differences from control excluding this animal were not considered remarkable. Other organ weight differences were attributed to body weight differences or were sporadic and were not considered to be related to the administration of glyphosate.

Table 5.5-133: A chronic feeding study of glyphosate (Roundup® technical) in mice ([redacted] 1983): Group mean organ weights – oncogenicity study

Dose group	Nominal dose (ppm)	Organ weight (abs.)	Organ weight (rel. to body weight)	Organ weight (rel. to brain weight)
Testes (males)				
1 (control)	0	0.157 ± 0.056	4.97 ± 1.80	3.22 ± 1.17
2 (low)	1000	↓0.153 ± 0.058	↓4.71 ± 1.87	↓3.05 ± 1.18
3 (mid)	5000	↑0.158 ± 0.059	↑5.23 ± 2.10	↑3.26 ± 1.17
4 (high)	30000	↑0.168 ± 0.046	↑5.84 ± 1.58	↑3.46 ± 0.90
Ovaries (females)				
1 (control)	0	0.026 ± 0.034	9.40 ± 11.20	5.21 ± 7.26
2 (low)	1000	↓0.016 ± 0.010	↓5.46 ± 3.21	↓3.05 ± 1.92
3 (mid)	5000	↓0.021 ± 0.023	↓3.98 ± 7.45	↓4.35 ± 4.86
4 (high)	30000	↑0.041 ± 0.046	↑13.41 ± 14.81	↑8.17 ± 9.13
Brain (males)				
1 (control)	0	0.489 ± 0.023	1.55 ± 0.12	1.00 ± 0.0
2 (low)	1000	↑0.501 ± 0.033	↓1.54 ± 0.13	1.00 ± 0.0
3 (mid)	5000	↓0.480 ± 0.022	↑1.58 ± 0.15	1.00 ± 0.0
4 (high)	30000	↓0.486 ± 0.025	**↑1.69** ± 0.17**	1.00 ± 0.0
Brain (females)				
1 (control)	0	0.511 ± 0.044	2.01 ± 0.32	1.00 ± 0.0

Table 5.5-133: A chronic feeding study of glyphosate (Roundup® technical) in mice ([redacted] 1983): Group mean organ weights – oncogenicity study

Dose group	Nominal dose (ppm)	Organ weight (abs.)	Organ weight (rel. to body weight)	Organ weight (rel. to brain weight)
2 (low)	1000	0.511 ± 0.026	↓1.83 ± 0.17	1.00 ± 0.0
3 (mid)	5000	↓0.502 ± 0.33	**↓1.66** ± 0.18**	1.00 ± 0.0
4 (high)	30000	↓0.501 ± 0.032	**↓1.69** ± 0.22**	1.00 ± 0.0
Adrenals (males)				
1 (control)	0	0.0055 ± 0.0035	1.76 ± 1.07	1.13 ± 0.70
2 (low)	1000	↓0.0038 ± 0.0017	↓1.18 ± 0.49	↓0.77 ± 0.36
3 (mid)	5000	↓0.0049 ± 0.0023	↓1.59 ± 0.71	↓1.02 ± 0.49
4 (high)	30000	0.0055 ± 0.0020	↑1.91 ± 0.72	1.13 ± 0.42
Adrenals (females)				
1 (control)	0	0.077 ± 0.0027	2.94 ± 0.83	1.51 ± 0.53
2 (low)	1000	↑0.0085 ± 0.0015	↑3.04 ± 0.40	↑1.67 ± 0.29
3 (mid)	5000	↑0.0081 ± 0.0031	↓2.69 ± 1.04	↑1.60 ± 0.57
4 (high)	30000	↓0.0074 ± 0.0021	↓2.48 ± 0.64	↓1.47 ± 0.40
Heart (males)				
1 (control)	0	0.192 ± 0.026	6.11 ± 0.93	3.95 ± 0.57
2 (low)	1000	↓0.191 ± 0.024	↓5.87 ± 0.73	↓3.82 ± 0.48
3 (mid)	5000	↓0.186 ± 0.022	↑6.13 ± 0.77	↓3.88 ± 0.45
4 (high)	30000	↓0.175 ± 0.025	↓6.03 ± 0.65	↓3.60 ± 0.50
Heart (females)				
1 (control)	0	0.156 ± 0.042	6.02 ± 1.73	3.05 ±0.79
2 (low)	1000	↑0.164 ± 0.24	↓5.84 ± 0.53	↑3.22 ± 0.49
3 (mid)	5000	↑0.169 ± 0.048	↓5.56 ± 1.41	↑3.39 ± 0.49
4 (high)	30000	↓0.155 ± 0.017	**↓5.23§§ ± 0.71**	↑3.12 ± 0.47
Kidneys (males)				
1 (control)	0	0.693 ± 0.144	2.19 ± 0.47	1.42 ± 0.30
2 (low)	1000	↓0.682 ± 0.080	↓2.09 ± 0.22	↓1.36 ± 0.19
3 (mid)	5000	↓0.666 ± 0.130	↑2.21 ± 0.46	↓1.39 ± 0.27
4 (high)	30000	↓0.635 ± 0.098	↑2.20 ± 0.29	↓1.31 ± 0.19
Kidneys (females)				
1 (control)	0	0.489 ± 0.082	1.90 ± 0.27	9.57 ± 1.54
2 (low)	1000	↑0.495 ± 0.068	↓1.77 ± 0.23	↑9.68 ± 1.18
3 (mid)	5000	↑0.513 ± 0.088	**↓1.68* ± 0.24**	↑10.23 ± 1.74
4 (high)	30000	↑0.511 ± 0.078	↓1.71 ± 0.23	↑10.24 ± 1.70
Liver (males)				
1 (control)	0	1.753 ± 0.483	5.60 ± 1.80	3.59 ± 0.95
2 (low)	1000	↑1.822 ± 1.156	↑5.83 ± 3.79	↑3.80 ± 2.47
3 (mid)	5000	↓1.488 ± 0.179	↓4.88 ± 0.52	↓3.10 ± 0.37
4 (high)	30000	**↓1.475§ ± 0.319**	↓5.08 ± 0.95	↓3.03 ± 0.63
Liver (females)				
1 (control)	0	1.339 ± 0.316	5.12 ± 0.85	2.62 ± 0.61
2 (low)	1000	↑1.521 ± 0.401	↑5.37 ± 1.10	↑2.97 ± 0.74
3 (mid)	5000	↑1.595 ± 0.443	↑5.19 ± 1.19	↑3.18 ± 0.89
4 (high)	30000	↑1.393 ± 0.13	↓4.69 ± 0.83	↑2.80 ± 0.52
Spleen (males)				
1 (control)	0	0.089 ± 0.060	2.84 ± 2.00	1.83 ± 1.22
2 (low)	1000	↑0.144 ± 0.217	↑4.43 ± 6.56	↑2.89 ± 4.34
3 (mid)	5000	↓0.067 ± 0.020	↓2.22 ± 0.63	↓1.41 ± 0.41
4 (high)	30000	↓0.064 ± 0.019	↓2.22 ± 0.69	↓1.32 ± 0.40

Table 5.5-133: A chronic feeding study of glyphosate (Roundup® technical) in mice ([redacted] 1983): Group mean organ weights – oncogenicity study

Dose group	Nominal dose (ppm)	Organ weight (abs.)	Organ weight (rel. to body weight)	Organ weight (rel. to brain weight)
Spleen (females)				
1 (control)	0	0.099 ± 0.056	3.81 ± 2.05	1.96 ± 1.12
2 (low)	1000	↓0.091 ± 0.043	↓3.20 ± 1.44	↓1.78 ± 0.82
3 (mid)	5000	↑0.136 ± 0.090	↑4.37 ± 2.81	↑2.69 ± 1.80
4 (high)	30000	↑0.100 ± 0.064	↓2.29 ± 1.98	↑2.02 ± 1.30

* Significantly different from control (Dunnett's test; $p \leq 0.05$); ** Significantly different from control (Dunnett's test; $p \leq 0.01$); §§ Significantly different from control (Dunn's Rank Sum; $p \leq 0.01$)

Histopathology

Non-neoplastic changes

The only non-neoplastic alteration in the urinary tract that occurred with an increased frequency over controls was slight-to-mild epithelial hyperplasia of the urinary bladder in males. The incidence was 6 %, 6 %, 20 % and 16 % controls through high-dose, respectively. Even though the increase at the mid- and high-dose was present is considered test substance related.

All other tissue alterations occurred sporadically or were considered to have been spurious in their distribution. Most occurred with approximately equal frequency and severity in control and treated animals, and were judged to be unrelated to glyphosate administration.

Table 5.5-134: Chronic study of glyphosate administered in feed to Albino rats ([redacted] 1983): Summary of selected non-neoplastic findings

ppm	Males				Females			
	0	1000	5000	30000	0	2000	6000	20000
Heart; Myofiber degeneration	2 (4 %)	2 (4 %)	5 (10 %)	1 (2 %)	1 (2 %)	0 (0 %)	1 (2 %)	0 (0 %)
Lungs; Lymphocytic infiltrates, perivascular	4 (8 %)	3 (6 %)	2 (4 %)	0 (0 %)	4 (8 %)	4 (8 %)	9 (18 %)	4 (8 %)
Lungs; Lymphocytic infiltrates, parabronchial	1 (2 %)	2 (4 %)	2 (4 %)	0 (0 %)	0 (0 %)	4 (8 %)	4 (8 %)	1 (2 %)
Lungs; Parabronchical lymphoid aggregates	29 (60 %)	31 (62 %)	31 (62 %)	26 (52 %)	24 (49 %)	36 (72 %)	36 (72 %)	32 (64 %)
Lungs; Interstitial pneumonitis	4 (8 %)	5 (10 %)	8 (16 %)	3 (6 %)	4 (8 %)	2 (4 %)	1 (2 %)	2 (4 %)
Lungs; Foamy macrophages in alveoli	5 (10 %)	1 (2 %)	2 (4 %)	0 (0 %)	7 (14 %)	1 (2 %)	8 (16 %)	6 (12 %)
Lungs; Chronic passive congestion	5 (10 %)	2 (4 %)	2 (4 %)	4 (8 %)	2 (4 %)	5 (10 %)	3 (6 %)	4 (8 %)
Lungs; Congestion	2 (4 %)	14 (28 %)	13 (26 %)	2 (4 %)	5 (10 %)	7 (14 %)	2 (4 %)	4 (8 %)
Liver; Central lobular hepatocyte hypertrophy	9 (18 %)	5 (10 %)	3 (6 %)	17 (34 %)	3 (6 %)	5 (10 %)	1 (2 %)	1 (2 %)
Liver; Hepatocyte binucleation	45 (92 %)	47 (94 %)	48 (96 %)	44 (88 %)	48 (98 %)	47 (94 %)	47 (96 %)	43 (88 %)
Liver; Karyomegaly and cytomegaly	48 (98 %)	49 (998 %)	50 (100 %)	48 (96 %)	49 (100 %)	46 (92 %)	48 (98 %)	46 (94 %)
Liver; Lymphocytic infiltrates, intralobular	15 (31 %)	16 (32 %)	21 (42 %)	21 (42 %)	29 (59 %)	21 (42 %)	22 (45 %)	25 (51 %)

Table 5.5-134: Chronic study of glyphosate administered in feed to Albino rats ([redacted] 1983): Summary of selected non-neoplastic findings

ppm	Males				Females			
	0	1000	5000	30000	0	2000	6000	20000
Liver; Microabcesses	10 (20 %)	16 (32 %)	15 (30 %)	18 (36 %)	23 (47 %)	11 (22 %)	20 (41 %)	16 (33 %)
Liver; Intranuclear inclusions	8 (16 %)	18 (36 %)	16 (32 %)	17 (34 %)	1 (2 %)	4 (8 %)	1 (2 %)	2 (4 %)
Liver; Coagulation necrosis	3 (6 %)	3 (6 %)	5 (10 %)	5 (10 %)	0 (0 %)	1 (2 %)	6 (12 %)	3 (6 %)
Liver; Microgranuloma	15 (31 %)	2 (4 %)	8 (16 %)	4 (8 %)	16 (33 %)	9 (18 %)	3 (6 %)	7 (14 %)
Liver; Lipoid degeneration of hepatocytes	20 (41 %)	12 (26 %)	22 (44 %)	25 (50 %)	31 (63 %)	26 (52 %)	23 (47 %)	16 (33 %)
Liver; Vacuolation, Kupffer cells	32 (65 %)	36 (72 %)	41 (82 %)	33 (66 %)	38 (78 %)	35 (70 %)	46 (94 %)	38 (78 %)
Liver; Hepatocellular atrophy	8 (16 %)	0 (0 %)	6 (12 %)	9 (18 %)	9 (18 %)	11 (22 %)	3 (6 %)	3 (6 %)
Liver; Necrosis, scattered hepatocytes	6 (12 %)	1 (2 %)	5 (10 %)	0 (0 %)	2 (4 %)	7 (14 %)	1 (2 %)	1 (2 %)
Mesenteric LN (LN0); Lymphoid hyperplasia	7 (15 %)	3 (6 %)	9 (20 %)	9 (20 %)	13 (27 %)	0 (0 %)	0 (19 %)	7 (15 %)
Mesenteric LN (LN0); Plasma cell hyperplasia	4 (8 %)	7 (14 %)	2 (4 %)	2 (4 %)	2 (4 %)	0 (0 %)	3 (6 %)	2 (4 %)
Salivary glands; Periductal lymphocytic infiltrates	17 (35 %)	20 (40 %)	16 (33 %)	13 (26 %)	14 (28 %)	10 (20 %)	20 (40 %)	13 (28 %)
Spleen; Increased extramedullary haematopoiesis	7 (15 %)	18 (37 %)	9 (18 %)	11 (22 %)	16 (32 %)	23 (48 %)	22 (45 %)	15 (31 %)
Spleen; Lymphoid depletion	2 (4 %)	7 (14 %)	6 (12 %)	5 (10 %)	3 (6 %)	7 (15 %)	0 (0 %)	1 (2 %)
Colon; Nematodiasis	7 (15 %)	13 (28 %)	7 (15 %)	8 (17 %)	5 (10 %)	7 (16 %)	3 (6 %)	3 (6 %)
Kidneys; Perivascular lymphocytic infiltrates	14 (29 %)	2 (4 %)	0 (0 %)	9 (18 %)	10 (20 %)	1 (2 %)	0 (0 %)	4 (8 %)
Kidneys; Dilated cortical tubules (B)	17 (35 %)	17 (35 %)	27 (54 %)	21 (42 %)	14 (28 %)	17 (34 %)	18 (36 %)	23 (46 %)
Kidneys; Proximal tubule epithelial basophilia and hypertrophy	15 (31 %)	10 (20 %)	15 (30 %)	7 (14 %)	0 (0 %)	2 (4 %)	4 (8 %)	9 (18 %)
Kidneys; Interstitial mononuclear infiltrates (B)	25 (51 %)	36 (73 %)	34 (68 %)	31 (62 %)	21 (42 %)	32 (64 %)	34 (68 %)	31 (62 %)
Kidneys; Mineralised concretions, cortex	2 (4 %)	7 (14 %)	11 (22 %)	4 (8 %)	1 (2 %)	0 (0 %)	1 (2 %)	2 (4 %)
Kidneys; Cortical cyst(s) (B)	11 (22 %)	4 (8 %)	12 (24 %)	4 (8 %)	7 (14 %)	2 (4 %)	1 (2 %)	7 (14 %)
Kidneys; Chronic interstitial nephritis (B)	5 (10 %)	1 (2 %)	7 (14 %)	11 (22 %)	3 (6 %)	8 (16 %)	2 (4 %)	4 (8 %)
Kidneys; Thickening of glomerular and/or tubular basement membranes	8 (16 %)	9 (18 %)	17 (34 %)	14 (28 %)	5 (10 %)	7 (14 %)	5 (10 %)	8 (16 %)
Kidneys; Mesangial thickening	7 (14 %)	2 (4 %)	7 (14 %)	6 (12 %)	5 (10 %)	9 (18 %)	14 (28 %)	10 (20 %)
Testis; Germinal epithelial degeneration	16 (33 %)	15 (31 %)	22 (44 %)	12 (24 %)	-	-	-	-
Testis; Oligospermia	13 (27 %)	11 (23 %)	16 (32 %)	11 (22 %)	-	-	-	-
Epididymides; Cellular debris in ducts (B)	13 (27 %)	11 (23 %)	15 (30 %)	10 (20 %)	-	-	-	-

Table 5.5-134: Chronic study of glyphosate administered in feed to Albino rats ([redacted] 1983): Summary of selected non-neoplastic findings

ppm	Males				Females			
	0	**1000**	**5000**	**30000**	**0**	**2000**	**6000**	**20000**
Prostate; Interstitial prostatitis	5 (10 %)	11 (23 %)	5 (10 %)	5 (10 %)	-	-	-	-
Urinary bladder; Epithelial hyperplasia	**3 (6 %)**	**3 (7 %)**	**10 (21 %)**	**8 (17 %)**	0 (0 %)	0 (0 %)	2 (4 %)	0 (0 %)
Urinary bladder; Cystitis	4 (8 %)	5 (11 %)	3 (6 %)	2 (4 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)
Ovaries; Follicular cyst(s) (B)	-	-	-	-	27 (57 %)	27 (57 %)	29 (58 %)	32 (68 %)
Ovaries; Follicular cyst(s) (U)	-	-	-	-	6 (13 %)	10 (21 %)	9 (18 %)	2 (4 %)
Ovaries; Atrophy (B)	-	-	-	-	8 (17 %)	6 (13 %)	16 (32 %)	12 (26 %)
Ovaries; Corpora lutea	-	-	-	-	24 (51 %)	23 (49 %)	26 (52 %)	21 (45 %)
Ovaries; Follicular development	-	-	-	-	16 (34 %)	14 (30 %)	14 (28 %)	22 (47 %)
Ovaries; Blood filled cysts (B)	-	-	-	-	2 (4 %)	7 (15 %)	6 (12 %)	5 (11 %)
Uterus; Cystic endometrial hyperplasia	-	-	-	-	45 (92 %)	37 (77 %)	45 (92 %)	42 (84 %)
Uterus; Fibrovascular polyp	-	-	-	-	5 (10 %)	5 (10 %)	6 (12 %)	7 (14 %)
Adrenal glands; Atrophy, zona fasciculate	13 (27 %)	9 (18 %)	9 (18 %)	19 (40 %)	1 (2 %)	0 (0 %)	0 (0 %)	0 (0 %)
Adrenal glands; Brown degeneration, cortex (B)	12 (25 %)	15 (31 %)	13 (26 %)	10 (21 %)	17 (34 %)	25 (53 %)	29 (59 %)	13 (27 %)
Skin; Ulceration	10 (20 %)	12 (25 %)	3 (6 %)	9 (19 %)	4 (9 %)	4 (9 %)	8 (16 %)	5 (10 %)
Eyes; Lens, Swelling of subcapsular fibers (U)	10 (24 %)	6 (15 %)	8 (18 %)	9 (20 %)	9 (22 %)	2 (5 %)	12 (29 %)	7 (17 %)
Bone; Ossification of cartilage	29 (67 %)	28 (62 %)	28 (62 %)	34 (71 %)	24 (51 %)	25 (50 %)	23 (47 %)	22 (54 %)
Seminal vesicles; Inspissated secretion	7 (50 %)	0 (0 %)	7 (54 %)	6 (46 %)	-	-	-	-
Mammary; Secretory activity	-	-	-	-	19 (50 %)	25 (69 %)	30 (75 %)	21 (55 %)
Mammary; Ductal hyperplasia	-	-	-	-	7 (18 %)	14 (39 %)	17 (43 %)	11 (29 %)
Mammary; Dilated Ducts	-	-	-	-	12 (32 %)	18 (50 %)	19 (48 %)	8 (21 %)

Neoplastic findings

Neoplastic findings were of the type commonly encountered in mice. Bronchiolar-alveolar tumours of the lungs, hepatocellular neoplasms and tumours of the lymphoreticular system accounted for the majority of those encountered. There were no suspected test substance-associated trends in the incidence of these tumours or of any of the other spontaneously occurring neoplasms.

Lymphoreticular tumours tended to be more frequent in treated animals, particularly the females. The numbers were relatively small and differences from the control population failed to provide any supportive evidence that the neoplasms had a test substance relationship.

The only other neoplasms that occurred with any frequency in treated mice only, were renal tubule adenomas, which occurred in males. Three were present at the high-dose and one at the mid-dose level. The distribution of these benign tumours was considered spurious and unrelated to treatment due to the absence of other renal lesions suggestive of or supportive of an effect on the urinary system.
All other neoplasms occurred sporadically and were considered to have had no treatment relationship.
Non-neoplastic tissue alterations were of the type and severity expected in long term mouse studies. Actually fewer tissue lesions were encountered than anticipated.

The only liver finding which may have been test substance associated was central lobular hepatocyte hypertrophy which occurred in 34 % of high-dose males compared to 18 %, 10 % and 6 % in control through mid-dose males, respectively. This change may have represented a hepatocellular adaptation to metabolism of the test substance.

Table 5.5-135: Chronic study of glyphosate administered in feed to Albino rats ([redacted] 1983): Summary of selected neoplastic findings

ppm	Males				Females			
	0	1000	5000	30000	0	2000	6000	20000
Heart; Lymphoblastic lymphosarcoma with leukemic manifestations	0 (0 %)	1 (2 %)	2 (4 %)	1 (2 %)	0 (0 %)	0 (0 %)	2 (4 %)	0 (0 %)
Lungs; Bronchiolar-alveolar adenoma	5 (10 %)	9 (18 %)	9 (18 %)	9 (18 %)	10 (20 %)	9 (18 %)	10 (20 %)	1 (2 %)
Lungs; Bronchiolar-alveolar adenocarcinoma	4 (8 %)	3 (6 %)	2 (4 %)	1 (2 %)	1 (2 %)	3 (6 %)	4 (8 %)	4 (8 %)
Lungs; Lymphoblastic lymphosarcoma with leukemic manifestations	1 (2 %)	4 (8 %)	3 (6 %)	1 (2 %)	1 (2 %)	2 (4 %)	5 (10 %)	1 (2 %)
Liver; Hepatocellular adenocarcinoma	5 (10 %)	6 (12 %)	6 (12 %)	4 (8 %)	1 (2 %)	2 (4 %)	1 (2 %)	0 (0 %)
Liver; Lymphoblastic lymphosarcoma with leukemic manifestations	1 (2 %)	4 (8 %)	2 (4 %)	2 (4 %)	1 (2 %)	4 (8 %)	4 (8 %)	1 (2 %)
Mediastinal LN (LN1); Lymphoblastic lymphosarcoma with leukemic manifestations (S)	1 (2 %)	2 (4 %)	1 (2 %)	2 (4 %)	1 (2 %)	1 (2 %)	3 (6 %)	0 (0 %)
Mediastinal LN (LN1); Lymphoblastic lymphosarcoma with leukemic manifestations (M)	0 (0 %)	0 (0 %)	2 (5 %)	0 (0 %)	0 (0 %)	3 (6 %)	1 (2 %)	1 (2 %)
Mediastinal LN (LN0); Lymphoblastic lymphosarcoma with leukemic manifestations (S)	0 (0 %)	0 (0 %)	1 (2 %)	2 (4 %)	0 (0 %)	1 (2 %)	3 (6 %)	0 (0 %)
Mediastinal LN (LN0); Lymphoblastic lymphosarcoma with leukemic manifestations (M)	1 (2 %)	2 (4 %)	1 (2 %)	0 (0 %)	1 (2 %)	1 (2 %)	2 (5 %)	0 (0 %)
Kidneys; renal tubule adenoma	0 (0 %)	0 (0 %)	1 (2 %)	3 (6 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)
Kidneys; Lymphoblastic lymphosarcoma with leukemic manifestations	1 (2 %)	3 (6 %)	2 (4 %)	2 (4 %)	1 (2 %)	2 (4 %)	3 (6 %)	1 (2 %)
Adrenal glands; Cortical adenoma	1 (2 %)	2 (4 %)	0 (0 %)	1 (2 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)
Bone marrow; Lymphoblastic lymphosarcoma with leukemic manifestations	1 (2 %)	2 (4 %)	1 (2 %)	1 (2 %)	0 (0 %)	1 (2 %)	3 (6 %)	1 (2 %)
Uterus; Leiomysarcoma	-	-	-	-	2 (4 %)	3 (6 %)	2 (4 %)	3 (6 %)

Table 5.5-135: Chronic study of glyphosate administered in feed to Albino rats ([redacted] 1983): Summary of selected neoplastic findings

ppm	Males				Females			
	0	1000	5000	30000	0	2000	6000	20000
Uterus; Lymphoblastic lymphosarcoma with leukemic manifestations	-	-	-	-	0 (0 %)	3 (6 %)	1 (2 %)	0 (0 %)

3. Assessment and conclusion

Assessment and conclusion by applicant: In conclusion, glyphosate technical was not carcinogenic in male and female CD-1 mice following continuous dietary exposure of up to 30000 ppm (equivalent to 4841 mg/kg bw/day for males and 5874 mg/kg bw/day for females) for 104 weeks. Based on non-neoplastic histological changes affecting urinary bladder epithelium in male mice at 5000 ppm glyphosate in diet (814 mg/kg bw/day) and higher, the chronic mouse NOAEL is considered the low dose of 1000 ppm (157 mg/kg bw/day).

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.5/024
Report author	[redacted]
Report year	1982 (original report) 1992 (revised translated version)
Report title	18-Month Carcinogenicity Study of Glyphosate in Mice
Report No	8010
Document No	Not reported
Guidelines followed in study	No guideline followed; similar to OECD 451 (1981)
Deviations from current test guideline (OECD 451, 2018)	No data on test item purity provided; animal weight at dosing, acclimatisation period and diet were reported; no in-life dates were reported; only two dose levels were tested; body weight were measured only monthly; the number of animals surviving up to scheduled termination and subjected to pathological examination was too small for meaningful evaluation; the mice which had died inter-currently were not examined and the cause of death is unknown; histopathology was performed without determining cervix, coagulating glands, harderian gland, lacrimal gland, pituitary, thymus and vagina. No historical control data.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No (pre-GLP)
Acceptability/Reliability:	Invalid Due to several limitations the study is not considered reliable for hazard and risk assessment.
Category study in AIR 5	Category 3b

dossier (L docs)	

2. Full summary

Glyphosate (Technical) was administered to groups of 50 male and female CFLP mice (bred in a facility in [redacted]; 26 - 30 days old at study initiation) per dose at dietary levels of 0, 100 and 300 ppm (equivalent to 0, 12.6 and 37.7 mg/kg bw/day for males and 0, 16.3 and 44.5 mg/kg bw/day for females). The administration period was 18 months.

Animals were kept under continuous observation for behaviour, general condition and lethality. External examinations were performed periodically. Body weight gains were measured monthly and food consumption was measured weekly. All surviving animals were necropsied after the treatment period and examined for gross pathological and histopathological findings.

No toxic symptoms were noted during the study regarding body weight changes and food consumption. Mortality was high in all groups (11-23 of 50 mice). In-life, necropsy and histopathology data excluded the dose-related toxicity of the test item at the applied dose levels. The number of tumours detected partly by gross pathology and identified by histopathology showed a similar distribution in treated and control groups.

This was the case with the number of tumour bearing animals, too. The various kinds of tumours showed a certain difference in their occurrence in treated and control groups without convincing and statistically significant signed of dose dependence. Consequently, the available study data were against the carcinogenicity of glyphosate in mice in the given experimental conditions.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate
Description:	No data given in the report
Lot/Batch #:	14/980-03090380
Purity:	No data given in the report
Stability of test compound:	No data given in the report

2. Vehicle and/or positive control:

Diet / none

3. Test animals:

Species:	Mouse
Strain:	CFLP
Source:	[redacted]
Age:	28 ± 2 days
Sex:	Males and females
Weight at dosing:	No data given in the report
Acclimation period:	No data given in the report
Diet/Food:	No data given in the report
Water:	Tap water, *ad libitum*
Housing:	Groups of 10 per sex in OMKER III shoebox type cages

Environmental conditions:	Temperature:	21 ± 3 °C
	Humidity:	50 ± 5 %
	Air changes:	10 times/hour

B: Study design and methods

In life dates: Not reported, but issued in 1982

Animal assignment and treatment:
In a carcinogenicity study groups of 50 CFLP mice per sex received daily dietary doses of 0, 100 or 300 ppm (equivalent to 0, 12.6 or 37.7 mg/kg bw/day for males and 0, 16.3 or 44.5 mg/kg bw/day for females) glyphosate for 18 month.

Clinical observations
Animals were kept under continuous observation for behaviour, general condition and lethality. External examinations were performed periodically.

Body weight
Individual body weights were recorded on monthly.

Food consumption
Food consumption was recorded once weekly.

Sacrifice and pathology
Necropsy was performed on all animals that died during the observation period and all animals at scheduled termination.

Tissue samples for histopathology were taken from the following organs of all animals: adrenals, aorta, bone with bone marrow, brain, duodenum, epididymides, eyes with optic nerve, gall bladder, heart, ileum, jejunum, large intestine, kidneys, lungs, lymph nodes (mesenteric and submandibular), muscle (femoral), oesophagus, ovaries, pancreas, prostate salivary glands, sciatic nerve, seminal vesicles, skin with mammary tissue, spleen, spinal cord, stomach, testes, trachea, thyroid, tongue, tumours, urinary bladder and uterus.

Statistics
For analysing the incidence of tumours and bearing animals χ^2 test was used.

Results

A. ANALYSIS/PREPARATION OF DOSE FORMULATIONS
One week before study initiation, than monthly, three different pre-mixes were prepared from glyphosate substance and soya. Premixes were submitted monthly to [redacted] where they were used for diet preparation. The prepared food samples were given to the animals for one month.

B. MORTALITY
The numbers of pre-terminal deaths in the main group are displayed in Table 5.5-136:

Table 5.5-136: 18-Month Carcinogenicity Study of Glyphosate in Mice ([redacted], 1982/1992): Mortality

Sex	Dose group [ppm]					
	0		100		300	
	Males	Females	Males	Females	Males	Females
Month 1 – 3	0	1	1	0	0	0

Month 4 – 6	0	2	1	7	0	2
Month 7 – 9	2	7	7	7	3	2
Month 10 – 12	3	7	15	56	5	7
Month 13 – 15	21	7	4	6	4	13
Month 16 – 18	13	12	8	9	15	12
Combined	39/50	36/50	36/50	34/50	37/50	36/50

Both the time dependent incidence and the cumulative values of deaths excluded the negative influence of test substance and dose on the life expectance of mice. The males of the 100 ppm dose group died somewhat earlier, while the survival rate was the highest in the 300 ppm male group with 25/50 overall mortality. Conclusively, test substance exerted no impairment on the health condition of mice as expressed by the mortality values.

C. CLINICAL OBSERVATIONS

Mice had a symptom-free general health condition during the study.

D. BODY WEIGHT

The kinetics of body weight change were of similar pattern in the treated and control groups. Maximal weights were measured mostly between month 6-12 with a slight declination by the end of the study period. The rate of decrease was slightly stronger in 100 and 300 ppm males, but was milder than in the corresponding control, in 100 ppm females.

Table 5.5-137: 18-Month Carcinogenicity Study of Glyphosate in Mice ([redacted], 1982/1992): Group mean body weights

Time point	Sex	Males			Females		
	Dose [ppm]	0	100	300	0	100	300
	No. of rats	50	50	50	50	50	50
Month 1		29.5	↑29.7	29.5	24.4	↑24.6	↑25.1
Month 2		43.2	↓41.4	↓41.0	33.1	↑33.6	↓28.0
Month 3		47.4	↓45.9	↓46.5	35.5	↑36.7	↑37.1
Month 4		51.8	↓49.8	↓51.0	35.9	↑40.0	↑41.2
Month 5		53.6	↓51.4	↓51.3	38.0	↑39.8	↑41.8
Month 6		55.3	↑55.4	↑53.8	40.4	↓40.3	↑44.3
Month 7		59.7	↓57.4	↓57.3	42.3	42.3	↑46.8
Month 8		61.4	↓59.6	↓58.8	42.7	↓41.1	↑47.3
Month 9		64.1	↓62.4	↓60.3	46.2	↓45.4	↑51.5
Month 10		62.8	↑67.8	↓60.2	46.4	↑47.6	↑52.9
Month 11		64.7	↓64.5	↓57.9	48.2	↓47.7	↑55.7
Month 12		62.8	↓54.8	↓57.7	45.0	↑46.9	↑49.3
Month 13		54.1	↓53.1	↑55.2	42.9	↑46.0	↑45.2
Month 14		49.4	↑52.9	↑55.5	42.3	↑47.6	↑44.1
Month 15		47.9	↑51.1	↑51.6	41.7	↑46.8	↑44.7
Month 16		47.5	↑50.5	↑51.2	43.4	↑46.4	↑43.5
Month 17		48.0	↓46.2	↑48.6	45.0	↑45.3	↓44.0

E. FOOD CONSUMPTION AND COMPOUND INTAKE

After the initially higher consumption values a steady state level was formed in all study groups with only minor and temporary deviations. The group mean achieved doses are summarised below.

Table 5.5-138: 18-Month Carcinogenicity Study of Glyphosate in Mice ([redacted], 1982/1992): Group mean achieved dose levels

Dose group	Dietary concentration [ppm]	Mean achieved dose level [mg/kg bw/day]	
		Males	Females
low	100	12.6	16.3
high	300	37.7	44.5

Table 5.5-139: 18-Month Carcinogenicity Study of Glyphosate in Mice ([redacted], 1982/1992): Group mean food consumption

Time point	Sex	Males			Females		
	Dose [ppm]	0	100	300	0	100	300
	No. of rats	50	50	50	50	50	50
Month 0		10.0	↓8.8	↓8.2	7.5	↑8.2	↑8.7
Month 1		11.1	↓10.8	↓10.5	10.9	↑11.3	↓10.4
Month 2		6.45	↓6.1	↓6.2	6.1	↑6.6	↑6.5
Month 3		5.9	↓5.7	5.9	5.6	↑6.1	↑5.7
Month 4		5.6	↓5.5	↑5.9	5.7	↓5.3	↓5.3
Month 5		6.3	↓5.9	↓5.9	5.9	↓5.3	↓5.7
Month 6		6.2	↓5.8	↓5.9	5.7	↓5.3	↓5.6
Month 7		6.6	↓6.1	↓6.4	6.2	↓5.3	6.2
Month 8		6.3	↓6.0	↓6.1	6.3	↑6.9	6.3
Month 9		-	-	-	-	-	-
Month 10		7.0	↑8.4	↓6.4	6.5	↑7.9	↓5.7
Month 11		7.0	↓6.1	↓6.0	5.9	↑6.8	↓5.7
Month 12		6.3	↑6.4	↓6.0	5.6	↑6.4	↓4.8
Month 13		5.9	↓5.4	↓5.6	5.3	↑6.0	↓4.6
Month 14		5.9	↓5.7	5.9	5.6	↑6.4	↑5.7
Month 15		5.2	↓4.9	↓5.1	5.3	↓5.1	5.3
Month 16		5.0	↓4.3	↓4.7	5.1	↑6.6	↑5.3
Month 17		5.1	↑5.8	↑5.4	6.1	↑6.4	↓6.0

F. NECROPSY

At terminal sacrifice necropsy findings referred first of all to regular or irregular enlargements in (liver, lung, lymph nodes, uterus and ovary) or on the surface of some organs, like intestines (M+F). Since no distinction was made in respect of their nature (tumor, inflammation, cyst or functional status), therefor, the seemingly dose-related incidence of alterations might refer to, but do not evidence the real treatment dependence of any of the lesions.

Table 5.5-140: 18-Month Carcinogenicity Study of Glyphosate in Mice ([redacted], 1982/1992): Group mean food consumption

Parameter	Sex	Males/Females		
	Dose [ppm]	0	100	300
	No. of rats	25	30	37
Liver: Focal enlargement		3 (12)	3 (10)	8 (21)
Spleen: Enlargement		2 (8)	2 (7)	6 (16)
Small intestines: round shaped formation		3 (12)	4 (13)	8 (21)
Lymph nodes: Enlargement		6 (24)	5 (17)	6 (16)
Lung: Focal enlargement		7 (28)	6 (20)	9 (24)

() Number in parenthesis are % of the respective group/parameter

Histopathology

Non-neoplastic changes

They were chiefly inflammatory or age related alterations. The inflammatory ones were mostly chronic with acute exacerbations, like in several lungs and some ovaries. In other organs like liver, kidney and glandular organs only focal and mild signs of inflammation were seen.

Liver degeneration of parenchymal or hydropic type was regarded as an age related lesion similarly to focal or multifocal fibrous processes in liver, lung, intestines, etc.

Thymus, and especially female reproductive organs revealed definite signs of atrophy, while the mucosa of the urinary bladder was frequent hypertrophic, in some cases reaching the level of benign papilloma.

Neoplastic changes

The incidence of tumour findings are summarised in the following table:

Table 5.5-141: 18-Month Carcinogenicity Study of Glyphosate in Mice ([redacted] 1982/1992): Neoplastic lesions

Organ	Tumour
Liver	Hepatoma, haemangioma (benign, malignant, unspecified)
Lung	Bronchial, broncho-alveolar adenoma (benign or malignant)
Lymphoid tissue	Malignant lymphoma (lymphoid or reticular type)
Urinary bladder	Papilloma (always benign)
Uterus	Adenoma, leiomyoma (benign or malignant)
Ovary	Cystoma, cystadenoma, carcinoma
Eye, thyroid, pancreas, skin, muscle, lacrimal gland adenoma, carcinoma, insuloma, dermoid cyst, haemangioma	Only occasionally encountered tumours
Brain, aorta, heart, tongue, oesophagus, stomach, small intestines, large intestines, salivary gland, adrenals, kidneys, testicle, epididymides, seminal vesicles	No tumour observed

Accordingly, and also owing to the relatively low and different number of animals per dose group, some tumours revealed an uneven distribution, while most were seen in similar incidence in dosed and control groups. Percentage frequency was therefore calculated for the most significant tumours, the result of which are as follows:

Table 5.5-142: 18-Month Carcinogenicity Study of Glyphosate in Mice ([redacted], 1982/1992): Most significant tumours

Parameter	Sex	Males			Females			Combined sex		
	Dose [ppm]	0	100	300	0	100	300	0	100	300
Liver hepatoma		1 (9)	0 (0)	5 (22)	0 (0)	0 (0)	1 (7)	4.5	0	16.2
Pulmonary tumour (adenoma, carcinoma)		1 (9)	1 (7)	4 (17)	4 (29)	4 (25)	2 (14)	22.5	16	16
Malignant lymphoma		1 (9)	1 (7)	1 (4)	1 (7)	2 (12)	1 (7)	8	10	5.4

(): Number in parenthesis are % of the respective group/parameter

Several uteri revealed marked structural transformation by the end of study period. Apart from a few cases of physiological atrophy, many of them showed endometrial cystic-glandular hyperplasia. Very probably this was in close relation with the development of endometrial adenoma and perhaps of leiomyoma, too. In the ovarian tumours the proliferation of both granulosa and transformed epithelial elements was detectable; they were always combined with extensive inflammatory reaction.

Table 5.5-143: 18-Month Carcinogenicity Study of Glyphosate in Mice ([redacted], 1982/1992): Female genital tumours

Parameter	Sex	Females		
	Dose [ppm]	0	100	300
Uterus		2/14 (14)	1/16 (6)	2/14 (14)
Ovary		1/14 (7)	2/16 (12)	1/14 (7)

() Number in parenthesis are % of the respective group/parameter

Owing to the relatively high number and even distribution of most tumours evaluation and concluding seem possible, in spite of the relatively low and different number of animals available per group and sex.

Non-tumorous changes were of partly related to inflammatory-parasitic processes. These were the cause of some focal or extended enlargements described at necropsy in some of the livers, lungs, etc. They were related occasionally to the physiological state of organ, like enlarged Peyer's plaques appearing as focal enlargements in or on the small intestines.

The most frequent tumours were those of the liver, lung and the lymphatic tissue. The combined incidence the three tumours was similar in the three study groups, still, certain tumours showed a higher incidence in one of the groups, like liver tumours in 300 ppm males and lung tumours in 0 ppm females.

It has still to be stated that neither the individual tumours, nor their overall incidence, nor the number of animals with tumour(s) revealed a statistically significant, dose-dependent distribution.

This is valid even if we add to the facts, that certain tumours found only in single animals, occurred in dosed and not in control. This can be regarded as incidental finding, they were taken into account at the overall tumour distribution.

3. Assessment and conclusion

Assessment and conclusion by applicant:
Glyphosate did not show any significant increase in tumour formation or toxicological effects when fed

to CFLP mice up to the highest tested dose of 300 ppm (dietary admix) for 18 months. Consequently the available study data do not reveal the carcinogenicity of glyphosate in mice in the given experimental conditions. The NOAEL in this study is 300 ppm (equivalent to 37.7 mg/kg bw/day for males and 44.5 mg/kg bw/day for females).

Due to several limitations the study is not considered reliable for hazard and risk assessment.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.5/025
Report author	[redacted]
Report year	2010
Report title	Spontaneous neoplastic lesions in the CRl:CD1 (ICR) mouse in control groups from 18 months to 2 year studies
Report No	ASB2015-2529
Document No	Not reported
Guidelines followed in study	Not applicable
Deviations from current test guideline (OECD 451, 2018)	Not applicable
Previous evaluation	Accepted in RAR (2015)
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Summary

Historical control data CRl:CD1 (ICR) mouse already submitted in 2015 are included in this document.

3. Assessment and conclusion

Assessment and conclusion by applicant:
Historical control data CRl:CD1 (ICR) mouse already submitted in 2015 are included in this document.

Assessment and conclusion by RMS:

Epidemiology

Notwithstanding the conclusion of the EU 2001 evaluation, the opinion of the glyphosate renewal task force and of several other recent reviews of Glyphosate (EFSA 2015, ECHA 2016, JMPR/WHO 2016, FSC Japan 2016, PMRA Canada, 2017, U.S. EPA 2019) where it was concluded that glyphosate is not a carcinogen,

in 2015, a review of the scientific evidence by the International Agency for Research on Cancer (IARC) concluded that the evidence for the carcinogenicity of glyphosate was limited in humans but sufficient in experimental animals (rats and mice). IARC further concluded that glyphosate was probably carcinogenic in humans.

The IARC conclusion has triggered a number of experts to investigate why there should be different conclusions from different investigating bodies. Crump *et al* (2020) reports one such investigation.

In his paper Crump points out that the animal carcinogenicity data on glyphosate are unusually extensive (≥15 long term rodent oral bioassays of glyphosate identified by U.S. EPA (2016), EFSA (2016) and IARC (2015). Each bioassay was conducted in both sexes, with each sex potentially having 40-60 unique tumour types, resulting in over 1000 potential statistical tests, which could easily result in many significant ($p \leq 0.05$) tumour increases occurring by chance alone – roughly 5 %. Crump K. *et al* (2020) have assessed the probability of false positives using a modification of the permutation approach of Farrar and Crump (1988 and 1990). The statistical method requires access to individual animal data on histopathological information and tumours, the length of time each animal was on test, and their doses. These criteria were met in 10 bioassays (4 mouse and 6 rat), which included all the bioassays cited by IARC as showing evidence of carcinogenicity.

The present analysis provides new information on the potential carcinogenicity of glyphosate by being the first to provide results from statistical tests with correct false positive rates. These tests found no strong or convincing evidence that glyphosate is an animal carcinogen.

Portier (2020) also provided an additional revised statistical evaluation and trend test analysis. The author asserts that his updated analyses in the publication support the IARC's conclusion of evidence of cancer in experimental animals. Crump (2020), however, determined that statistically a significant number of false positives across the very large glyphosate database supports all previous regulatory reviews around the world, that glyphosate does not demonstrate any carcinogenic potential in rats or mice.

During the last evaluation in 2015, a number of epidemiology studies were evaluated and summaries provided within the dossier. The summaries of these studies (beyond the scope of a 10 year literature search) were additionally included in the dossier in order to allow a comprehensive evaluation (Appendix 1, MCA 5). In order to trace back the publications in the Appendix, the ASB reference numbers are still included in the text below and additionally in the Appendix 1.

A number of epidemiology studies over the last decade have focused on pesticide exposure and associated health outcomes. Publications vary in the scope of their conclusions regarding either pesticides in general, certain classes of pesticides and in some cases individual insecticides, herbicides or fungicides. While some of these publications specifically mention glyphosate, few draw tenable associations with any specific cancer outcome. Publications suggesting glyphosate is associated with any cancer outcome are discussed in Appendix 1.

An essential consideration in both, risk assessment and interpreting the relevance of toxicology data, is exposure assessment. An inherent low level of confidence exists for epidemiological studies where tenuous links to exposure exist. Suggested associations between health outcomes and any possible causative agent are merely speculative if exposure cannot be confirmed and quantified.

The largest epidemiological study of pesticide exposure and health outcomes in the United States was the Agricultural Health Study (AHS) that also addressed and included glyphosate. Dozens of publications have resulted from data generated in this study of approximately 57000 enrolled farmers (applicators). Blair *et al*. (2009, ASB2012-11566) provided an overview of cancer endpoints associated with different agricultural chemicals reported in earlier AHS publications. Glyphosate was not reported to be associated with leukaemia, melanoma, or cancers of the prostate, lung, breast, colon or rectum. De Roos *et al*. (2005, ASB2012-11605) reported AHS data evaluating glyphosate use and multiple cancer endpoints. No

association was noted for glyphosate with all cancers, including cancer of the lung, oral cavity, colon, rectum, pancreas, kidney, bladder, prostate, melanoma, all lymphohematopoietic cancers, non-Hodgkin's lymphoma (NHL) and leukemia. In an earlier publication based on another data set, however, De Roos *et al*. (2003, ASB2012-11606) had reported an association between NHL and glyphosate use. Likewise, McDuffie *et al*. (2001, ASB2011-364) mentioned a non-significant positive association between self-reported glyphosate exposure and NHL in a Canadian study. Blair *et al*. (2009, ASB2012-11566), in contrast, did not report an association between glyphosate use and NHL in the AHS data but a "possible association" between glyphosate use and multiple myeloma was mentioned making reference to a "suggested association" between glyphosate use and multiple myeloma suggested by De Roos *et al*. (2005, ASB2012-11605). However, in this paper, no significant increase in relative risk for multiple myeloma was demonstrated. Both papers by De Roos *et al*. will be discussed in more detail in Appendix 1. Interestingly, a subsequent AHS review paper for the President's Cancer Panel (Freeman, 2009, ASB2012-11623) specifically referenced De Roos *et al*. (2005 ASB2012-11605) to provide no evidence of cancers of any type to be associated with glyphosate. With regards to multiple myeloma Presutti *et al*. (2016) analysed a subset of three NAPP studies (Iowa, Nebraska and Canada) where multiple myeloma (MM) cases were recruited. Self-reported information on pesticide use, farming activities and demographic characteristics was collected and the odds ratios (OR) were calculated for "ever/never" exposure, years of exposure and cumulated lifetime days of exposure to glyphosate with and without exclusion of proxy respondents. The result is that no statistically significant increases in risk of multiple myeloma (MM) associated with self-reported exposure to glyphosate were observed. This publication is considered relevant for glyphosate risk assessment but reliable with restrictions because it concerns pooled case control studies which are subject to recall bias and in which confounding factors could not be ruled out.

Sorahan *et al*. (2015) calculated the relative risk estimates for exposed and non-exposed applicators using Poisson regression and subjects with missing data were not excluded from the main analyses. When using the full dataset adjusted for age and gender the analysis produced a RR close to unity for ever-use of glyphosate. Additional adjustment for lifestyle factors and use of ten other pesticides had little effect. This study found no statistically significant trends of multiple myeloma risk with reported cumulative days of glyphosate use and unexceptional point estimates of risk for ever-use of glyphosate. This was irrespective of whether the analyses had adjustment for a few basic variables (age and gender) or adjustment for many other lifestyle factors or pesticide exposures, as long as data on all available pesticide applicators were used. The suspiciously elevated RRs reported previously in De Roos *et al*. (2005) arose from the use of restricted data sets that were unrepresentative of the study cohort.

Andreotti *et al*. 2018 updated the 2005 AHS publication by DeRoos *et al*. (2005), extending cancer incidence follow-up through 2012 in North Carolina and 2013 in Iowa and incorporating additional exposure information from a follow-up questionnaire. The authors also dealt with missing information through imputation and conducted sensitivity analyses to address the potential for various types of bias in their primary analyses. This 2018 publication includes a total of 7,290 cancers, 3.6 times as many as in the earlier publication. The median lifetime days of glyphosate use for cohort members who reported glyphosate use (83 % of the cohort) was 48 days (interquartile range (IQR) 20 to 166 days). The authors found no evidence of an association between glyphosate use and risk of any solid tumour, NHL (RR 0.87 (95 % CI 0.64, 1.20 in the highest intensity weighted exposure quartile, p_{trend} 0.95), or multiple myeloma (RR 0.87, 95 % CI 0.45, 1.69 highest quartile,, p_{trend} 0.84). They found a stastically non-significant elevated RR for acute myelogenous leukaemia (RR 2.44, 95 % CI 0.94, 6.32, p_{trend} = 011). The lack of stastically significant findings for cancer types were consistent across different exposure metrics, in various sensitivity analyses, and for lagged exposure analyses meant to address cancer induction-latency.

The purpose of the analysis by Crump (2019) was to evaluate the evidence for recall bias in the overall pattern of results in five case control studies and two cohort studies that comprise the main part of the glyphosate-NHL literature. In evaluating the case control studies, Crump reasoned that the percentage of odds ratios > 1 for non-glyphosate exposures should be approximately 50 % if recall bias was not operative and those exposures did not cause NHL. Yet, it turned out that the percentages of ORs >1 for non-glyphosate exposures were 90 % for Hardell *et al*. (2002), 90 % for Erikson *et al*. (2008), 93 % for

McDuffie *et al*. (2001), 76 % for Orsi *et al*. (2009), and 53 % for DeRoos *et al*. (2003). These extreme departures from 50 % for 4 of the 5 case control studies is consistent with recall bias, perhaps augmented by a type of selection bias in the analyses by Hardell *et al*. (2002) and Eriksson *et al*. (2008). In contrast, in the most recent publication from the Agricultural Health Study (Andreotti *et al*. 2018), only 48 % of the relative risks (RR) calculated were >1 – a percentage in the range expected with a true probability of 50 %. While the evaluation of Andreotti *et al*. (2018) concerned glyphosate and other cancer sites and not other exposures and NHL, the principle is the same: under the null hypothesis the proportion of ORs or RRs > 1 should be roughly 50 % absent bias.

Pahwa *et al*. (2019) performed a pooled reanalysis of the data from 2 published non-Hodgkin's lymphoma (NHL) case control studies was conducted: McDuffie *et al*. 2001 and DeRoos *et al*. 2003. The reanalysis sought to evaluate associations for glyphosate use and NHL overall and by histological sub-type. In addition, the pooled analysis implemented more extensive control of confounding factors than in the original publications and considered the impact of excluding pesticide information provided by next-of-kin or proxy respondents. The OR for NHL overall for ever using glyphosate was 1.4 (95 % CI 1.1, 1.8). After adjustment for other pesticides, the OR was reduced to 1.1 (95 % CI 0.8, 1.5). For NHL subtypes, consistent patterns of association across exposure metrics were not seen, with the possible exception of SLL, though SLL findings were not statistically significant. In general, exclusion of proxy respondents reduced ORs to a minor extent with the exception of the analyses for other NHL subtypes. For NHL overall, ever use and analyses that considered duration of use per se or lifetime days of use did not show a relationship with glyphosate. There was a moderate association seen for glyphosate use for the metric > 2 days/year. There was some limited evidence of an association between glyphosate use and SLL across exposure metrics, but the small number of SLL cases resulted in very imprecise OR estimates as evidenced by wide 95 % CIs. The major limitation of the study is likely case recall bias in the reporting of prior pesticide use.

Lee *et al*. (2005, ASB2012-11882) reported a glyphosate association with gliomas, with the odds ratio differing between self-respondents (OR = 0.4) and proxy respondents (OR = 3.1). The authors expressed concern about higher positive associations observed for proxy respondents with glyphosate and several other pesticides. They suggested perhaps more accurate reporting of proxies for cases and underreporting by proxies for controls. Monge *et al*. (2007, ASB2012-11909) investigated associations between parental pesticide exposures and childhood leukaemia in Costa Rica. Results are not interpretable for glyphosate as exposure was estimated with "other pesticides", including paraquat, chlorothalonil and "others". No association was noted for paternal exposures, but elevated incidence of leukaemias was associated with maternal exposures to "other pesticides" during pregnancy.

Some further epidemiological studies have focused on an association between pesticide exposure and Non-Hodgkin`s Lymphoma (NHL). Hardell and Eriksson (1999, ASB2012-11838) investigated in a case-control study the incidence of NHL in relation to pesticide exposure in Sweden. 404 cases and 741 controls have been included. The authors discussed an increased risk for NHL especially for phenoxyacetic acids. Glyphosate was included in the univariate and multi-variate analyses. However, only 7 of 1145 subjects in the study gave exposure histories to this agent. The authors reported a moderately elevated odds ratio (OR) of 2.3 for Glyphosate. This OR was not statistically significant and was based on only 4 "exposed" cases and 3 "exposed" controls. The major limitations of this study were: the reliance on reported pesticide use (not documented exposure) information, the small number of subjects who reported use of specific pesticides, the possibility of recall bias, the reliance on secondary sources (next-of-kin interviews) for approximately 43 % of the pesticide use information, and the difficulty in the controlling for potential confounding factors given the small number of exposed subjects.

A further study was submitted by Hardell *et al*. (2002, ASB2012-11839). This study pools data from the above mentioned publication by Hardell and Eriksson (1999, ASB2012-11838) with data from a previously submitted publication from Nordström *et al*. (1998, TOX1999-687). The authors found increased risks in an uni-variate analysis for subjects exposed to herbicides, insecticides, fungicides and impregnating agents. Among herbicides, significant associations were found for glyphosate and MCPA. However, in multi-variate analyses, the only significantly increased risk was found with a heterogenous category of "other

herbicides" and not for glyphosate. No information is given about exposure duration, exposure concentration, as well as medical history, lifestyle factors (e.g. smoking, use of prescribed drugs etc.). In all, the above mentioned limitations of the publication of Hardell and Eriksson (1999, ASB2012-11838) are also applicable to the publication by Hardell *et al*. (2002, ASB2012-11839).

Fritschi *et al*. (2005, ASB2012-11624) submitted a case-control study with 694 cases of NHL and 694 controls in Australia. Substantial exposure to any pesticide was associated with an increase of NHL. However, no association between NHL and glyphosate can be made on basis of this study. No information was given about exposure duration, used glyphosate products, and application rates. Therefore, the documentation is considered to be insufficient for assessment. Eriksson *et al*. (2008, ASB2012-11614) reported a case-control study which included 910 cases of NHL and 1016 controls living in Sweden. The highest risk was calculated for MCPA. Glyphosate exposure was reported by 29 cases and 18 controls, and the corresponding odds ratio (OR) was 2.02. Results and reliability of the study are discussed in the Appendix 1.

Alavanja *et al*. (2013, ASB2014-9174) reviewed studies on cancer burden among pesticide applicators and others due to pesticide exposure. In this article, the epidemiological, molecular biology, and toxicological evidence emerging from recent literature assessing the link between specific pesticides and several cancers including prostate cancer, NHL, leukaemia, multiple myeloma, and breast cancer were integrated. Glyphosate was reported to be the most commonly used conventional pesticide active substance worldwide. However, the only association between the use of glyphosate and cancer burden mentioned in this review was the observation of Eriksson *et al*. (2008, ASB2012-11614, see above).

The following epidemiological studies did not reveal an association between glyphosate and specific cancer types.

· Alavanja *et al*. (2003, ASB2012-11535) reported on prostate cancer associations with specific pesticide exposures in the AHS; glyphosate did not demonstrate a significant exposure-response association with prostate cancer.

· Multigner *et al*. (2008, ASB2012-11917) also reported a lack of association between glyphosate use and prostate cancer. This data appears to have also been reported by Ndong *et al*. (2009, ASB2012-11922).

· The lack of association between glyphosate use and prostate cancer was also supported recently in an epidemiology study in farmers in British Columbia, Canada, by Band *et al*. (2011, ASB2012-11555).

· Lee *et al*. (2004, ASB2012-11883) reported a lack of association between glyphosate use and stomach and esophageal adenocarcinomas.

· Carreon *et al*. (2005, ASB2012-11585) reported epidemiological data on gliomas and farm pesticide exposure in women; glyphosate had no association with gliomas.

· Engel *et al*. (2005, ASB2012-11613) reported AHS data on breast cancer incidence among farmers' wives, with no association between breast cancer and glyphosate.

· Flower *et al*. (2004, ASB2012-11620) reported AHS data on parental use of specific pesticides and subsequent childhood cancer risk among 17,280 children, with no association between childhood cancer and glyphosate.

· Andreotti *et al*. (2009, ASB2012-11544) reported AHS data where glyphosate was not associated with pancreatic cancer.

· Landgren *et al*. (2009, ASB2012-11875) reported AHS data on monoclonal gammopathy of undetermined significance (MGUS), showing no association with glyphosate use.

· Karunanayake *et al*. (2011, ASB2012-11865) reported a lack of association between glyphosate and Hodgkin's lymphoma.

· Pahwa *et al*. (2011, ASB2012-11987) reported a lack of association between glyphosate and multiple myeloma.

Schinasi and Leon (2014, ASB2014-4819) published the results of epidemiologic research on the relationship between non-Hodgkin lymphoma (NHL) and occupational exposure to pesticides. Phenoxy herbicides, carbamate insecticides, organophosphorus insecticides and lindane were positively associated with NHL. However, no association between NHL an glyphosate was reported.

· Kachuri *et al*. (2013, ASB2014-8030) investigated an association between lifetime use of multiple

pesticides and multiple myeloma in Canadian men. Excess risks of multiple myeloma were observed among men reported using at least one carbamate pesticide, one phenoxy herbicide and ≥ organochlorines. However, no excess risk was observed for glyphosate.
· Cocco *et al*. (2014, ASB2014-7523) investigated the role of occupational exposure to agrochemicals in the aetiology of lymphoma overall, B cell lymphoma and its most prevalent subtypes. No increased CLL risk in relation to glyphosate was evidenced.
· Alavanja and Bonner (2012, ASB2014-9173) reviewed studies on occupational pesticide exposure and cancer risk. Twenty one pesticides identified subsequent to the last IARC review showed significant exposure-response associations in studies of specific cancers. No significant association was observed for glyphosate.
· El-Zaemey and Heyworth (2013, ASB2014-9473) reported a case control study on the association between pesticide spray drift from agricultural pesticide application areas and breast cancer in Western Australia. The findings support the hypothesis that woman who ever noticed spray drift or who first noticed spray drift at a younger age had increased risk of breast cancer. However, it was no possible to examine whether the observed associations are the result of a particular class of pesticides.
· Pahwa *et al*. (2011, ASB2014-9625) investigated the putative association of specific pesticides with soft-tissue sarcoma (STS). A Canadian population-based case-control study conducted in six provinces was used on this analysis. The incidence of STS was associated with insecticides aldrin and diazinon after adjustment for other independent predictors. However, no statistically significant association between STS and exposure to glyphosate or other herbicides was observed.
· Koutros *et al*. (2011, ASB2014-9594) studied associations between pesticide and prostate cancer. No statistically significant positive association between pesticides and prostate cancer were observed. There was suggestive evidence on an increased risk (OR>1.0) with an increasing number of days of use of petroleum oil/petroleum distillate used as herbicide, terbufos, fonofos, phorate and methyl bromide. However, no increased risk (OR>1.0) was observed for glyphosate.

In a comprehensive review of the AHS publications and data, Weichenthal *et al*. (2010, ASB2012-12048) noted that increased rates in the following cancers were not associated with glyphosate use: overall cancer incidence, lung cancer, pancreatic cancer, colon or rectal cancer, lymphohematopoietic cancers, leukaemia, NHL, multiple myeloma, bladder cancer, prostate cancer, melanoma, kidney cancer, childhood cancer, oral cavity cancers, stomach cancer, oesophagus cancer and thyroid cancer.

Mink *et al*. (2012, ASB2014-9617) submitted a comprehensive review of epidemiologic studies of glyphosate and cancer. To examine potential cancer risks in humans they reviewed the epidemiologic literature to evaluate whether exposure to glyphosate is associated causally with cancer risk in humans. They also reviewed relevant methodological and biomonitoring studies of glyphosate. The review found non consistent pattern of positive associations indicating a causal relationship between total cancer (in adults or in children) or any site-specific cancer and exposure to glyphosate.

Literature summaries

A literature search for the active substance glyphosate was performed in accordance to the provisions of the EFSA Guidance "Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) 1107/2009" and updated Appendix to this Guidance document. The following publications were found relevant and reliable for this section and the summaries are thus presented below and are part of the general discussion at the beginning of the section.

Table 5.5-144: Overview on literature found relevant for evaluation of glyphosate in section 5.5

Annex Point	Study	Study type	Substance(s)	Reliability & restriction comments	Result
CA 5.5/026	Crump, 2020	Statistical analysis of available bioassays Continuity corrected poly-3 test (a survival-adjusted Cochran-Armitage test)	Not applicable	Reliable without restrictions	No strong or convincing evidence that glyphosate is an animal carcinogen
CA 5.5/027	Portier, 2020	Statistical analysis of available bioassays Use of Cochran-Armitage (C-A) linear trend test	Not applicable	Reliable with restrictions	Sufficient evidence that glyphosate causes cancer in experimental animals
CA 5.5/028	Wozniak, 2020	*In vitro* assay, effect on epigenetic parameters and major cell cycle drivers in PBMCs	Glyphosate (Purity. 95 %)	Reliable without restrictions	No indication of glyphosate to impact the measured parameters was demonstrated
CA 5.5/029	Biserni, 2019	*In vitro*, lipid accumulation in adipocytes	Glyphosate (Purity: ≥ 96 %)	Reliable without restrictions	No lipid accumulation
CA 5.5/030	Crump, 2019	Methodologic evaluation of recall bias in existing studies	Not applicable	Reliable without restrictions	Recall bias not reliable
CA 5.5/031	Duforestel, 2019	*In vitro*, mammary cells (MCF10A)	Glyphosate (Purity not reported)	Reliable with restrictions Glyphosate not sufficiently characterized Only one dose tested, 10^{-11}M	Glyphosate primes mammary cells for tumorigenesis
CA 5.5/032	Hao, 2019	*In vitro*, human A549 cells	Glyphosate isopropylamine salt (Purity: ≥ 95 %)	Reliable with restrictions No positive control Only one dose tested	No activation of the AMPK/mTOR signalling pathway, no role in autophagy
CA 5.5/033	Pahwa, 2019	Reanalysis of case control studies	Not applicable	Reliable with restrictions Recall bias unresolved Missing data	Inconclusive effect of glyphosate to non-Hodgkin

Table 5.5-144: Overview on literature found relevant for evaluation of glyphosate in section 5.5

Annex Point	Study	Study type	Substance(s)	Reliability & restriction comments	Result
					lymphoma
CA 5.5/034	Wang, 2019	*In vivo*, 7-day and 72-week study, mice (C57bl/6 and Vk*MYC)	Glyphosate (Purity not reported)	Reliable with restrictions Glyphosate uncharacterized Only one dose considered in chronic study Insufficient number of animals in acute study and number unreported in chronic study	Glyphosate induces MGUS and promotes disease progression to multiple myeloma
CA 5.5/035	Andreotti, 2018	Epidemiologic evaluation of US Agricultural Health Study (AHS) Data	Glyphosate formulation (no further details)	Reliable with restrictions Dose not known, only frequency of use	No evidence of an association between glyphosate use and risk of any solid tumor, NHL, or multiple myeloma
CA 5.5/036	Presutti, 2016	Epidemiologic evaluation	Glyphosate formulation	Reliable with restrictions Pooled case control studies, thus recall bias with confounding factors	No statistically significant increases in risk of multiple myeloma
CA 5.5/037	Sorahan, 2015	Statistical re-analysis of US Agricultural Health Study (AHS) Data	Glyphosate formulation	Reliable without restrictions	No statistically significant trends of multiple myeloma risk with reported cumulative days of glyphosate use

2. Information on the study

Data point:	CA 5.5/026
Report author	Crump, K. et al.
Report year	2020
Report title	Accounting for Multiple Comparisons in Statistical Analysis of the Extensive Bioassay Data on Glyphosate.
Document No	https://doi.org/10.1093/toxsci/kfaa039
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	-/Reliable without restrictions

6. Full summary of the study according to OECD format

Abstract

Glyphosate is a widely used herbicide worldwide. In 2015, the International Agency for Research on Cancer (IARC) reviewed glyphosate cancer bioassays and human studies and declared that the evidence for carcinogenicity of glyphosate is sufficient in experimental animals. We analyzed ten glyphosate rodent bioassays, including those in which IARC found evidence of carcinogenicity, using a multi-response permutation procedure that adjusts for the large number of tumors eligible for statistical testing and provides valid false-positive probabilities. The test statistics for these permutation tests are functions of p-values from a standard test for dose-response trend applied to each specific type of tumor. We evaluated three permutation tests, using as test statistics the smallest p-value from a standard statistical test for dose-response trend and the number of such tests for which the p-value is less than or equal to 0.05 or 0.01. The false-positive probabilities obtained from two implementations of these three permutation tests are: smallest p-value: 0.26, 0.17, p-values ≤ 0.05: 0.08, 0.12, p-values ≤ 0.01: 0.06, 0.08. In addition, we found more evidence for negative dose-response trends than positive. Thus, we found no strong evidence that glyphosate is an animal carcinogen. The main cause for the discrepancy between IARC's finding and ours appears to be that IARC did not account for the large number of tumor responses analyzed and the increased likelihood that several of these would show statistical significance simply by chance. This work provides a more comprehensive analysis of the animal carcinogenicity data for this important herbicide than previously available.

Materials and methods

Analysis of bioassay data - Of the bioassays identified by U.S. EPA, EFSA, or IARC, 10 glyphosate cancer studies of sufficient quality were selected which allowed the analysis of individual animal data. The characteristics of the selected studies are summarized in Table 1.

Table 5.5-145: Characteristics of the bioassays selected for analysis.

Bioassay	Species	Strain	# Dose Groups	Sex	Animals/Dose	Maximum Dose[a] (mg/kg/d)		Maximum Weeks on test	Sites Where Histopathology was Conducted in All Dose Groups[b]
						Males	Females		
Atkinson et al 1993b[c]	Mouse	CD-1	4	50		988	1000	105	Kidney, Liver, Lung, Vascular System
Knezevich and Hogan 1983[c]	Mouse	CD-1	4	50		4841	5873	102	(all)
Wood et al 2009b	Mouse	CD-1	4	51		810	1081	81	Kidney, Liver and Lung
Sugimoto 1997	Mouse	CD-1-ICR	4	50		4348	4116	78	(all)
Atkinson et al 1993a[c]	Rat	SD[d]	5	50		1007	1018	105	Kidney, Liver, Lung and Salivary Glands: Parotid, Mandibular and Sublingual
Lankas 1981[c]	Rat	SD	4	50		31.49	34.02	111	(all)
Stout and Ruecker 1990[c]	Rat	SD	4	60		940	1183	105	(all)
Brammer 2001[c]	Rat	Wistar	4	64		1214	1498	104	(all)
Suresh 1996	Rat	Wistar	4	50		595.2	886	107	(none)
Wood et al 2009a	Rat	Wistar	4	51		1077	1382	105	Kidney, Liver, Lung and Bone Marrow

[a] All doses in each bioassay are listed in Table 2.
[b] Systemic tumors are assumed to have been searched for if at least one tissue in an animal was given a histopathological examination.
[c] These six studies were evaluated by IARC. IARC (2015) also reviewed two additional studies in which they identified shortcomings, but which they did not claim were "inadequate": Chruscielska et al (2000) and JMPR (2006). No glyphosate-related tumor responses were noted in either of these studies.
[d] SD: Sprague Dawley.

Eight of the 10 studies were assigned a Klimisch code 1 (reliable without restrictions). The Knezevich and Hogan (1983) study was assigned a Klimisch code 2 (reliable with restrictions) mainly because this study

was initiated before the implementation of the OECD test guidelines and GLP, and the study of Lankas (1981) also a pre-GLP and pre-guideline study was assigned a Klimisch code 3 (not reliable) mainly because of the low power of the study due to the low dose range selected.

Statistical tests - The statistical tests applied in the analysis were functions of p-values obtained from conventional continuity-corrected poly-3 tests (survival-adjusted Cochran-Armitage test) for trend applied to each type of tumor or combination of tumor types in each bioassay. In the present analysis, the continuity-corrected version of the poly-3 test used was copied from a key portion of the computer program used by the NTP. Direct comparisons have shown that the author's implementation gives the same results as the version used by the NTP. Throughout this paper, all implementations of the poly-3 test, are one-sided, as are the NTP implementations of the test.

Results from 3 multi-response permutation tests are presented. In the simplest test, referred to as the "min-test", the test statistic is the smallest p-value obtained from applying the poly-3 test to all tumor types in all of the 10 bioassays. In the simplest implementation of this test, animals are randomly reassigned to dose groups (permuted among dose groups) in a Monte Carlo analysis, keeping the total numbers of animals in each dose group equal to the number in the original data. The tumors in each such reassignment are analyzed using the poly-3 test in exactly the same way as in the original data. Males and females are permuted separately. The false positive rate is the proportion of random reassignments that result in a smallest poly-3 p-value that is smaller than or equal to the smallest poly-3 p-value obtained from the original data. In addition to the min-test, two additional permutation tests were computed. The test statistics for these tests were the number of poly-3 tests of tumors in the original data for which the p-value is less than or equal to the critical value of 0.05 (05-test) or 0.01 (01-test). The false positive rates for these tests are the proportion of random permutations of the data for which the number of poly-3 p-values from the permuted data that are less than or equal to the critical value equal or exceed the number from the original data. The min-test could have enhanced power in a situation in which a test agent causes cancer at a single site, whereas the 05-test could have enhanced power when a test agent causes detectable cancer of several types. The permutation tests described above are conditional, not just on the total number of tumors, but also on the patterns of tumors occurring in individual animals.

In addition to conducting conventional poly-3 tests on specific types of tumors, tests were also conducted on combinations of tumor types thought to have a common origin such as liver adenomas and carcinomas. Since including these combinations resulted in the same tumors being present in multiple analyses, it was decided to perform two analyses, one (primary analysis) that included all of the individual tumors and combinations, and one (reduced analysis) in which individual tumors and combinations of tumors were removed from the analysis if they were part of a more inclusive tumor combination (e.g. the individual tumor categories of liver adenoma and liver carcinoma were removed and only the combination was used in the analysis).

For 5 of the glyphosate bioassays, all tissues listed for histopathological examination were scheduled for a histological examination in all animals of all dose groups. In the remaining 5 bioassays, control and high dose animals were all given a complete histopathological examination, along with the animals that died before the final sacrifice in the intermediate dose groups. In addition, certain tissues in all animals were scheduled for a histopathological examination regardless of when they died.

Simple randomization suffers from a potential bias due to dose-related differential survival, and, for studies with incomplete histopathology, a problem of data comparability.

In each of the 10 bioassays, dose-related effects on survival were tested using a Cochran-Armitage test for negative trend on the proportions of animals surviving to final sacrifice in the various dose groups. Regardless of the outcome of this test, to control for potential dose-related differences in survival each randomization of the data maintained the same number of survivors and non-survivors in each group as was seen in the actual data.

For other tissues than the mandatory tissues in the studies with incomplete histopathology, only the non-survivors in the intermediate dosed groups could be used in the trend analyses. For mandatory tissues, the survivors and non-survivors were separately permuted. Mandatory tissues and other tissues had to be separately randomized in studies with incomplete histopathology to make sure that all pathological information routinely collected in this type of studies is included.

In all applications of the poly-3 test, the test is applied only to data from one sex in a single study and the p-values from the poly-3 tests of all the studies are combined to create the "global" tests (min-test, 05-test and 01-test) to give the correct false positive rates. In addition to the randomization procedures for testing for positive dose-response trends in tumor incidence, the same procedures were repeated after reconfiguring the poly-3 test for negative trends.

Results

When the frequency of poly-3 p-values for positive trend computed from all tumors in all 10 bioassays in which at least two tumors occurred are considered there is an excess of large p-values (close to 1.0) compared to small p-values (close to 0.0). Since the version of the poly-3 trend test applied is a one-sided test for a positive trend, p-values close to 1.0 would translate into p-values near 0.0 for one-sided trend tests for anti-carcinogenicity.

Results of tests for a dose-related decrease in survival in each study show that in none of the bioassays analyzed this test was statistically significant. Moreover, 4 of the datasets had p-values in excess of 0.95 which indicates a significant positive trend in survival with increasing dose.

The 24 tumors for which the poly-3 test for a positive dose-related trend was significant at the 0.05 level in the primary analysis are shown in Table 2. Pancreatic islet-cell adenoma in male rats reported in the Stout and Ruecker (1990) study is not listed because it did not have a significant dose-related trend. In an identical analysis with the poly-3 test configured to test for a negative dose-related trend, there were 26 tumors for which the dose-response trend was significantly negative at the 0.05 level.

Table 5.5-146: Tumors with a significant positive trend (poly-3 p ≤ 0.05)

Bioassay	Species/ Sex	Tumor	Summary Tumor Incidence				Poly-3 p-value	Cited by IARC[a]
Atkinson et al. 1993b	M/M	Haemangiosarcoma	0/50	0/50	0/50	4/50	0.0013	IARC
Lankas 1981	R/F	Thyroid: C-cell Carcinoma	1/47	0/49	2/50	6/47	0.0015	
Sugimoto 1997	M/F	Hemangioma	0/50	0/50	2/50	5/50	0.0028	
Sugimoto 1997	M/F	Hemangioma, Hemangiosarcoma	0/50	0/50	3/50	5/50	0.0062	
Stout and Ruecker 1990	R/F	Adrenal: Cortical Carcinoma	0/60	0/60	0/60	3/60	0.0073	
Sugimoto 1997	M/F	Osteoma, Osteosarcoma	0/50	0/50	0/50	3/50	0.0074	
Wood et al. 2009b	M/M	Lymphoma	0/51	1/51	2/51	5/51	0.0076	
Brammer 2001	R/M	Liver: Hepatocellular Adenoma	0/64	2/64	0/64	5/64	0.014	
Lankas 1981	R/M	Testis: Interstitial Cell Tumor	0/50	3/50	1/50	6/50	0.021	
Stout and Ruecker 1990	R/M	Liver: Hepatocellular Adenoma	3/60	2/60	3/60	8/60	0.022	IARC[b]
Atkinson et al. 1993a	R/F	Lipoma	0/50	0/50	0/50	2/50	0.022	
Wood et al. 2009b	M/M	Lung: Adenocarcinoma	5/50	5/51	7/51	11/51	0.025	
Knezevich and Hogan 1983	M/M	Kidneys: Renal Tubal Adenoma	0/49	0/49	1/50	3/50	0.034	IARC
Lankas 1981	R/F	Lipoma	0/50	0/50	0/50	2/50	0.036	
Sugimoto 1997	M/M	Malignant Lymphoma	2/50	3/50	0/50	6/50	0.038	
Knezevich and Hogan 1983	M/F	Lymphoblastic Lymphosarcoma	0/50	1/50	0/50	3/50	0.041	
Sugimoto 1997	M/F	Osteosarcoma	0/50	0/50	0/50	2/50	0.041	
Sugimoto 1997	M/M	Kidney: Adenoma	0/50	0/50	0/50	2/50	0.042	
Sugimoto 1997	M/M	Hemangiosarcoma	0/50	0/50	0/50	2/50	0.043	
Stout and Ruecker 1990	R/M	Neurofibroma, Neurofibrocarcinoma	0/60	0/60	0/60	2/60	0.045	
Sugimoto 1997	M/F	Harderian Gland: Adenoma	1/50	0/50	0/50	5/50	0.046	
Stout and Ruecker 1990	R/F	Thyroid Gland: C-cell Adenoma	1/60	2/60	6/60	6/60	0.047	IARC
Suresh 1996	R/M	Lymphoma	0/50	0/50	0/50	2/50	0.049	
Stout and Ruecker 1990	R/F	Thyroid Gland: C-cell Adenoma or Carcinoma	1/60	2/60	7/60	6/60	0.049	

[a] Indicates tumor responses cited by IARC (2015) as evidence of carcinogenicity. Pancretic islets in male rats in Stout and Ruecker (1990) was also cited by IARC (1/58, 8/57, 5/60 and 7/59) but this response did not give a p-value ≤ 0.05 by the Poly-3 trend test.
[b] IARC (2015) resported tumor responses of 2, 2, 3, 7.

The most significant poly-3 trend in all 10 bioassays was for hemangiosarcoma in male mice in the Atkinson *et al*. (1993) study with a p-value of 0.0013. The actual significance of this smallest p-value, which is the false positive rate for the min-test, was 0.26 based on the primary analysis, rather than the naive value of 0.0013. This means that 26 % of the randomizations of the 10 datasets gave a smallest p-value less than or equal to the smallest p-value obtained from the original data.

The false positive rate for the 05-test was 0.08, which means that 8 % of randomizations of the 10 datasets found at least 24 sites for which the poly-3 p-value was ≤ 0.05. The results from all permutation tests based on the reduced data were similar to those based on the primary data. The false positive rate for the 01-test was 0.06 in the primary analysis and 0.08 in the reduced analysis. Overall, these findings suggest that, after accounting for the number of statistical tests performed, there was no clear evidence of a positive dose-related trend in tumor occurrence.

The evidence for negative trends is greater than that for positive trends in all analyses. The smallest poly-3 p-value for a negative trend was 0.0008 whereas the smallest p-value for a positive trend was 0.0013. The 01-test for a negative trend was highly significant in both the primary and reduced analyses with a p-value of 0.002 for each. These findings suggest stronger evidence for negative rather than positive dose-response trends in tumor occurrence.

Discussion and conclusions

The highest doses given to any animal groups in the 10 bioassays analyzed were 5,873 mg/kg bw/day and 4,841 mg/kg bw/day in female and male mice, respectively. Despite the extremely high doses, there was no evidence of reduced survival in this study. On the contrary, there was a statistically significantly

enhanced survival in male mice in this study, as well as in male animals in several other bioassays. The use of the individual animal data allowed the authors to distinguish between an adenoma and a carcinoma occurring in separate animals and both tumors occurring together in a single animal. Knowledge of the age at death of each individual animal is required for the conduct of the poly-3 test. In addition to the application of the poly-3 test, which is an age-adjusted Cochran-Armitage test, age was also controlled by keeping the numbers of animals surviving to final sacrifice in each dose group the same in all permutations as in the original data.

In the primary analysis of all bioassays 525 poly-3 analyses were conducted of individual tumor responses, of which a total of 174 were on combinations of individual tumor types that may have similar etiologies. In the primary analysis, individual tumors can appear in more than one poly-3 analysis. Since this will happen in the original data and the permuted data with equal frequency, it will not bias the analysis. Also a reduced analysis was conducted in which individual tumors and combinations of tumors were removed from the analysis if they were part of a more inclusive tumor combination. This reduced analysis involved 304 poly-3 analyses. Results from these two analyses were quite similar.

The smallest poly-3 p-value (0.0013) found in the analysis of all the datasets was that for hemangiosarcoma in male mice in the Atkinson *et al*. study. The analysis showed that the actual false positive rate for this finding after accounting for multiple comparisons was 0.26 in the primary analysis and 0.17 in the reduced analysis. Neither the 05-test nor the 01-test gave a false positive rate that was clearly less than 0.05 in the primary and reduced analysis. The statistically significant ($p = 0.0013$) response of 8 % in CD-1 males in the Atkinson *et al*. study resulted from 4 hemangiosarcomas at a dose level of 1,000 mg/kg bw/day, with no hemangiomas or hemangiosarcomas reported at the 3 lower doses. The CD-1 male mice in the Knezevich and Hogan study were exposed to 4,831 mg/kg bw/day, a dose nearly 5 times that used in the Atkinson *et al*. study with no hemangiomas or hemangiosarcomas. Moreover, the incidence of 8 % was still within the historical control range reported for male CD-1 mice by Atkinson *et al*. (0-8 %) and Giknis and Clifford (2005) (0-12 %). The 10 % incidence in hemangioma/ hemangiosarcoma in female CD-1 mice reported in the Sugimoto (1997) study is within the historical control range of 0-12 % of hemangiosarcoma reported by Giknis and Clifford (2005). The lack of a consistent dose response in either males or females suggests that finding significant responses in hemangioma and hemangiosarcoma in both sexes of mice may be attributable to chance, especially considering that this represents the "worst case" of more than 100 tumor sites/types in these bioassays that could have shown evidence of carcinogenicity.

The only other tumor in the mouse studies that the IARC regarded as being clearly related to glyphosate exposure was the marginally significant increase (0/49, 0/49, 1/50, 3/50) in kidney adenoma in male mice observed in the Knezevich and Hogan study. However, additional step sectioning of kidneys in the dosed and control groups revealed one kidney adenoma in the control group, but no additional kidney tumors in the dosed groups. Since the new data provided did not identify this control animal, this additional tumor could not be taken into account in the analysis of this paper. When this tumor-bearing control animal is taken into account in the trend analysis the trend is not anymore statistically significant, adding to the evidence that the tumor increases reported in the glyphosate studies are due to chance.

Comparing the results of negative dose-related trends with those testing for a positive trend, the evidence for an effect was stronger for negative than for positive trends. The smallest p-value for a positive trend was 0.0013 versus 0.0008 for a negative trend, although the corresponding false positive rates after correcting for multiple comparisons were 0.26 and 0.11 demonstrating how adjusting for multiple comparisons can change the interpretation of analyses of individual tumors. The only clearly significant results for any of the 3 permutation tests were the highly significant 01-tests for negative trend in both the primary and reduced analysis.

In all 10 bioassays, the analysis made in this paper identified 24 tumors that exhibited a poly-3 positive trend with a p-value ≤ 0.05. Nevertheless, after accounting for the multitude of statistical tests this analysis did not find that number statistically significant ($p = 0.08$). The statistical analysis of 10 glyphosate bioassays presented in this paper found no strong statistical evidence that glyphosate is carcinogenic. The

main cause for the discrepancy between the analysis made by IARC and that of the authors appears to be that IARC failed to consider the large number of statistical tests performed in the multiple bioassays they reviewed and the resulting multiple comparison problem. IARC and other organizations involved with interpreting results from large data sets to which a large number of statistical tests have been applied should consider applying analyses of the type used in this paper to make informed and reasonable decisions. The present analysis provides new information on the potential carcinogenicity of glyphosate by being the first to provide results from statistical tests with correct false positive rates. These tests found no strong or convincing evidence that glyphosate is an animal carcinogen.

7. Assessment and conclusion

Assessment and conclusion by applicant:

Ten cancer bioassays of sufficient quality and which allowed the analysis of individual animal data was selected for the application of a multi-response permutation procedure that adjusts for the large number of tumors eligible for statistical testing and provides valid false-positive probabilities. The statistical tests applied in the analysis were functions of p-values obtained from conventional continuity-corrected poly-3 tests for trend applied to each type of tumor or combination of tumor types in each bioassay. Results from 3 multi-response permutation tests are reported and discussed: the "min-test", the "05-test" and the "01-test". In the min-test, the test statistic is the smallest p-value obtained from applying the poly-3 test to all tumor types in all bioassays investigated. Animals are randomly reassigned to dose groups in a Monte Carlo analysis, keeping the total numbers of animals in each dose group equal to the number in the original data. The tumors in each such reassignment are analyzed using the poly-3 test in exactly the same way as in the original data. Males and females are permuted separately. The false positive rate is the proportion of random reassignments that result in a smallest poly-3 p-value that is smaller than or equal to the smallest poly-3 p-value obtained from the original data. The test statistics for the 05-test and the 01-test are the number of poly-3 tests of tumors in the original data for which the p-value is less than or equal to the critical value of 0.05 or 0.01, respectively. In all applications of the poly-3 test, the test is applied only to data from one sex in a single study and the p-values from the poly-3 tests of all the studies are combined to create the "global" tests (min-test, 05-test and 01-test) to give the correct false positive rates. In addition to the randomization procedures for testing for positive dose-response trends in tumor incidence, the same procedures were repeated after reconfiguring the poly-3 test for negative trends. When the frequency of poly-3 p-values for positive trend computed from all tumors in all 10 bioassays in which at least two tumors occurred are considered there is an excess of large p-values (close to 1.0) compared to small p-values (close to 0.0). Since the version of the poly-3 trend test applied is a one-sided test for a positive trend, p-values close to 1.0 would translate into p-values near 0.0 for one-sided trend tests for anti-carcinogenicity. Results of tests for a dose-related decrease in survival in each study show that in none of the bioassays analyzed this test was statistically significant. Moreover, 4 of the datasets had p-values in excess of 0.95 which indicates a significant positive trend in survival with increasing dose. The most significant poly-3 trend in all 10 bioassays was found in the Atkinson *et al.* (1993) study for hemangiosarcoma in male mice with a p-value of 0.0013. The actual significance of this smallest p-value, which is the false positive rate for the min-test, was 0.26 based on the primary analysis, rather than the naive value of 0.0013. This means that 26 % of the randomizations of the 10 datasets gave a smallest p-value less than or equal to the smallest p-value obtained from the original data. Besides, the incidence in hemangiosarcomas (8 %) remained within the historical control range and no such tumors were identified in another mouse study at a dose level nearly 5 times of that used in the Atkinson *et al.* (1993) study. Overall, these findings suggest that, after accounting for the number of statistical tests performed, there was no clear evidence of a positive dose-related trend in tumor occurrence. The 01-test for a negative trend was highly significant with a p-value of 0.002. These findings suggest stronger evidence for negative rather than positive dose-response trends in tumor occurrence. In all 10 bioassays investigated, the analysis made in this paper identified 24 tumors that exhibited a poly-3 positive trend with a p-value of less than or equal to 0.05. Nevertheless, after accounting for the multitude of statistical tests this analysis did not find that number statistically significant ($p = 0.08$). The statistical analysis of 10 glyphosate bioassays presented in this paper found no strong statistical evidence that glyphosate is carcinogenic. This publication is considered relevant for

the risk assessment of glyphosate and reliable without restrictions because state-of-the-art statistical methods were employed to a selected set of cancer bioassays to demonstrate false-positive probabilities.

Assessment and conclusion by RMS:

Reliability criteria for *in vivo* toxicology studies

Publication: Crump *et al*., 2020.	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N.A.	
Study performed according to GLP	N.A.	
Study completely described and conducted following scientifically acceptable standards	Y	Statistical re-analysis of 10 selected bioassays for which individual animal data are available.
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Provided in the original bioassays.
Only glyphosate acid or one of its salts is the tested substance	Y	
AMPA is the tested substance	N	
Study		
Test species clearly and completely described	Y	Provided in the original bioassays.
Test conditions clearly and completely described	Y	Provided in the original bioassays.
Route and mode of administration described	Y	Oral *via* the diet.
Dose levels reported	Y	Provided in the original assays. The maximum doses reported are 5,873 mg/kg bw/day and 4,841 mg/kg bw/day in female and male mice, respectively.
Number of animals used per dose level reported	Y	50 – 64 animals per dose group.
Method of analysis described for analysis test media	N	Should be provided in original bioassays.
Validation of the analytical method	N	
Analytical verifications of test media	N	
Complete reporting of effects observed	N.A.	Statistical re-analysis of all tumor sites.
Statistical methods described	Y	Application of a multi-response permutation procedure providing valid false-positive probabilities.
Historical control data of the laboratory reported	Y	For some tumors.
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions	Y	

Reliable with restrictions		
Reliability not assignable		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate and reliable without restrictions because state-of-the-art statistical methods were employed to a selected set of cancer bioassays to demonstrate false-positive probabilities.		

1. Information on the study

Data point:	CA 5.5/027
Report author	Portier, C.J.
Report year	2020
Report title	A comprehensive analysis of the animal carcinogenicity data for glyphosate from chronic exposure rodent carcinogenicity studies
Document No	Environ Health (2020) Vol. 19, 18. https://doi.org/10.1186/s12940-020-00574-1
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	-/Reliable with restrictions

2. Full summary of the study according to OECD format

Abstract

Since the introduction of glyphosate-tolerant genetically-modified plants, the global use of glyphosate has increased dramatically making it the most widely used pesticide on the planet. There is considerable controversy concerning the carcinogenicity of glyphosate with scientists and regulatory authorities involved in the review of glyphosate having markedly different opinions. One key aspect of these opinions is the degree to which glyphosate causes cancer in laboratory animals after lifetime exposure. In this review, twenty-one chronic exposure animal carcinogenicity studies of glyphosate are identified from regulatory documents and reviews; 13 studies are of sufficient quality and detail to be reanalyzed in this review using trend tests, historical control tests and pooled analyses. The analyses identify 37 significant tumour findings in these studies and demonstrate consistency across studies in the same sex/species/strain for many of these tumours. Considering analyses of the individual studies, the consistency of the data across studies, the pooled analyses, the historical control data, non-neoplastic lesions, mechanistic evidence and the associated scientific literature, the tumour increases seen in this review are categorized as to the strength of the evidence that glyphosate causes these cancers. The strongest evidence shows that glyphosate causes hemangiosarcomas, kidney tumours and malignant lymphomas in male CD-1 mice, hemangiomas and malignant lymphomas in female CD-1 mice, hemangiomas in female Swiss albino mice, kidney adenomas, liver adenomas, skin keratoacanthomas and skin basal cell tumours in male Sprague-Dawley rats, adrenal cortical carcinomas in female Sprague-Dawley rats and hepatocellular adenomas and skin keratoacanthomas in male Wistar rats.

Materials and methods

Bioassay data - The animal carcinogenicity data analyzed in this publication were derived from the published literature, the EPA review, the addendum to the EFSA review prepared by the German Institute for Risk Analysis, the JMPR review, the review of the carcinogenicity of glyphosate by a panel of scientists on behalf of industry, and full laboratory reports. The 13 cancer bioassays considered acceptable for this

evaluation are presented in Table 1.

Table 5.5-147: Characteristics of the cancer bioassays of glyphosate analyzed in this study

Study Reference	Duration (months)	Strain		Dietary exposure dose levels (mg/kg/day)	Animals per Group	Purity (%)	Comments on survival and weight
		Mouse	Rat				
A: Knezevich and Hogan (1983) [11]	24	CD-1		M: 0, 157, 814, 4841 F: 0, 190, 955, 5874	50	99.8	No survival differences, slight weight reduction in high dose (M)
B: Atkinson et al. (1993) [12]	24	CD-1		M: 0, 98, 297, 988 F: 0, 102, 298, 1000	50	> 97.0	No survival differences, no weight differences
C: Sugimoto (1997) [13]	18	CD-1		M: 0, 165, 838.1, 4348 F: 0, 153.2, 786.8, 4116	50	94.6–95.7	No survival differences, slight weight reduction in mid (M) & high dose (M + F)
D: Wood et al. (2009) [14]	18	CD-1		M: 0, 71.4, 234.2, 810 F: 0, 97.9, 299.5, 1081.2	51	95.7	No survival differences, no weight differences
E: Takahashi (1999a) [15]	18	CD-1		M: 0, 167.6, 685, 7470 F: 0, 93.2, 909, 8690	50	97.5	Reduced survival high dose (M), slight weight reduction in mid (M) & high dose (M + F). This study was only mentioned by JMPR [7] and provides limited tumor data.
F: Kumar (2001) [16]	18	S-A[a]		M: 0, 85.5, 285.2, 1077.4 F: 0, 104.5, 348.6, 1381.9	50	> 95.0	No survival differences, no weight differences
G: Lankas (1981) [17]	26		SD[b]	M: 0, 3.05, 10.3, 31.49 F: 0, 3.37, 11.22, 34.02	50	98.7	No survival differences, no weight differences
H: Stout and Ruecker (1990) [18]	24		SD[b]	M: 89, 362, 940 F: 0, 113, 457, 1183	50	98.7	No survival differences, slight weight reduction in high dose (F)
I: Atkinson (1993) [19]	24		SD[b]	M: 0, 11, 112, 320, 1147 F: 0, 12, 109, 347, 1134	50	98.9	No survival differences, slight weight reduction in high dose (M + F)
J: Enemoto (1997) [20]	24		SD[b]	M: 0, 104, 354, 1127 F: 0, 115, 393, 1247	50	95.7	Reduced survival high dose (M), slight weight reduction in high dose (M + F)
K: Suresh (1996) [21]	24		W[c]	M: 0, 6.3, 59.4, 595.2 F: 0, 8.6, 88.5, 886	50	96.8	No survival differences, no weight differences
L: Brammer (2001) [22]	24		W[c]	M: 0, 121, 361, 1214 F: 0, 145, 437, 1498	53	97.6	High dose survived longer (M), reduced weight highest dose (M + F)
M: Wood et al. (2009) [23]	24		W[c]	M: 0, 165, 838.1, 4348 F: 0, 153.2, 786.8, 4116	51	94.7–95.8	No survival differences, no weight differences

[a]Swiss Albino mouse; [b]Sprague-Dawley rat; [c]Wistar rat

For twelve of these studies full study reports were available and most of them were conducted in accordance with the appropriate regulatory guidelines. A full study report was not available for the Takahashi (1999) study but the data on kidney tumours in males and malignant lymphomas in females could be obtained from a JMPR review.

Statistical tests - Individual tumour counts for the individual studies were re-analyzed using the exact Cochran-Armitage one-sided linear trend test in proportions. Re-analyses were conducted on all primary tumours where there were at least 3 tumours in all of the animals in a sex/species/strain combination. In addition, any tumour where a significant positive trend ($p \leq 0.05$) was seen in at least one study was also evaluated in all studies of the same sex/species/strain, regardless of the number of animals with the tumour. When adenomas and carcinomas were observed in the same tissue, a combined analysis of adenomas and carcinomas was also conducted. Pairwise comparisons between individual exposed groups and the control group are conducted using Fisher's exact test. To evaluate the consistency of a tumour finding across multiple studies using the same sex-species-strain combinations, logistic regression with individual background responses and dose trends are fit to the pooled data using maximum likelihood estimation. A common positive trend is seen in the pooled analysis when the null hypothesis that the slope is 0 is rejected (statistical p-value ≤ 0.05 using a likelihood-ratio test) in favor of the alternative that the slope is greater than 0. Heterogeneity is seen in the pooled analysis when the null hypothesis that the slopes are equal is rejected (statistical p-value ≤ 0.05 using a likelihood-ratio test) in favor of the alternative that at least one of the slopes is different. For CD-1 mice analyses were conducted separately for 18 month and 24 month studies and then a combined analysis was performed. Similar grouped analyses were conducted for SD rats with one study of 26 months and 3 studies of 24 months. Only the combined analysis over all study durations is provided. The same methods of analysis were used to evaluate the incidence of non-neoplastic toxicity in tissues where tumours were observed. In cases of rare tumours where the increase in incidence didn't reach statistical significance the test proposed by Tarone (1982) was applied using an appropriate historical control group. All analyses were done using MATLAB, version R2017b.

Results

The purpose of this analysis is to understand the potential of glyphosate to produce tumours across all studies and not one study at a time. Thus, rather than presenting the results of each study separately, this review focuses on the tumours that are seen as positive in any one study and compares the findings across all studies of the same tumour in the same sex/species/strain combination.

Re-analysis of the data from CD-1 mice - From Table 2 it can be derived that a significant ($p \leq 0.05$) positive trend was found in males for kidney adenomas and kidney adenomas/carcinomas in the Takahashi study, malignant lymphomas in the Sugimoto and the Wood studies, hemangiosarcomas in the Atkinson study, and alveolar-bronchiolar carcinomas in the Wood study. When Tarone's test was applied using historical control data then a significant increase was found for kidney adenomas in the Sugimoto study, kidney carcinomas in the Knezevich and Hogan study, kidney adenomas/carcinomas in the Knezevich and Hogan and the Sugimoto study, and hemagiosarcomas in the Sugimoto study. In females a significant ($p \leq 0.05$) positive trend was found for hemangiomas, harderian gland adenomas and adenomas/carcinomas in the Sugimoto study, alveolar-bronchiolar adenomas/carcinomas in the Atkinson study, and malignant lymphomas in the Takahashi study. A significant common trend was found for kidney adenomas, carcinomas and adenomas/carcinomas, hemangiosarcomas in males and hemangiomas and malignant lymphomas in females.

Table 5.5-148: P-values for the exact Cochran-Armitage trend test and pooled logistic regression analysis for tumours with at least one significant trend test ($p \leq 0.05$) or Fisher's exact test ($p \leq 0.05$) in male and female CD-1 mice.

Tumor	Individual study *p*-values for trend[a]					Common Trend	Heterogeneity Test
Males	A	B	C	D	E		
Kidney Adenomas	0.442 (0.138)[d]	0.993	0.062 (**0.009**)[d]	---[b]	**0.019**	**0.006**	0.268
Kidney Carcinomas	0.063 (**< 0.001**)[d]	0.938	---[b]	---[b]	0.250	**0.031**	0.546
Kidney Adenomas and Carcinomas	0.065 (**0.008**)[d]	0.981	0.062 (**0.009**)[d]	---[b]	**0.005**	**< 0.001**	0.106
Malignant Lymphomas	0.754	0.087	**0.016**	**0.007**	ND[c]	0.093	0.007
Hemangiosarcomas	0.501	**0.004**	0.062 (**0.005**)[d]	---[b]	ND[c]	**0.033**	0.007
Alveolar-Bronchiolar Adenomas	0.494	0.231	0.513	0.924	ND[c]	0.384	0.409
Alveolar-Bronchiolar Carcinomas	0.078	0.456	0.148	**0.028**	ND[c]	0.407	0.083
Alveolar-Bronchiolar Adenomas and Carcinomas	0.586	0.231	0.294	0.336	ND[c]	0.346	0.826
Females	A	B	C	D	E		
Hemangiomas	0.631	---[b]	**0.002**	0.438	ND[c]	**0.031**	0.155
Harderian Gland Adenomas	0.877	ND[c]	**0.040**	0.155	ND[c]	0.155	0.052
Harderian Gland Carcinomas	---[b]	ND[c]	---[b]	1.000	ND[c]	0.500	1.00
Harderian Gland Adenomas and Carcinomas	0.877	ND[c]	**0.040**	0.372	ND[c]	0.184	0.110
Alveolar-Bronchiolar Adenomas	0.999	0.144	0.800	0.656	ND[c]	0.996	0.211
Alveolar-Bronchiolar Carcinomas	0.183	0.110	0.623	0.601	ND[c]	0.268	0.544
Alveolar-Bronchiolar Adenomas and Carcinomas	0.985	**0.048**	0.842	0.688	ND[c]	0.982	0.241
Malignant Lymphomas	0.070[e]	0.484	0.294	0.353	**0.050**	**0.012**	0.995

[a] – Study A is Knezevich and Hogan [11] (Additional file 2: Table S1), Study B is Atkinson et al. [12] (Additional file 2: Table S2), Study C is Sugimoto [13] (Additional file 2: Table S3), Study D is Wood [14] (Additional file 2: Table S4), Study E is Takahashi [15] (Additional file 2: Table S5); [b] – three dashes "---" indicates all tumor counts are zero; [c] – ND indicates there is no data available for this tumor in this study; [d] – using historical control data (see text for details) and Tarone's test; [e] – Spleen composite lymphosarcomas (malignant lymphomas) are also significantly increased in female mice in this study (see Additional file 2: Table S1)

Re-analysis of the data from Swiss albino mice - The single study with Swiss albino mice (Kumar, 2001) shows a significant increase in hemangiomas in female mice ($p = 0.004$).

Re-analysis of the data from SD rats - From Table 3 it can be derived that a significant ($p \leq 0.05$) positive trend was found in males for testicular interstitial cell tumours in the Lankas study, hepatocellular adenomas and adenomas/carcinomas in the Stout and Ruecker study, kidney adenomas in the Enemoto study, skin

keratoacanthomas in the Stout and Ruecker, Atkinson and Enemoto studies, and skin basal cell tumours in the Enomoto study. In females a significant (p ≤ 0.05) positive trend was found for thyroid C-cell adenomas in the Stout and Ruecker study, C-cell carcinomas in the Lankas study, and adrenal cortical carcinoma in the Stout and Ruecker study. When Tarone's test was applied using historical control data then a significant increase was found for pancreatic islet cell adenomas in males in the Stout and Ruecker study, and thyroid C-cell adenomas/carcinomas in females in the Lankas study. A significant common trend was found for hepatocellular adenomas, kidney adenomas, skin keratoacanthomas and skin basal cell tumours in males. In females the common trend was statistically significant for adrenal cortical carcinoma.

Table 5.5-149: P-values for the exact Cochran-Armitage trend test and pooled logistic regression analysis for tumours with at least one significant trend test or Fisher's exact test (p ≤ 0.05) in male and female Sprague-Dawley rats

Tumor	Individual study *p*-values for trend[a]				Common Trend	Heterogeneity Test
Males	G	H	I	J		
Testicular Interstitial Cell Tumors	**0.009**	0.296	0.580	0.594	0.461	0.105
Pancreas Islet Cell Adenomas	0.512	0.147 (**0.007**)[c]	0.974	0.839	0.849	0.143
Pancreas Islet Cell Carcinomas	0.251	1.000	–	0.500	0.731	0.166
Pancreas Islet Cell Adenomas or Carcinomas	0.316	0.206	0.974	0.844	0.875	0.185
Thyroid C-cell Adenomas	0.743	0.089	0.578	0.831	0.210	0.532
Thyroid C-cell Carcinomas	0.505	0.442	0.495	0.565	0.322	0.898
Thyroid C-cell Adenomas and Carcinomas	0.748	0.097	0.19[illegible]	0.642	0.175	0.526
Thyroid Follicular-cell Adenomas	0.122	0.408	0.067	0.966	0.464	0.055
Thyroid Follicular-cell Carcinomas	---[b]	0.255	0.443	1.000	0.448	0.137
Thyroid Follicular-cell Adenoma and Carcinoma	0.122	0.332	0.099	0.986	0.446	0.031
Hepatocellular Adenomas	0.471	**0.015**	0.325	0.500	**0.029**	0.664
Hepatocellular Carcinomas	0.062	0.837	0.760	0.642	0.803	0.269
Hepatocellular Adenomas and Carcinomas	0.173	**0.050**	0.480	0.690	0.144	0.428
Kidney Adenomas	0.938	0.813	1.000	**0.004**	**0.039**	0.002
Skin Keratoacanthomas	---[b]	**0.042**	**0.047**	**0.029**	**< 0.001**	0.998
Skin Basal Cell Tumors	0.251	0.249	1.000	**0.004**	**< 0.001**	0.009
Females	G	H	I	J		
Thyroid C-cell Adenomas	0.079	**0.049**	0.207	0.912	0.287	0.150
Thyroid C-cell Carcinomas	**0.003 (< 0.001)**[c]	0.500	---[b]	---[b]	0.385	0.041
Thyroid C-cell Adenomas and Carcinomas	0.072 **(0.037)**[c]	0.052	0.207	0.912	0.275	0.071
Adrenal Cortical Adenoma	0.851	0.603	---[b]	0.626	0.713	0.750
Adrenal Cortical Carcinoma	0.386	**0.015**	0.493	---[b]	**0.031**	0.199
Adrenal Cortical Adenoma and Carcinoma	0.801	0.090	0.493	0.626	0.195	0.520

[a] – Study G is Lankas [17] (Additional file 2: Table S7), Study H is Stout and Ruecker [18] (Additional file 2: Table S8), Study I is Atkinson et al. [12] (Additional file 2: Table S9) and Study J is Enemoto [20] (Additional file 2: Table S10); [b] – three dashes "---" indicates all tumor counts are zero; [c] – using historical control data (see text for details) and Tarone's test

Re-analysis of the data from Wistar rats - From Table 4 it can be derived that a significant (p ≤ 0.05) positive trend was found in males for hepatocellular adenomas and adenomas/carcinomas in the Brammer study, pituitary adenomas in the Wood study, skin keratoacanthomas in the Wood study, and adrenal pheochromocytomas in the Suresh study. In females a significant (p ≤ 0.05) positive trend was found for mammary gland adenocarcinomas and adenomas/adenocarcinomas, pituitary adenomas and adenomas/carcinomas all in the Wood study. A significant common trend was found for hepatocellular adenomas and adenomas/carcinomas and skin keratoacanthomas in males. No statistically significant common trend was found for the tumours in females.

Table 5.5-150: P-values for the exact Cochran-Armitage trend test and pooled logistic regression analysis for tumours with at least one significant trend test or Fisher's exact test ($p \leq 0.05$) in male and female Wistar rats

Tumor	Individual study *p*-values for trend[a]			Common Trend	Homogeneity Test
Males	K	L	M		
Hepatocellular Adenomas	0.391	**0.008**	0.418	**0.048**	0.156
Hepatocellular Carcinomas	0.418	---[b]	1.000	0.492	0.242
Hepatocellular Adenomas and Carcinomas	0.286	**0.008**	0.610	**0.029**	0.194
Pituitary Adenomas	0.376	0.277	**0.045**	0.057	0.664
Pituitary Carcinomas	0.692	---[b]	1.000	0.771	0.956
Pituitary Adenomas and Carcinomas	0.454	0.277	0.059	0.073	0.700
Skin Keratoacanthomas	---[b]	0.387	**0.030**	**0.032**	0.823
Adrenal Pheochromocytomas	**0.048**	0.721	0.306	0.223	0.210
Females	K	L	M		
Mammary Gland Adenomas	0.539	0.941	0.062	0.448	0.015
Mammary Gland Adenocarcinomas	1.000	0.271	**0.042**	0.071	0.008
Mammary Gland Adenomas and Adenocarcinomas	0.729	0.590	**0.007**	0.113	0.064
Pituitary Adenomas	0.967	0.261	**0.014**	0.105	0.023
Pituitary Carcinomas	1.000	–	0.750	0.748	0.491
Pituitary Adenomas and Carcinomas	0.976	0.261	**0.017**	0.129	0.019

[a] – Study J is Suresh [21] (Additional file 2: Table S11), Study K is Brammer [22] (Additional file 2: Table S12), and Study L is Wood et al. [14] (Additional file 2: Table S13); [b] – three dashes "---" indicates all tumor counts are zero

False positive errors - The evaluation of any one animal cancer study involves a large number of statistical tests that could lead to false positives. To evaluate this issue, the probability that all of the results in any sex/species/strain could be due to false positive results is calculated. Overall, a total of 496 evaluations were done for these 13 studies including the few evaluations done against historical controls. There are 41 evaluations at 37 tumour/site combinations with a trend test $p \leq 0.05$. The probability that all of these are due to false positives is 0.001. Similarly, looking at the evaluations resulting in $p \leq 0.01$, the probability that all of the findings are due to false positives is < 0.001. The strongest evidence was found for male CD-1 mice where the probability for 11 positive findings to occur at $p \leq 0.05$ and 8 positive findings at $p \leq 0.01$ are both below 0.001.

Conclusions

Oral exposure of rats and mice to glyphosate *via* the diet in 13 separate carcinogenicity studies demonstrates that glyphosate causes a variety of tumours that differ by sex, species, strain and length of exposure. To summarize the results of the strength-of-evidence analysis, each tumour is placed in any of the following categories: Clear evidence (CE) when there is a causal link between glyphosate exposure and the tumour; Some evidence (SE) when there is a causal link between glyphosate exposure and the tumour but chance, although unlikely, cannot be ruled out; Equivocal evidence (EE) when there is a causal link between glyphosate exposure and the tumour but chance is as likely an explanation for the association as is exposure to glyphosate; No evidence (NE) indicates that any causal link between glyphosate exposure and the tumour is almost certainly due to chance. The factors used to place tumours into these categories include the analyses of the individual studies, the consistency of the data across studies (pooled analyses), the analyses using historical control data, the analyses of non-neoplastic lesions, mechanistic evidence and the associated scientific literature.

The weight-of-evidence analysis conducted in this study indicates that there is clear evidence (CE) that oral exposure to glyphosate *via* the diet produces adrenal cortical carcinoma in the female SD rat, hemangioma in the female mouse (CD-1 and Swiss albino), hemangiosarcoma in the male CD-1 mouse, kidney tumours in the male CD-1 mouse and SD rat, liver adenoma in male rats (SD and Wistar), malignant lymphoma in the male and female CD-1 mouse, skin basal cell tumour in the male SD rat and skin keratoacanthoma in male rats (SD and Wistar). Some evidence (SE) for a causal relationship was found for kidney tumours in

male Swiss albino mice, mammary tumours in female Wistar rats, malignant lymphoma in male and female Swiss albino mice, pituitary adenoma in the male and the female Wistar rat, and testicular interstitial cell tumours in the male SD rat. The results of the analyses conducted in this study are supportive of the conclusions of IARC that there is sufficient evidence to consider glyphosate as a rodent carcinogen.

6. Assessment and conclusion

Assessment and conclusion by applicant:

Thirteen glyphosate cancer bioassays considered acceptable for this re-analysis were selected from the published literature, the EPA review, the review from the German Institute for Risk Analysis, the JMPR review, and full laboratory reports. For twelve of them full study reports were available. Individual tumour counts for the individual studies were re-analyzed using the exact Cochran-Armitage one-sided linear trend test. Re-analyses were conducted on all primary tumours where there were at least 3 tumours in all of the animals in a sex/species/strain combination. In addition, any tumour where a significant positive trend ($p \leq 0.05$) was found in at least one study was also evaluated in all the other studies of the same sex/species/strain combination, regardless of the number of animals with the tumour. Pairwise comparisons between individual exposed groups and the control group were conducted using Fisher's exact test. To evaluate the consistency of a tumour finding across multiple studies using the same sex-species-strain combinations, logistic regression with individual background responses and dose trends are fit to the pooled data using maximum likelihood estimation. The same methods of analysis were used to evaluate the incidence of non-neoplastic lesions in tissues where tumours were observed. In cases of rare tumours where the increase in incidence didn't reach statistical significance the test proposed by Tarone (1982) was applied using an appropriate historical control group. To summarize the results of the strength-of-evidence analysis, each tumour is placed in any of the following categories: Clear evidence (CE), some evidence (SE), equivocal evidence (EE), and no evidence (NE). The factors used to place tumours into these categories include the analyses of the individual studies, the consistency of the data across studies (pooled analyses), the analyses using historical control data, the analyses of non-neoplastic lesions, and mechanistic evidence with the associated scientific literature. The author's weight-of-evidence analysis indicates that there is clear evidence (CE) that oral exposure to glyphosate *via* the diet produces adrenal cortical carcinoma in the female SD rat, hemangioma in the female mouse (CD-1 and Swiss albino), hemangiosarcoma in the male CD-1 mouse, kidney tumours in the male CD-1 mouse and SD rat, liver adenoma in the male rat (SD and Wistar), malignant lymphoma in the male and female CD-1 mouse, skin basal cell tumour in the male SD rat and skin keratoacanthoma in male rats (SD and Wistar). Some evidence (SE) for a causal relationship was put forth for kidney tumours in the male Swiss albino mouse, mammary tumours in the female Wistar rat, malignant lymphoma in the male and female Swiss albino mouse, pituitary adenoma in the male and the female Wistar rat, and testicular interstitial cell tumours in the male SD rat.

After thorough analysis and considering all factors that are important in the interpretation of cancer studies none of the tumours identified by the author as indicating clear evidence (CE) or some evidence (SE) of carcinogenicity were found relevant for reconsideration under the necessary due diligence of the European AIR5 review of glyphosate. Most of the tumours selected by the author were previously dismissed by the EU experts as not relevant even before the last review of glyphosate in 2017, and the applicant believes that there is no solid toxicological evidence for glyphosate exposure related carcinogenicity in the mouse and the rat that warrants any science-based concerns for human health. The discussion of each of the suspect tumours is given below.

Clear evidence (CE) for carcinogenicity:

Adrenal cortical carcinoma in the female SD rat (Stout and Ruecker study):

The tumour incidences were 0/60, 0/60, 0/60, 3/60 at 0, 113, 457, and 1183 mg/kg bw/day, respectively. This tumour has not been considered treatment related by the authors of the study. There is no dose-related increase in adrenal cortical adenoma (1/60, 3/60, 2/60, 1/60), no dose-related increase in pre-neoplastic lesions and this tumour was not found in the males of the same study or in other rat studies. Therefore, this tumour has been considered not relevant for the risk assessment of glyphosate and was

not discussed further in the previous EU review of glyphosate.

Hemangioma in female CD-1 mice (Sugimoto study):
In the Sugimoto study hemangiomas were observed in different tissues:
- In liver with an incidence of 0/50, 0/50, 1/50, 1/50;
- In the ovary with an incidence of 0/50, 0/50, 0/50, 1/50;
- In the uterus with an incidence of 0/50, 0/50, 1/50, 2/50;
- In the spleen with an incidence of 0/50, 0/50, 1/50, 0/50;
- In the abdominal cavity with an incidence of 0/8, 0/9, 0/9, 1/9;

At 0, 153.2, 786.8, and 4116 mg/kg bw/day, respectively. Taken together as systemic tumours a significant positive trend is obtained. However, hemangiomas have also been observed in males (liver and testes) but without any dose-response relationship and the highest incidence found was in the control group (1/50). These tumours have not been confirmed in the other carcinogenicity studies in the CD-1 mouse. Moreover, the dose level (4116 mg/kg bw/day) at which the incidence was statistically significantly increased when compared against the control, is more than 4-fold the limit dose for the testing of carcinogens in rodent species. If that dose is ignored there is no significative positive trend. Therefore, this tumour has been considered not relevant for the risk assessment of glyphosate and was not discussed further in the previous EU review of glyphosate.

Hemangioma in female Swiss albino mice (Kumar study):
In the re-analysis of the tumour data of the Kumar study by K. Weber (report submitted in 2017) no statistically significant trend was found for systemic neoplasms using the Peto test. When analyzed using the Fischer's exact test no statistically significant increase in incidence was found in pair-wise comparisons of all dose groups with the control group. It is important to emphasize that this study was compromised by non-identified ecto-and endoparasites in a large number of animals. Therefore, this tumour has been considered not relevant for the assessment of glyphosate.

Hemangiosarcoma in the male CD-1 mouse (Atkinson study):
The tumour incidences were 0/50, 0/50, 0/50, 4/50 (3 in liver and 1 in prostate) at 0, 98, 297, and 988 mg/kg bw/day, respectively. The incidence at the highest dose (8 %) was still within the historical control range of the test laboratory (0-8 %, 300 male mice in 6 studies up to 1993). This tumour was not confirmed in other mouse studies of which one (Knezevich and Hogan study) with a dose level nearly 5-fold that of the Atkinson study (4841 mg/kg bw/day). Therefore, this tumour has been considered not relevant for the assessment of glyphosate.

Kidney tumours in the male CD-1 mouse (Takahashi study, as reported by JMPR, 2016):
Renal cell adenoma (3/50) and renal cell carcinoma (1/50) were observed in males at 7470 mg/kg bw/day, but, according to the authors, there was no statistically significant difference with the control group. It is of note that the high dose considered in this study for males is extraordinarily high, more than 7-fold the limit dose for the testing of carcinogens in rodent species. If this dose is ignored there is no significant positive trend. These tumours were re-examined by the original study pathologist in 2012 because the Pesticide Expert Panel of the Food Safety Commission of Japan requested more information on historical control data and association with the non-neoplastic renal findings. After re-examination, the incidences for renal cell adenoma were 1/50, 1/50, and 1/50 at 167.6, 685, and 7470 mg/kg bw/day, showing no dose-response relationship. The incidence for renal cell carcinoma was confirmed to be 1/50 at 7470 mg/kg bw/day. The historical control data for the Takahashi study were not available, but the historical control values described in the re-examination document for renal cell carcinoma were 1/725 (0.13 %) in males and 0/725 (0 %) in females and for renal cell adenoma were 3/564 (0.53 %) in males and 0/564 (0 %) in females. The re-examination report also provides reference data of 0-1.8 % in males and 0 % for all doses in females for renal cell carcinoma, and 0-1.8 % in males and females for renal cell adenoma. The results of the re-examination revealed also that the tubular epithelial cell hypertrophy was localized with an incidence in each treatment group that did not significantly differ from that in the control group. There was no association between the tubular epithelial cell hypertrophy and the

development of renal tumours. The renal cell tumours observed in this study are thus not relevant for the human risk assessment of glyphosate because (1) the incidence of renal tumours in males at 7470 mg/kg bw/day did not significantly differ from that in the control group upon re-evaluation; (2) none of the females had neoplastic or non-neoplastic lesions; and (3) the high dose considered in this study for males is more than 7-fold the limit dose for the testing of carcinogens in rodent species. Therefore, this tumour has been considered not relevant for the assessment of glyphosate.

Kidney tumours in the male SD rat (Enemoto study):
The incidences of kidney adenoma were 0/76, 0/75, 0/80, 4/78 at 0, 104, 354, and 1127 mg/kg bw/day. An increasing trend in the incidence of adenomas in the kidney was observed in males of the high dose group (animal 193: killed in extremis at week 92, animal 167: found dead at week 94, animal 159: found dead at week 101, and animal 169: killed by design after 104 weeks of treatment) and this incidence was greater than the historical control range referred to in the study report (0-2.9 %). However, according to the authors of this study, the increase observed was not statistically significant. No kidney tumours were found in the females and nearly all male rats at all dose levels suffered from chronic nephropathy (62/76, 63/75, 56/80, 67/78). This tumour in this study was not considered relevant for the risk assessment of glyphosate and was not discussed further in the previous EU review of glyphosate.

Hepatocellular adenomas in the male SD rat (Stout and Ruecker study):
The tumour incidences for adenomas were 3/60, 2/60, 3/60, 8/60 and of carcinomas were 3/60, 2/60, 1/60, 2/60 at 0, 89, 362, and 940 mg/kg bw/day, respectively. The incidence of adenomas at the high dose (13.3 %) is still within the historical control range of the test laboratory (1.4-18.3 %). Foci of cellular alteration were observed at all dose levels without any dose-response relationship and there were no signs of hepatocellular hypertrophy, a prerequisite for hepatocellular carcinogenesis. Beside the Brammer study no increase in hepatocellular adenomas was noted in the other rat studies. Therefore, this tumour was not considered relevant for the assessment of glyphosate.

Hepatocellular adenomas in the male Wistar rat (Brammer study):
The tumour incidences were 0/64, 2/64, 0/64, 5/64 at 0, 121, 361, 1214 mg/kg bw/day, respectively. The positive trend is significant and the incidence at the high dose is significantly different from the control. However, the incidence at the high dose (7.8 %) is still within the historical control range of the test laboratory (0-11.5 %, 26 studies in 1984-2003). There were no histopathological signs of liver enzyme induction or pre-neoplastic lesions. The high dose animals in this study survived longer when compared to the other groups. This may also influence the spontaneous tumour rate. Beside the Stout and Ruecker study no increase in hepatocellular adenomas was noted in the other rat studies. Therefore, this tumour was not considered relevant for the assessment of glyphosate.

Malignant lymphoma in the male CD-1 mouse (Sugimoto study):
The tumour incidences were 2/50, 2/50, 0/50, 6/50 at 0, 165, 838, 4348 mg/kg bw/day, respectively. The positive trend is significant but the incidence at the high dose is not significantly different from the control. Moreover, the incidence at the high dose (12 %) is still within the historical control range of the test laboratory (3.6-19.2 %, 458 male mice in 12 studies in 1993-1998). The trend has been found significantly positive because of the elevated incidence at a dose level that is over 4-fold the limit dose for carcinogenicity studies in rodents. If this dose is ignored the trend is not positive. Therefore, this tumour was not considered relevant for the assessment of glyphosate.

Malignant lymphoma in the female CD-1 mouse (Takahashi study):
The tumour incidences were 3/50, 1/50, 4/50, 6/50 at 0, 93.2, 909, and 8690 mg/kg bw/day, respectively. The increased incidence of lymphoma at the high dose was statistically significant in the trend test but not in a pairwise comparison. The trend has been found significantly positive because of the elevated incidence at an extraordinarily high dose level, more than 8-fold the limit dose for carcinogenicity studies in rodents. If this dose is ignored the trend is no longer significant. Therefore, this tumour was not considered relevant for the assessment of glyphosate.

Skin basal cell tumour in the male SD rat (Enemoto study):
The tumour incidences were 0/78, 0/75, 0/80, 3/78 for adenoma and 0/78, 0/75, 0/80, 1/78 for carcinoma at 0, 104, 354, and 1127 mg/kg bw/day. No increased incidence of this tumour was observed in the females or other rat studies and may be associated with other skin lesions (follicular hyperkeratosis and/or folliculitis/follicular abscess) observed in this study. Although there is a significant positive trend for the adenomas, the increase in incidence at the high dose level was not considered relevant for the risk assessment of glyphosate by the authors of this study. This tumour was not discussed further in the previous EU review of glyphosate.

Skin keratoacanthoma in the male SD rat (Stout and Ruecker study):
The tumour incidences were 1/60, 3/60, 4/60, 5/60 at 0, 89, 362, and 940 mg/kg bw/day. Although there is a significant positive trend the incidence at the high dose was not statistically significantly different from the control and considered not related to treatment. Skin keratoacanthoma is one of the most common spontaneous benign neoplasms in male Sprague Dawley rats. Therefore, this tumour was not considered relevant for the risk assessment of glyphosate by the authors of this study. This tumour was not discussed further in the previous EU review of glyphosate.

Skin keratoacanthoma in the male SD rat (Atkinson study):
The combined incidences of intracutaneous cornifying epithelioma (keratoacanthoma) were 1/50, 2/25, 0/19, 0/21, 5/50 at 0, 11, 112, 320, and 1147 mg/kg bw/day. Although the trend was significant, the incidence at the high dose was not statistically significantly different from the control and considered not related to treatment by the authors of this study. Skin keratoacanthoma is one of the most common spontaneous benign neoplasms in male Sprague Dawley rats. This tumour was not discussed further in the previous EU review of glyphosate.

Skin keratoacanthoma in the male SD rat (Enemoto study):
The incidences of the tumour were 4/76, 3/75, 0/80, 7/78 at 0, 104, 354, and 1127 mg/kg bw/day. The increased incidence of this skin tumour at the high dose may be associated with other skin lesions (follicular hyperkeratosis and/or folliculitis/follicular abscess) observed in this study. Although there is a significant positive trend for this tumour, the increase in incidence at the high dose level was not statistically significantly different from the control. Skin keratoacanthoma is one of the most common spontaneous benign neoplasms in male Sprague Dawley rats and considered by the authors of this study not relevant for the risk assessment of glyphosate. This tumour was not discussed further in the previous EU review of glyphosate.

Skin keratoacanthoma in the male Wistar rat (Wood study):
There were no treatment-related conditions seen in the skin or in subcutaneous tissues, but several spontaneous lesions were observed. Epidermal ulceration and scab formation, inflammatory lesions, abscess formation, focal acanthosis, focal mineralisation, focal dermal thickening, and focal necrosis were seen, occasionally or rarely and without significance. This tumour was not discussed further in the previous EU review of glyphosate.

Some evidence for carcinogenicity (SE)
Kidney tumours in the male Swiss albino mouse (Kumar study):
In the re-analysis of the tumour data of the Kumar study by K. Weber (submitted in 2017) no statistically significant trend was found for systemic neoplasms in the Peto test. When analyzed using the Fischer's exact test no statistically significant increase in incidence was found in pair-wise comparisons of all dose groups with the control group. It is important to emphasize that this study was compromised by non-identified ecto-and endoparasites in a large number of animals. Therefore, this tumour was not considered relevant for the assessment of glyphosate.

Mammary tumour in the female Wistar rat (Wood study):
At interim and terminal sacrifice combined mammary neoplasia was seen in 6 female mice. There were no mammary neoplasms in the control group but carcinomas were seen with incidences of 2/51, 3/51,

and 1/51 at 153.2, 786.8, and 4116 mg/kg bw/day, respectively. All neoplasms were adenocarcinomas with the exception of one adenosquamous carcinoma seen in a low dose group animal. No increase in the incidence of these tumours was reported in the females of other rat studies. The authors concluded that there was no effect of treatment upon the incidence of mammary neoplasia in this study. Therefore, this tumour was not considered relevant for the assessment of glyphosate.

Malignant lymphoma in the male Swiss albino mouse (Kumar study):
In the re-analysis of the tumour data of the Kumar study by K. Weber (2017) no statistically significant trend was found for systemic neoplasms in the Peto test. When analyzed using the Fischer's exact test no statistically significant increase in incidence was found in pair-wise comparisons of all dose groups with the control group. It is important to emphasize that this study was compromised by non-identified ecto-and endoparasites in a large number of animals. Therefore, this tumour was not considered as relevant for the assessment of glyphosate.

Pituitary adenomas in the male and the female Wistar rat (Wood study):
Pituitary adenomas were only seen in female mice with incidences of 0/51, 1/51, 0/51, 2/52 at 0, 104.5, 348.6, and 1381.9 mg/kg bw/day. The group distribution was unrelated to treatment. Therefore, this tumour was not considered relevant for the assessment of glyphosate.

Testicular interstitial cell tumour in the male SD rat (Lankas study):
The incidences of this tumour were 0/50, 3/50, 1/50, 6/50 at 0, 3.05, 10.30, and 31.49 mg/kg bw/day, respectively. The positive trend is statistically significant and the incidence at the high dose level (12 %) is statistically significantly different from the control and greater that the historical control rate of the test laboratory (3.4-6.6 %). However, there was no dose-response relationship for interstitial cell hyperplasia (1/50, 1/50, 1/50, 0/50). Since the dose range considered in this study (0-31.5 mg/kg bw/day) is approximately at least 30-fold lower than that of all the other studies in rats where no increase of such tumours was found this finding should be considered as spontaneous in nature. Therefore, this tumour was not considered relevant for the risk assessment of glyphosate and was not discussed further in the previous EU review of glyphosate.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because some of the statistical methods employed were not described in sufficient detail. Besides, the results of this study are not in agreement with the findings of Crump *et al*. 2020 in relation to the estimation of false positives and the overall evaluation of the significance of the tumours by the EU regulatory authorities. All the tumours that were identified by the author as providing clear evidence for the carcinogenicity of glyphosate have been previously dismissed in the EU regulatory process.

Assessment and conclusion by RMS:

Reliability criteria for *in vivo* toxicology studies

Publication: Portier, 2020.	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N.A.	
Study performed according to GLP	N. A.	Most of the study reports analysed were GLP compliant.
Study completely described and conducted following scientifically	Y	Re-analysis of the tumour data of

acceptable standards		13 selected glyphosate cancer bioassays.
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity of glyphosate used in every cancer bioassay mentioned.
Only glyphosate acid or one of its salts is the tested substance	Y	
AMPA is the tested substance	N	
Study		
Test species clearly and completely described	Y	
Test conditions clearly and completely described	N.A.	Described in the test reports of 12 of the selected studies. The data from one study were derived from a JMPR review.
Route and mode of administration described	Y	Oral *via* the diet.
Dose levels reported	Y	Dose range from 71.4 to 8690 mg/kg bw in the mouse and from 3.05 to 4348 mg/kg bw in the rat.
Number of animals used per dose level reported	Y	About 50 per dose group.
Method of analysis described for analysis test media	N.A.	Described in the original test reports.
Validation of the analytical method	N.A.	
Analytical verifications of test media	N.A.	
Complete reporting of effects observed	Y	
Statistical methods described	Y	All statistical methods used in the re-analysis of the tumour data were reported, however sometimes not in sufficient detail.
Historical control data of the laboratory reported	N.A.	
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Reliability not assignable		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because some of the statistical methods employed were not described in sufficient detail. Besides, the results of this study are not in agreement with the findings of Crump *et al*. 2020 in relation to the estimation of false positives and the overall evaluation of the significance of the tumours by the EU regulatory authorities. All the tumours that were identified by the author as providing clear evidence for the carcinogenicity of glyphosate have been dismissed in the EU regulatory process.		

1. Information on the study

Data point:	CA 5.5/028
Report author	Wozniak, E. *et al.*
Report year	2020
Report title	Glyphosate affects methylation in the promoter regions of selected tumor suppressors as well as expression of major cell cycle and apoptosis drivers in PBMCs (*in vitro* study)
Document No	doi.org/10.1016/j.tiv.2019.104736 E-ISSN: 1879-3177
Guidelines followed in study	None
Deviations from current test guideline	Not applicable

GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes/Reliable without restrictions

2. Full summary of the study according to OECD format

The effect of glyphosate on selected epigenetic parameters and major cell cycle drivers in human peripheral blood mononuclear cells (PBMCs) was determined. The cells were incubated with glyphosate at 0.5, 10 and 100 µM. The analysis included: global DNA methylation, methylation in the promoter regions of tumor suppressor genes (P16, P21, TP53) and proto-oncogenes (BCL2, CCND1) by the Real-Time PCR and the expression profile of the indicated genes by Real-Time PCR. The obtained results have revealed significant reduction of global DNA methylation level in PBMCs exposed to glyphosate. Tested compound changed methylation pattern of the P21 and TP53 suppressor gene promoters, but in case of other analysed genes: P16, BCL2 and CCND1 we did not identify any statistically significant changes. Gene profiling showed that glyphosate changed the expression of genes involved in the regulation of cell cycle and apoptosis. Glyphosate decreased expression of P16 and TP53 as well as an increase in the expression of BCl2, CCND1 and P21. Summing up, our results have shown a potential disturbance in methylation processes and gene expression in human PBMCs exposed to glyphosate, but the observed changes do not prejudge about the final metabolic effects, which are depended on many other factors.

Materials and methods

Chemicals; N-(phosphonomethyl)glycine (glyphosate) (purity 95 %), fetal bovine serum (FBS), penicillin – streptomycin, TRIzol™ and using primers were bought from Sigma-Aldrich, (USA). RPMI 1640 medium with L-glutamine and lymphocyte separation medium (LSM) (1.077 g/cm^3) were purchased in Cytogen (Germany). Invisorb Spin Tissue Mini Kit was bought in Stratec (Germany). Methylated DNA Quantification Kit was bought in Abcam (United Kingdom). Transcriptor First Strand cDNA Synthesis Kit and FastStart Essential DNA Green Master was purchased from Roche (Basel, Switzerland). EZ DNA Methylation™ Kit was bought from Zymo Research (USA). Methyl Primer Express®, v.1.0 was obtained from Life Technologies. TRIzol™ Reagent was purchased from Thermo Fischer Scientific, Waltham, MA, USA. Other chemicals were from Roth (Germany) and POCh (Poland) and were of analytical grade.

Cells isolation; PBMCs were isolated from leucocyte-buffy coat obtained from blood purchased in Blood Bank in Lodz, Poland. Blood was obtained from four healthy volunteers (aged 18–55), who showed no signs of infection disease symptoms at the time the blood samples were collected. The investigation was approved by the Bioethics Committee of the University of Lodz No. 1/KBBN-UŁ/II/2017. Cells isolation was determined. The final PBMCs density used in the experiments (after addition of glyphosate) was 1 × 10^6 cells/mL.

Cells treatment; Glyphosate was dissolved in PBS, pH 7.4. The concentrations of glyphosate were from 0.5 to 100 µM (0.085–17 mg/L). In a previous study, no changes in cell viability after treatment of PBMCs with glyphosate were observed at the above mentioned concentrations. The cells were incubated with investigated xenobiotic for 24 h in four independent experiments (four blood donors). During incubation, the cells were resuspended in RPMI supplemented with 10 % FBS and penicillin/streptomycin solution (50 U/mL and 50 µg/mL, respectively) at 37 °C, 5 % CO_2. After incubation, the cells were centrifuged, glyphosate was discarded, and the cells were resuspended in RPMI medium.

Methylation levels

Global DNA methylation; Genomic DNA from human PBMCs was isolated using Invisorb Spin Tissue Mini Kit (Stratec Molecular GmbH, Berlin, Germany). Global DNA methylation was determined by colorimetric measurement of 5-methylcytosine in DNA using Methylated DNA Quantification Kit (Abcam). For global DNA methylation analysis, 100 ng of genomic DNA was used, following the protocol provided by the manufacturer. Methylation levels were calculated relatively to the methylated control DNA

(included in the kit) and expressed as a percentage of total methylated DNA using the following formula:

$$5-\mathrm{mC}\% = \frac{(\text{Sample OD}-\text{Negative Control OD}) \div \mathrm{S})}{\text{Positive Control OD}-\text{Negative Control OD}) \times 2 \div \mathrm{P}} \times 100\%$$

where:

S – the amount of input sample DNA in ng; P – the amount of input positive control in ng; 2 – a factor to normalize 5-mC in the positive control to 100 %, as the positive control contains only 50 % of 5-mC.

Methylation at promoter regions of tumor suppressor genes (P16, P21, TP53) and proto-oncogenes (BCL2, CCND1); Chemical modification of 500 ng of genomic DNA was analysed using EZ DNA Methylation™ Kit (Zymo Research, Irvine, CA, US), according to manufacturer's instruction. For methylation analysis, methylation-specific real-time PCR assay (MSP-PCR) was conducted with FastStart SYBR Green Master (Roche, Basel Switzerland). Bioinformatic analysis of the potential methylation sites within the promoter regions of the proto-oncogens BCL2 and CCND1 as well as methylated and unmethylated primers were designed by utilizing of Methyl Primer Express®, v.1.0 (Life Technologies, Carlsbad, CA, US). The DNA sequences around the transcription start sites (from -1000 to 300 bp) of both genes, were obtained from the DBTSS. The primer list is presented in Supplementary Table 1. All samples were amplified in duplicate and in the presence of positive control (CpG methylated Jurkat genomic DNA, fully methylated), negative control (5-Azadc-treated Jurkat genomic DNA) (NEB, Ipswich, MA, US) and blank control (water). The methylation status of a particular gene is expressed as methylation index (MI):

$$\Delta\mathrm{Ct} = \mathrm{CtU} - \mathrm{CtM}$$

$$MI = \frac{1}{1 + 2^{-\Delta\mathrm{Ct}}} \times 100\%$$

where: MI - methylation index [%]; Ct values of the methylated gene (M) were compared with the Ct values of the unmethylated gene (U).

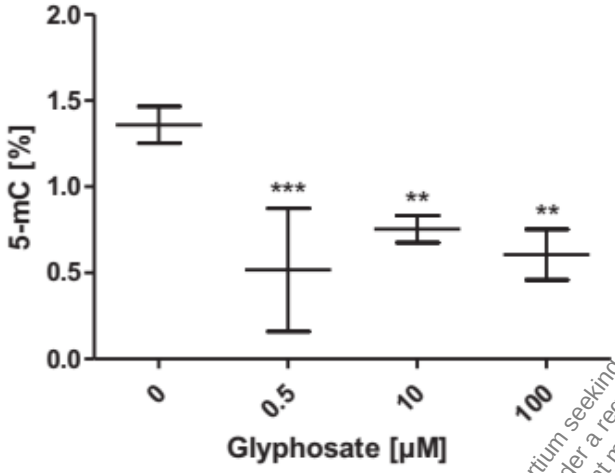
Gene expression: RNA was extracted with TRIzol™ Reagent (Thermo Fischer Scientific, Waltham, MA, USA). RNA samples with a 260/280 nm ratio in the range of 1.8–2.0 were used for further analysis. cDNA synthesis was performed using Transcriptor First Strand cDNA Synthesis Kit (Roche, Basel Switzerland). The cDNA was quantified by real-time PCR using FastStart SYBR Green Master (Roche, Basel Switzerland). Gene expression was normalized to the mean expression of all three housekeeping genes (GAPDH, RPL13, RPLP0) and was presented as a relative gene expression. The 2ΔCt (Ctgene–Ct mean from GAPDH, RPL13, RPLP0) method was used to calculate the expression levels of studied genes. The $2^{-\Delta Ct} \times 100$ values were re-calculated into relative copy number values.

Statistical analysis; The statistical analysis was performed with STATISTICA 13.1 data analysis software (2000 Stat-Soft, Inc., Tulsa, USA). Statistical analysis was conducted using the one-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparisons procedure. The difference was considered to be significant for $P < .05$. The individual analysis was performed on blood from four donors, while each experiment (conducted for blood from one donor) was repeated twice or three times depending on the method.

Results

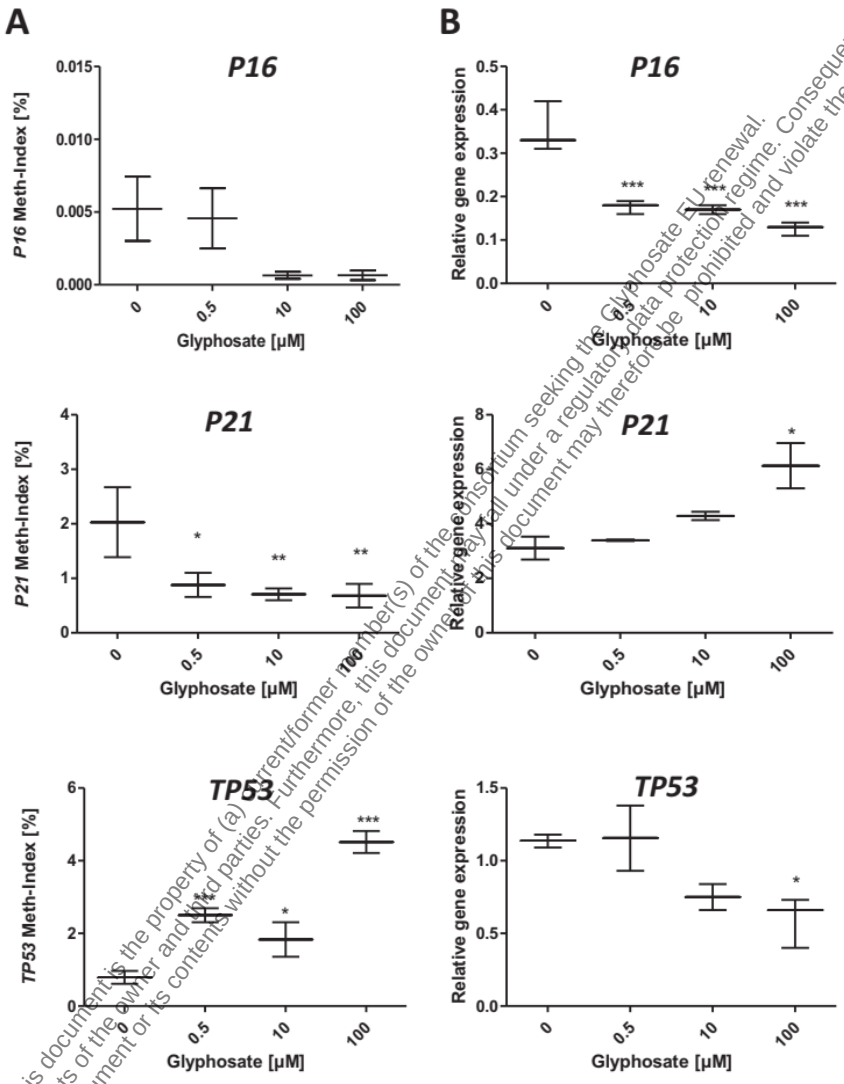
Analysis of global DNA methylation level; Statistically significant changes in 5-mC level were observed in PBMCs treated with glyphosate (see figure below). As compared to control cells, the level of global DNA methylation was significantly decreased after glyphosate treatment at all concentrations tested: 0.5 µM, 10 µM and 100 µM.

Figure 5.5.2-2: 5-methylcytosine (%) in human PBMCs incubated with glyphosate (0.5-100 µM) for 24 h. Mean ± SD was calculated for four individual experiments. Statistically different from control at **p < 0.01; * < 0.0001. Statistical analysis was conducted using one-way ANOVA and a posteriori Tukey test.**



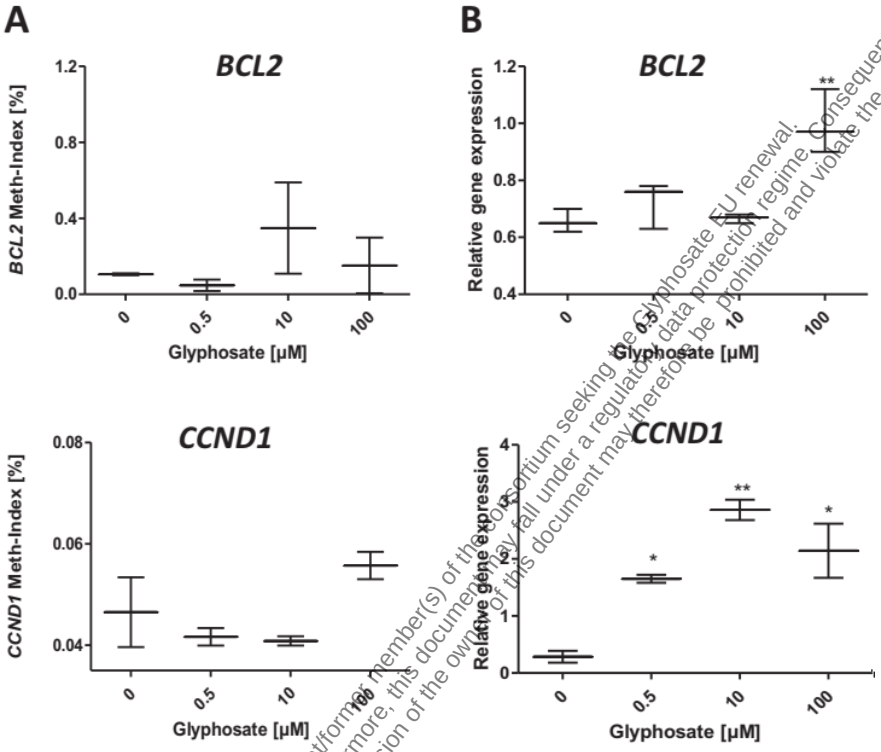
Analysis of methylation at promoter regions of the selected tumor suppressor genes (P16, P21, TP53) and proto-oncogenes (BCL2, CCND1); Statistically significant (p <.05, one-way ANOVA) decrease of methylation within P21 gene promoter was found in PMBCs treated with glyphosate from its lowest concentration of 0.5 µM (Figure 5.5.2-3A). The opposite response to the investigated xenobiotic (0.5 µM) such as hypermethylation of gene promoter, was observed on TP53 tumor suppressor gene (Figure 5.5.2-3A). In the case of other analyzed genes: P16, BCL2 and CCND1 we did not determine statistically significant changes in gene promoter methylation level (Figure 5.5.2-3A and Figure 5.5.2-4A).

Figure 5.5.2-3: Methylation (A) and expression (B) of suppressor genes (P16, P21 and TP53) in human PBMCs incubated with and glyphosate (0.5-100 µM) for 24 h. Mean ± SD was calculated for four individual experiments. Statistically different from control at *p < 0.05, **p < 0.01; *** < 0.0001. Statistical analysis was conducted using one-way ANOVA and a posteriori Tukey test.



Analysis of gene expression of the selected tumor suppressor genes (P16, P21, TP53) and proto-oncogenes (BCL2, CCND1): The analysis of methylation of the selected gene promoters using Real-Time PCR let us assess their expression at the transcript level. A statistically significant decrease of P16 expression in PBMCs treated with all concentrations of glyphosate were identified (Figure 5.5.2-3B). Glyphosate induced significant decrease in TP53 expression only at the highest concentration of 100 µM (p <.05, one-way ANOVA). Expression of other cell cycle drivers like P21 demonstrated a significant increase only at the highest concentration of glyphosate (p <.05, one-way ANOVA; Figure 5.5.2-4B), which was also effective in increasing of BCL2 expression (Figure 5.5.2-4B). Expression of the cyclin was significantly increased by two tested concentrations (10 µM and 100 µM) of glyphosate (p <.05, oneway ANOVA) (Figure 5.5.2-4B).

Figure 5.5.2-4: Methylation (A) and expression (B) of proto-oncogenes (BCL2 and CCND1) in human PBMCs incubated with and glyphosate (0.5-100 µM) for 24 h. Mean ± SD was calculated for four individual experiments. Statistically different from control at *p < 0.05, **p < 0.01. Statistical analysis was conducted using one-way ANOVA and a posteriori Tukey test.



Discussion

The knowledge that pollutants may influence the epigenome raises grave concerns concerning their long-term effects on chronic diseases development. A study showed that glyphosate can predispose breast cells to tumorigenesis *via* epigenetic reprogramming, which occur through TET3-mediated global and local DNA hypomethylation. Just recently, changes in the DNA methylation machinery due to glyphosate treatment were also identified in the fish model of Japanese medaka (Oryzias latipes), where upregulation of DNMT1, DNMT3a, Tet1 and Tet3 gene expression was shown. The current results have shown that treatment of PBMCs with glyphosate changed global DNA methylation profile. Decreased 5-mC level in PBMCs treated with low concentration of glyphosate that is comparable to that determined in human body after environmental exposure (0.5 µM) were found. Moreover, the obtained results are in agreement with previous findings, showing that glyphosate at high concentrations (determined in blood during glyphosate acute poisoning at 250–500 µM), reduces total DNA methylation in PBMCs. Recently, a study observed that glyphosate causes global DNA hypomethylation in non-neoplastic mammary epithelial MCF10A cells and triggers tumorigenesis in a "two-hit oncogenic model". Also a specific DNA hypomethylation signature of genes (i.e. local DNA hypomethylation), which was linked to the TET3 pathway that potentially can be used as an epimark to glyphosate exposure, was identified. Similar findings i.e. global hypomethylation of DNA were found in blood cells of tobacco farmers (n = 40) using pesticides and other xenobiotics during plant cultivation. However, literature data regarding potential epigenetic effects exerted by herbicides became contradictory. Recent epidemiological studies point to an increase of DNA methylation level under the combined effects of various pesticides, including glyphosate. It was found that long-term exposure of farmers (n = 137), employed at growing soybeans cultivation, to complex mixtures of pesticides, resulted in DNA hypermethylation and micronuclei formation in their blood cells probably due to impairment of

DNA repair mechanisms. Nevertheless, the farmers were exposed to the mixture of different pesticides and heavy metals that are known to induce DNA hypermethylation and to disturb the cell cycle progression. These findings were confirmed in a study on children living in an industrial areas. In blood samples of young subjects exposed to increased heavy metals and polycyclic aromatic hydrocarbons concentrations, DNA hypermethylation was observed. Detailed analysis of selected genes promoters involved in cellular metabolism regulation including cell cycle, i.e. TP53, P21, P16, CCND1 or BCL2, revealed significant changes in their methylation profiles. Beside global DNA hypomethylation we have found an increased methylation within the CpG islands of the TP53 gene promoter in PBMCs treated with glyphosate in all range of concentrations. The lowest concentration (0.5 μM) of glyphosate induced 5-mC methylation of the TP53 gene promoter. This result is in agreement with previous studies, where observed statistically significant increase of CpG islands methylation within the TP53 gene promoter after treatment of PBMCs with 250 μM and 500 μM glyphosate were identified. TP53 is known to be genome guard and tumor suppressor gene. It has been shown that hypermethylation of CpG islands within its promoter results in the silencing of gene transcription, but it is not clear-cut relationship in this case. Glyphosate reduced TP53 expression only from the concentration of 100 μM. Changes in TP53 expression and identified functions of p53 protein in the regulation of cell cycle directed us towards the findings of an author, who have shown that only Roundup but not glyphosate changed cell cycle of sea urchin embryonic cells due to delayed activation of the CDK1/cyclin B complex, acting downstream from p53. The effect of glyphosate on cell cycle was also excluded in the experiments on human lymphocytes, however the tested concentrations range of this compound was between 0.0125 and 0.5 μg/mL (0.07–2.9 μM), while the current study showed that glyphosate at 100 μM caused downregulation of TP53 expression in PBMCs. Beside hypermethylation of TP53 gene promoter and down regulation of its transcript level in PMBCs treated with glyphosate (100 μM), hypomethylation within P21 gene promoter was detected for all glyphosate concentrations. Changes in methylation of the P21 gene promoter were correlated with increased expression of the cell cycle regulator at 100 μM of glyphosate, that would suggest inhibition of PBMCs cycling in G0/G1 phase. Further analysis of molecular drivers of the cell cycle revealed a decreased expression of the P16 gene encoding the cell cycle inhibitor in glyphosate-treated cells, staring from its lowest concentration comparable to that occurring during environmental exposure. However, the analysis carried out did not show any changes in the methylation level within the P16 gene promoter region in PBMCs due to glyphosate treatment. These results correlate with previous findings, in which higher concentrations of glyphosate (250 μM and 500 μM) also did not cause any changes in methylation of P16 gene promoter. The protein product of P16 gene, p16, is an inhibitor of cyclin D. Literature data suggests that low expression of P16 may result in overexpression of cyclin D1 (CCND1), activation of its complexes with CDK4 and CDK6 complexes (cyclin D1/CDK4, cyclinD1/CDK6) and overcoming the G0/G1 checkpoint of the cell cycle. In the tested experimental conditions, an increased expression of the CCND1 due to glyphosate treatment at its whole tested range of the concentrations (0.5–100 μM) was identified, but no statistically significant changes in the methylation level of the gene promoter has been shown. Beside of changes in the expression level of the major cell cycle regulators and/or methylation pattern within their gene promoters, significant upregulation of the BCL2 expression at the mRNA level at the highest tested concentration of glyphosate (100 μM) was also identified. Therefore, this data may suggest a disturbance of apoptosis induction, due to sustained antiapoptotic abilities of BCL2. In contrast, *in vitro* studies on the effect of glyphosate on apoptosis in mature rat testicular cells and PBMCs showed that glyphosate only at very high concentrations (29.57 mM/0.5 mM, in rat testicular cells and PBMCs; respectively) induced apoptosis. Thus, the obtained data may not result in specific metabolic effects until the exposure to high doses or cumulative long-term exposure of humans to glyphosate will be preceded.

Conclusion

This study has attempted to assess epigenetic mechanisms of action of glyphosate in human PBMCs, which has been poorly studied in cellular models including blood cells. The conducted analysis have shown that glyphosate significantly affected global DNA methylation of PBMCs as well as methylation in the promoter regions of selected tumor suppressors (P21 and TP53) as well as expression of major cell cycle and apoptosis drivers (P16, TP53, BCl2, CCND1 and P21). Changes in the DNA methylation profile were minimally correlated with gene expression level, however, regulation of transcription process is performed at many different levels and further and more global analysis (genome-wide based on) are necessary to give

clear answer about epigenetic-transcriptomic changes induced by glyphosate. It should be noted that glyphosate induced changes in concentrations corresponding to environmental or occupational exposure. Conducting of further *in vitro* studies on various epigenetic modifications caused by glyphosate with different target cell cultures is still warranted.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The objective was to assess epigenetic mechanisms of action of glyphosate in human PBMCs, which has been poorly studied in cellular models including blood cells. The study was conducted using an *in vitro* test system. The ability of glyphosate to impact the measured parameters was demonstrated (global DNA methylation of PBMCs, methylation in the promoter regions of selected tumor suppressors (P21 and TP53), and expression of major cell cycle and apoptosis drivers (P16, TP53, BCl2, CCND1 and P21). However, a positive control was not used, and a clear dose-response was not established for all of the measured parameters. Additionally, the measured effects *in vitro* are not clearly linked to an adverse outcome *in vivo*. While it is stated that the concentrations used are comparable to environmental exposure, external exposure was not linked to a corresponding internal concentration. Therefore, it is not possible to calculate a dose for risk assessment purposes. The study is useful for supplemental information on *in vitro* effects resulting from glyphosate exposure, but, is not appropriate for derivation of an endpoint in human health risk assessment.

This publication is considered relevant and reliable without restrictions because the assays conducted comply in general with the quality criteria for *in vitro* testing.

Assessment and conclusion by RMS:

Reliability criteria for *in vitro* toxicology studies

Publication: Wozniak *et al.*, 2020	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity of 95 %. Source: Sigma-Aldrich, USA.
Only glyphosate acid or one of its salts is the tested substance	Y	
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	Y	PBMC.
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	N	
Test concentrations in physiologically acceptable range (< 1 mM)	Y	0.5 to 100 µM
Cytotoxicity tests reported	Y	Ref. to earlier paper.
Positive and negative controls	Y	Methylated control DNA as positive control.

Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions	Y	
Reliable with restrictions		
Not reliable		
This publication is considered relevant and reliable without restrictions because the assays conducted comply in general with the quality criteria for *in vitro* testing.		

1. Information on the study

Data point:	CA 5.5/029
Report author	Biserni, M. *et al.*
Report year	2019
Report title	Quizalofop-p-ethyl induces adipogenesis in 3T3-L1 Adipocytes
Document No	doi: 10.1093/toxsci/kfz097 ISSN: 1096-0929
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes/Reliable without restrictions

2. Full summary of the study according to OECD format

In this study glyphosate, among other pesticide active substances, was investigated for its effect on lipid accumulation in differentiated adipocytes *in vitro* at concentrations ranging from 0.1 to 1000 µM. The results indicated that at the concentrations tested glyphosate scored negative for lipid accumulation.

Materials and methods

Chemicals – Glyphosate (purity ≥ 96 %) purchased from Sigma-Aldrich, Gillingham, UK. Stock solutions of glyphosate were prepared in serum-free medium and adjusted to pH 7.2.

Cell culture - The murine fibroblast 3T3-L1 cell line was purchased from ZenBio (Cambridge Bioscience, Cambridge, UK) and was not used past passage 10. Undifferentiated 3T3-L1 cells were grown at 37 °C under 5 % CO_2 in a maintenance medium composed of phenol red free Dulbecco's Modified Eagle Medium (DMEM), 10 % newborn calf serum, 2 mM glutamine and 10 µg/mL penicillin/streptomycin. Cells were released from the flask substratum using 0.05 % trypsin-EDTA and counted using a hemocytometer prior to seeding.

Adipocyte differentiation - For the differentiation of murine 3T3-L1 cell cultures to adipocytes, cells were seeded into 96-well plates at a density of 20,000 cells per well in 100 µL maintenance medium. Following a 2-day stabilization period, cells were switched to differentiation medium consisting of DMEM, 2 mM glutamine, 10 µg/mL penicillin/streptomycin, 10 % fetal bovine calf serum, 500 µM 3-isobutyl-1-methyl-xanthine and 100 nM insulin from bovine pancreas. After a further 2 days of culture, the medium was refreshed to start the incubation with concentrations of glyphosate ranging from 0.1 to 1000 µM.

Dexamethasone was used as a positive control. Media were replenished every 2 days for a further 6 days. Lipid accumulation was visualized on day 8 using fluorescent Nile Red staining in accordance with the manufacturer's instructions and quantified using a microplate reader. The fluorescence was measured with a filter giving an excitation at 490 nm and emission at 510-570 nm. The adipogenic effect was expressed as a fold change in emission signal intensity between untreated differentiated and treated differentiated 3T3-L1 cells.

Cell viability assay - Cell viability was assessed using a colorimetric 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay, which indirectly measures cell number by testing for activity of mitochondrial succinate dehydrogenase. The 3T3-L1 cells were seeded into 96-well plates and differentiated as described above for 8 days. Cells were then incubated with 100 mL of MTT solution (1 mg/mL) for 2 hours and the test terminated by adding 100 µL DMSO. As a measure of cell number, the optical density of the cell lysate was determined at 570 nm using a GloMax Multi Microplate Multimode Reader. The number of cells was directly proportional to the intensity of the signal. Cell viability was expressed as a percentage of the control samples.

Intracellular lipid staining - The 3T3-L1 cells were seeded into 96-well clear bottom black tissue culture treated plates and differentiated as described above for 8 days. Medium was then removed and cells fixed by addition of 100 µL 4 % paraformaldehyde. Cells were then stained for intracellular lipid accumulation by adding 50 µL of 1 mg/mL Nile Red and 1 µg/mL 4',6-diamidino-2-phenylindole (DAPI) in 0.2 % Triton X-100-PBS for 15 minutes in the dark. Nile Red staining for lipid droplets and DAPI staining for cell nuclei were imaged at 530 and 405 nm, respectively, using fluorescence imaging on a Nikon Eclipse Ts2 microscope at 40 x.

Statistical analysis - The statistical analysis of the dose-response results from the adipogenesis assay was performed by ANOVA. Pair-wise comparisons were made using a Mann-Whitney test. Nonlinear regression analysis was performed using 5-parameter logistic dose-response curve models. These statistical analyses were performed using GraphPad Prism version 7.00 for MAC OS X.

Results

With dexamethasone as the positive control the adipogenic assay using murine 3T3-L1 cells to undergo differentiation to adipocytes was shown to be a sensitive assay system causing a maximum of 21-fold increase in lipid accumulation when compared to untreated differentiated cells. The dose response relationship of dexamethasone was used to determine the concentration that caused a 50 % response (EC_{50}). The EC_{50} for dexamethasone was 9.4 pM and some of the tested compounds showed an effect on lipid accumulation with different dose response patterns. Treatment with glyphosate scored negative.

Discussion

Commonly used herbicide active substances were tested in an adipogenesis assay to evaluate their obesogenic potential. Using the 3T3-L1 cell assay system, which measures lipid accumulation following differentiation to adipocytes, glyphosate scored negative at all concentrations tested. This study uses the well-established murine 3T3-L1 cell line model system for obesogenic screening. However, this cell line can only address a limited number of possible modes of action. The 3T3-L1 cells consist of unipotent pre-adipocytes, which can only differentiate into mature adipocytes. Another important limitation of 3T3-L1 cells is that they are of murine origin, and may not fully be representative of human metabolism. In addition, stocks of 3T3-L1 cells from different sources can have different metabolic capabilities.

Conclusion

Amongst the pesticide active substances tested for effects on lipid accumulation in differentiated adipocytes glyphosate scored negative.

3. Assessment and conclusion

Assessment and conclusion by applicant:
In this study glyphosate, among other pesticide active substances, were investigated for their effect on lipid accumulation in differentiated adipocytes *in vitro* at concentrations ranging from 0.1 to 1000 µM. The results indicated that at the concentrations tested, glyphosate scored negative for lipid accumulation. This publication is considered relevant for the risk assessment of glyphosate and reliable without restrictions.

Assessment and conclusion by RMS:

Reliability criteria for *in vitro* toxicology studies

Publication: Biserni *et al*., 2019	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity of ≥ 96 %. Source: Sigma-Aldrich, Gillingham, UK
Only glyphosate acid or one of its salts is the tested substance	N	Other pesticide active substances were tested as well.
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	Y	
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	NA	
Test concentrations in physiologically acceptable range (< 1 mM)	Y	Concentration range *in vitro* from 0.1 to 1000 µM.
Cytotoxicity tests reported	Y	
Positive and negative controls	Y	
Complete reporting of effects observed	Y	Glyphosate was not tested in all tests described.
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	Y	No effect of glyphosate over the entire concentration range tested.
Overall assessment		
Reliable without restrictions	Y	

Reliable with restrictions		
Reliability not assignable		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate and reliable without restrictions.		

1. Information on the study

Data point:	CA 5.5/030
Report author	Crump, K.
Report year	2019
Report title	The potential effects of recall bias and selection bias on the epidemiological evidence for the carcinogenicity
Document No	DOI: 10.1111/risa.13440 E-ISSN: 1539-6924
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Yes/Reliable

2. Full summary of the study according to OECD format

The principal human data for glyphosate and non-Hodgkin's lymphoma (NHL) come from five case–control studies and two (related) cohort studies. The case–control studies are at risk of recall bias resulting from information on exposure to pesticides being collected from cases and controls based on their memories; cases being deemed likely by textbook authors to have a greater motivation than controls for remembering or reporting past exposures. In addition, two of the case–control studies are additionally at risk of a form of selection bias that can exacerbate the effect of recall bias. Both biases are in the direction of making glyphosate appear carcinogenic (viz. causing odds ratios (ORs) to be >1 in the absence of a true causal relationship). If ORs are not biased and a pesticide plays no role in causing NHL, the probability that an OR for that pesticide is greater than 1.0 is approximately 0.5. The fractions of ORs for pesticides other than glyphosate that are greater than 1.0 in the five case–control studies are 0.90 (n = 92), 0.90 (n = 152), 0.93 (n = 59), 0.76 (n = 140), and 0.53 (n = 54), the first two from studies that are at risk for both types of bias. In the two cohort studies, which are not subject to these biases, the comparable fractions for relative risks for all cancers are 0.51 (n = 70) and 0.48 (n = 158). Thus, this analysis provides evidence that at least four of the five case–control studies of glyphosate exposure and NHL are contaminated by statistical bias, likely stemming in the main from recall bias, exacerbated by selection bias in two of the studies. This suggests that these case control studies of glyphosate are not reliable evidence for a relationship between glyphosate and NHL.

Materials and methods

Two of the case–control studies exclude from some analyses of glyphosate (as well as analyses of other pesticides) the unexposed cases and controls who report exposures to pesticides not the subject of the analysis, which raises the possibility of selection bias if recall bias is also operating. The potential effect of selection bias in the presence of recall bias on ORs is illustrated by simulating sets of case–control data in which increasing amounts of recall bias are introduced, and the effects of adding selection bias to these analyses are noted. Each simulation involves 500 cases and 1,500 controls. Controls are randomly assigned exposure to pesticides according to the percentages of controls reporting exposure to nine pesticides reported by McDuffie *et al.* (2001) (Table II). Cases are randomly assigned exposures in the same way

except the exposed percentages are multiplied by increasing factors in different simulations, which introduces increasing amounts of recall bias. In each of six simulations, 10,000 sets of data are simulated and the 10,000 simulated ORs for glyphosate are averaged, (1): using all data so that recall bias but not selection bias is present and (2): after removing from the unexposed group both cases and controls that are exposed to any pesticide other than glyphosate (so that both recall bias and selection bias are present). In interpreting this simulation study, it needs to be understood that the McDuffie data and glyphosate are used only to make the simulations more realistic, and that the simulations say nothing about the McDuffie study or about the risk from glyphosate exposure, as any such data or a herbicide other than glyphosate could have been used to illustrate the same points. The likelihood that statistical bias (from any source) may be responsible for the elevated ORs for glyphosate is evaluated by tabulating ORs and RRs from the studies and cross-classifying them by pesticide groups and the fraction that exceed 1.0. If all types of pesticides have an elevated percentage of ORs greater than 1.0, this suggests that bias may be the cause of the elevations rather than any carcinogenic effect of the pesticides. The ORs and RRs included in the tabulations were selected from the original papers according to the following rules:

(1) In some instances, the exact same OR calculation is reported in two or more separate tables (e.g. the category "herbicide" in tables I, II, and V of Hardell *et al*., 2002). Only one of the identical calculations is tabulated.

(2) Similarly, some tables contain two sets of OR or RR calculations for testing the same hypotheses, but using different statistical methods (e.g. controlling for different sets of potential confounders). In these instances, only one set of calculations is tabulated, namely the set of ORs or RRs whose method of calculation agrees most closely with methods used in in the remainder of the article or in other articles. For example, De Roos *et al*. (2003) reported ORs calculated using both logistic regression and hierarchical regression. The ORs computed using logistic regression were selected for tabulation because this was the only study that employed hierarchical regression and logistic regression was the most common method used in the remaining studies.

(3) In De Roos *et al*. (2003), the category "potentially carcinogenic pesticides" apparently was formed post hoc and included those pesticides that gave greatest evidence of a carcinogenic effect in initial analyses. Such an approach would almost guarantee an OR greater than 1. In fact, the three ORs from this category were all greater than 1. ORs from this analysis were not included in order to avoid biasing the tabulation. (This does not imply that De Roos *et al*. erred in computing these ORs, only that they were not suitable for inclusion in our analysis.)

(4) Otherwise, all OR or RR calculations reported in the publications were tabulated. A complete listing of the ORs from each study contained in the tabulation is provided in the Supporting Information. The results of these tabulations are summarized in graphs and in tabular form.

Results
Specific results part not given in this article. Results and discussion are merged.

Discussion
Results of the simulation exercise to demonstrate the effect of selection bias are shown in Table I. The first row in the table verifies that, as expected, selection bias does not affect the expected OR in the absence of recall bias. The remaining rows assume increasing amounts of recall bias as indicated in the first column. The effect of that recall bias on the expected ORs for glyphosate are shown in the second column. The third column shows the expected ORs when selection bias is added to the recall bias present by removing from the unexposed (to glyphosate) groups cases and controls exposed to any herbicide other than glyphosate, just as was done in Hardell *et al*. (2002) and Eriksson *et al*. (2008).

Table I. Results of Simulations[a] to Demonstrate the Effect of Recall Bias[b] Alone and with Selection Bias[c] on Average OR

Recall Bias[b]	Average OR for Glyphosate with Recall Bias Only	Average OR for Glyphosate with Both Recall Bias and Selection Bias
0	1.01	1.01
0.05	1.07	1.10
0.1	1.12	1.18
0.3	1.35	1.59
0.5	1.59	2.10
1	2.24	3.95

[a]10,000 simulations, 500 cases, 1,500 controls, each row. Herbicides assumed present and the percentage of controls assumed to report exposure: (glyphosate, 8%), (2,4-D, 19%), (mecoprop, 5.4%), (MCPA, 3.1%), (diclofopmethyl, 1.7%), (thiocarbamates, 3.3%), (bromoxynil, 3.2%), (dicamba, 8.7%), (dinitroaniline, 2.1%). Thus, for example, the probability that a particular control claimed exposure to MCPA was 0.031. Exposures to different herbicides are assumed to occur independently.

[b]Recall bias is introduced into each simulation by computing the prevalence of each herbicide exposure among cases as [prevalence among controls] × [1+ Recall bias].

[c]Selection bias is introduced by eliminating from OR calculations both unexposed (to glyphosate) cases and controls exposed to herbicides other than glyphosate.

Crump notes that the effect of selection bias increases with the increase in recall bias. This simulation demonstrates that selection bias can cause ORs to be inflated by important amounts above that due solely to recall bias when recall bias is also present. Fig. 1 shows plots of the tabulated ORs or RRs by study, with the pesticide groupings upon which the ORs are based (including ORs derived for both individual pesticides and groups of pesticides), classified as to fungicides, herbicides not containing glyphosate, impregnating agents, insecticides, and pesticide groupings that include glyphosate. The individual pesticides and groupings for each study are listed in the footnote to Table II. The tabulated RRs from the two cohort studies are similarly classified into cancer groupings not containing NHL and groupings containing NHL. Since the logarithms of ORs are plotted, the focus is on the proportion of log-transformed ORs that are greater than 0.0 (equal to the proportion of untransformed ORs greater than 1.0). These figures show that ORs in McDuffie *et al*. (2001), Hardell *et al*. (2002), and Eriksson *et al*. (2008) are nearly all greater than 1.0.

Table II. Counts of ORs and RRs Categorized by Study, Pesticide, and Whether Greater than 1.0

Case–Control Studies	Fungicides		Herbicides (No Glyphosate)		Impregnating Agents		Insecticides		Glyphosate-Containing		Glyphosate Only		Totals (No Glyphosate)		Totals (Incl. Glyphosate)	
	N	% > 1	*N*	% > 1	*N*	% > 1	*N*	% > 1	*N*	% > 1	*N*	% > 1	*N*	% > 1	*N*	% > 1
Hardell et al. (2002)[a]			31	93.5%	42	84.5%	19	94.7%	13	96.2%	1	100.0%	92	89.7%	106	90.6%
Eriksson et al. (2008)[b]	10	100.0%	44	92.0%	43	86.0%	55	89.1%	22	100.0%	11	100.0%	152	89.8%	185	91.6%
McDuffie et al. (2001)[c]	18	83.3%	19	94.7%			22	100.0%	6	100.0%	3	83.3%	59	93.2%	68	93.4%
Orsi et al. (2009)[d]	27	83.3%	36	72.2%			77	75.3%	27	66.7%	9	66.7%	145	76.1%	176	74.1%
De Roos et al. (2003)[e]			20	47.5%			34	55.9%	6	33.3%	1	100.0%	54	52.8%	61	51.6%
Totals	55	86.4%	150	82.0%	85	85.3%	207	80.2%	74	81.8%	25	88.0%	497	82.3%	596	82.4%

Cohort Studies	No NHL		NHL + Subtypes		Totals	
	N	% > 0	*N*	% > 0	*N*	% > 0
De Roos et al. (2005)[f]	55	53.6%	15	43.3%	70	51.4%
Andreotti et al. (2018)[g]	86	51.2%	72	43.1%	158	47.5%
Totals	141	52.1%	87	43.1%	228	48.7%

[a]ORs for NHL and hairy cell leukemia computed for herbicides; phenoxyacetic acids; MCPA; 2,4,5-T + 2,4-D; glyphosate; other herbicides; insecticides; DDT; mercurial seed dressing; pyrethrins; fungicides; impregnating agents; chlorophenols; pentachlorophenol; arsenic; creosote; other impregnating agents; organic solvents.

[b]ORs for NHL, including subtypes, as well as several subcategories of NHL were computed for herbicides, total; phenoxyacetic acids; MCPA; 2,4,5-T and/or 2,4-D; other phenoxyacetic acids; herbicides except phenoxyacetic acids; glyphosate; other herbicides; insecticides, total; DDT; mercurial seed dressing; pyretrine; permetrine; other insecticides; fungicides; impregnating agents; chlorophenols; arsenic; creosote; tar; other impregnating agents; rodenticides.

[c]ORs for NHL computed for phenoxyherbicides; 2,4-D; mecoprop; MCPA; diclofopmethyl; phosphonic acid; glyphosate (Roundup); thiocarbamates; diallate; phenols: bromoxynil; dicamba; dicamba (Banvel or Target); dinitroaniline; trifluralin; carbamates; carbaryl; carbofuran; methomyl; organochlorine; chlordane; lindane; aldrin; organochlorine diphenylchlorides; DDT; organophosphorus; malathion; dimethoate; diazinon; amide; captan; phaval; aldehyde; formaldehyde; mercury containing; mercury dust; mercury liquid; sulfur compounds.

[d]ORs for NHL, including subtypes, and other categories of lymphoid neoplasms computed for occupational pesticide use, insecticides, organochlorine, organophosphate, pyrethrin, fungicides, carbamates, imide, triazole, herbicides, phenoline, phenoxy, picoline, triazine, amide, urea, quaternary ammonium, glyphosate, garden pesticide use, insecticides, fungicides, herbicides, domestic insecticide use.

[e]ORs for NHL computed for aldrin; bufencarb; carbaryl; carbofuran; chlordane; copper acetoarsenite; coumaphos; DDT; diazinon; dichlorvos; dieldrin; dimethoate; ethoprop; famphur; fly, lice, or tick spray; fonofos; heptachlor; lead; arsenate; lindane; malathion; methoxychlor; nicotine; phorate; pyrethrins; rotenone; tetrachlorvinphos; toxaphene; terbufos; alachlor; atrazine; bentazon; butylate; chloramben; cyanazine; 2,4-D; dicamba; EPTC (protectant; glyphosate; linuron; MCPA; metolachlor; metribuzen; paraquat; propachlor; sodium; chlorate; 2,4,5-T; trifluralin; any pesticide; any insecticide; any herbicide; chlordane and DDT; carbofuran and atrazine; diazinon and atrazine; alachlor and atrazine; atrazine and dicamba.

[f]RRs computed for glyphosate exposure in relation to the following cancers: all cancer, lung, oral cavity, rectum, pancreas, kidney, bladder, prostate, melanoma, lymphohematopoietic cancer, NHL, lukemia, and multiple myeloma.

[g]RRs computed for glyphosate exposure in relation to the following cancers: all cancer; oral cavity; colon; rectum; pancreas; lung; melanoma; prostate; testicular; bladder; kidney; lymphohematopoietic; Hodgkin lymphoma; non-Hodgkin lymphoma; non-Hodgkin lymphoma B cell; chronic lymphocytic lymphoma or small lymphocytic leukemia; diffuse large B cell lymphoma; marginal-zone lymphoma; follicular lymphoma; multiple myeloma and non-Hodgkin lymphoma T cell.

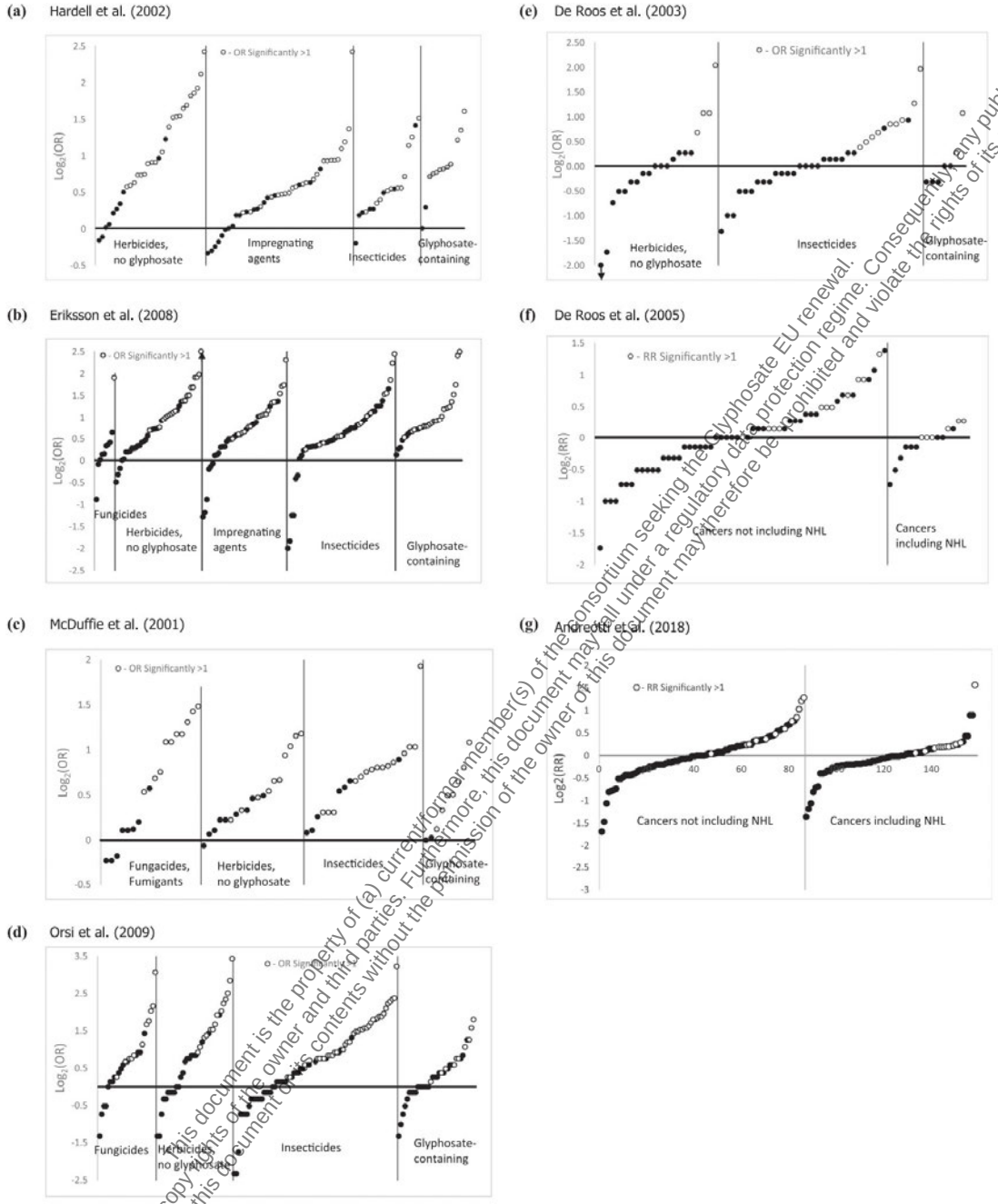


Fig. 1. Graphs of tabulated ORs and RRs. (a) Hardell et al. (2002), (b) Eriksson et al. (2008), (c) McDuffie et al. (2001), (d) Orsi et al. (2009), (e) De Roos et al. (2003), (f) De Roos et al. (2005), (g) Andreotti et al. (2018).

Also, there is an excess of ORs greater than 1.0 in Orsi *et al*. (2009). These excesses of ORs greater than 1.0 occur in all categories of pesticides considered in these studies. On the other hand, there appears to be roughly a balance between the numbers of ORs greater than and less than 1.0 in all categories of pesticides in the case–control study of De Roos *et al*. (2003). Similarly, in both cohort studies (Andreotti *et al*., 2018; De Roos *et al*., 2005), there seems to be roughly an equal number of RRs greater than and less than 1.0, both for cancer groupings that include NHL and those that do not. These graphs also display those ORs and RRs that are statistically significantly greater than 1.0, with statistical significance defined by the lower bound on the 95 % confidence interval being greater than or equal to 0.8 (apparently the decision rule used in De Roos *et al*., 2003 to define "potentially carcinogenic pesticides.") This shows that many statistically

significantly elevated ORs occur in every pesticide category with little notable difference between categories that contain glyphosate and those that do not. Table II, which summarizes the tabulated ORs and RRs, provides confirmation of the impressions obtained from the graphs. In this table OR and RR reported in the original papers as equal to 1.0 (which are mostly due to roundoff in reported values) each contribute 0.5 to the counts of ORs and RRs greater than 1.0. In the case–control studies of Hardell *et al.* (2002), Eriksson *et al*. (2008), and McDuffie *et al*. (2001), 90 % or more of all ORs from pesticide groups not containing glyphosate are greater than 1.0. In these three studies, the percentage of ORs greater than 1.0 exceeds 80 % in all pesticide groupings (fungicides, herbicides not including glyphosate, impregnating agents, and insecticides, as well as groupings that contain glyphosate). The percentages of ORs from Orsi *et al*. (2009) that exceed 1.0 are also elevated, although not to the same extent as in the other three studies. By contrast, the percentages of ORs from the case–control study of De Roos *et al.* (2003) that are greater than 1.0 are all fairly close to 50 % in all pesticide categories (52.8 % in categories combined than do not include glyphosate and 51.6 % if glyphosate-containing categories are included). Thus, of the five case control studies, the study of De Roos *et al*. (2003) presents considerably less evidence of recall bias resulting from an excess of ORs greater than 1.0. In the two cohort studies, the percentages of RRs greater than 1.0 in cancer groupings not containing NHL in both studies are 54 %, and overall, with NHL included, are 49.5 % (Table II). Thus, these results from the two cohort studies, which are not subject to recall bias or selection bias, are reasonably consistent with what would be expected if these studies are free of statistical bias and glyphosate has no effect upon cancer rates. If the ORs are not biased, the results in Table II pertaining to the case–control studies suggest that all types of pesticides investigated in these studies are having a role in causing NHL, including fungicides, herbicides other than glyphosate, impregnating agents and insecticides. It should also be kept in mind that the category NHL contains many types of lymphoma, not all of which are likely to share common risk factors. Thus, it seems unlikely (at least to this investigator) that pesticides within each of these types of pesticides would be causing NHL, and particularly to an extent to be responsible for the evidence seen in Table II. It seems much more likely that the preponderance of ORs greater than 1.0 seen in all pesticide categories in most of the case–control studies is simply the result of recall bias, which is a well-known problem with these types of case–control studies, possibly augmented in two studies by selection bias. Such a conclusion is further supported by the fact that in the two cohort studies, which are not subject to these biases, the overall percentage of RRs greater than 1.0 is 49.6 % that is in excellent agreement with the theoretical value of 0.5, assuming no bias and no effect of glyphosate on any cancer. Given this evidence, one could reasonably conclude that at least four of the case–control studies of glyphosate and NHL are contaminated by statistical bias, and consequently are not suitable for reaching conclusions about the potential ability of glyphosate to cause NHL.The potential for case–control studies to be affected by recall bias is well known and has been discussed in many publications. The potential for the case–control studies of glyphosate, in particular, to be subject to recall bias, along with concerns about glyphosate studies not controlling for exposure to farm animals, and for the use of proxy respondents were discussed previously. These same issues were raised by some panelists in an EPA FIFRA scientific advisory panel.

Conclusion

In summary, the potential for these types of case–control studies to be contaminated by bias from the use of exposure information based on the memories of both cases and controls (recall bias) is well known. This article provides evidence that at least four of the five case–control studies of glyphosate exposure and NHL are contaminated by statistical bias, likely stemming in the main from recall bias, exacerbated by selection bias in two of the studies. This suggests that the case control studies of glyphosate are not reliable for determining whether glyphosate is carcinogenic. However, the two cohort studies (Andreotti *et al*., 2018; De Roos *et al*., 2005) do not present evidence of bias. If further study of the potential relationship between glyphosate exposure and NHL is needed, it would best come from cohort or other studies that are not at risk of recall bias resulting from quantifying exposures by questioning subjects. Of course, cohort studies have other potential problems that must be evaluated, including incomplete follow-up, the healthy worker effect and poor information on exposures. The potential for recall bias identified herein could affect, not just case–control studies of the potential carcinogenicity of glyphosate, but any such study that involves quantifying exposures occurring in the distant past based on participant’s memories.

3. Assessment and conclusion

Assessment and conclusion by applicant:

It is well known that recall bias is a potentially important bias in cancer case control studies where study participants are asked to recall their past exposures. In an ideal study, information about exposures for cases and controls would be collected under exactly the same circumstances. However, circumstances are quite different for cases and controls. Cancer cases have suffered a grievous illness and it is only natural for them to be deeply introspective about what might have caused their cancers. Controls have no such motivation that would augment their recall (or reporting). So, the concern expressed in many textbooks is that recall bias tends to produce false positive results. The purpose of this analysis by Crump was to evaluate the evidence for recall bias in the overall pattern of results in five case control studies and two cohort studies that comprise the main part of the glyphosate-NHL literature.

In evaluating the case control studies, Crump reasoned that the percentage of odds ratios > 1 for non-glyphosate exposures should be approximately 50 % if recall bias was not operative and those exposures did not cause NHL. Yet, it turned out that the percentages of ORs >1 for non-glyphosate exposures were 90 % for Hardell *et al*. (2002), 90 % for Erikson *et al*. (2008), 93 % for McDuffie *et al*. (2001), 76 % for Orsi *et al*. (2009), and 53 % for DeRoos *et al*. (2003). These extreme departures from 50 % for 4 of the 5 case control studies is consistent with recall bias, perhaps augmented by a type of selection bias in the analyses by Hardell *et al*. (2002) and Eriksson *et al*. (2008). In contrast, in the most recent publication from the Agricultural Health Study (Andreotti *et al*. 2018), only 48 % of the relative risks (RR) calculated were >1 – a percentage in the range expected with a true probability of 50 %. While the evaluation of Andreotti *et al*. (2018) concerned glyphosate and other cancer sites and not other exposures and NHL, the principle is the same: under the null hypothesis the proportion of ORs or RRs > 1 should be roughly 50 % absent bias.

We agree with Crump's conclusion that the 4 case-control studies with a high proportion of ORs > 1 are "contaminated" by statistical bias and are not reliable as evidence of a relationship between glyphosate and NHL. Of course, there are also other types of bias that may contribute to the high proportion of positive ORs (e.g. lack of control for confounding, lower participation for controls than cases (traditional selection bias), proxy respondents, etc.) (see Acquavella *et al*. 2016). Nonetheless, Crump's point is well taken that ORs for glyphosate in 4 of the 5 case control studies should be interpreted as unreliable because the vast majority of ORs for other exposures are >1.

Assessment and conclusion by RMS:

Reliability Criteria: Epidemiology studies

Publication: Crump K., 2019	**Criteria met? Y/N/?**	**Comments**
Study Design		
Adequate study design given study objectives	Yes	For a methodologic evaluation of recall bias in existing studies
Appropriate study population to address potential glyphosate-related health outcomes	Not applicable	

Publication: Crump K., 2019	**Criteria met? Y/N/?**	**Comments**
Exposure studied		
Exposure to formulations with glyphosate as a.s.	Yes	
Exposure to formulations with other a.s.	Yes	
Exposure to other farm exposures	Yes	
Study Conduct/analysis		
Adequate description of study population	Not applicable	
Adequate description of exposure circumstances	Not applicable	
Comparable participation by groups being compared	Not applicable	
Information provided by proxy respondents	Not applicable	
Adequate statistical analysis	Yes	To illustrate bias
Adequate consideration of personal confounding factors	Not applicable	
Adequate consideration of potentially confounding exposures	Not applicable	
Overall assessment		
Reliable without restrictions	Yes	As methodologic work. Clearly illustrates recall bias in the glyphosate case control studies.
Reliable with restrictions	No	
Not reliable	No	

1. Information on the study

Data point:	CA 5.5/031
Report author	Duforestel, M. *et al.*
Report year	2019
Report title	Glyphosate primes mammary cells for tumorigenesis by reprogramming the epigenome in a TET3-dependent manner
Document No	doi: 10.3389/fgene.2019.00885 ISSN: 1664-8021
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary of the study according to OECD format

The herbicide glyphosate has been scrutinized for an impact on cancer incidence, but reports demonstrate the difficulty of linking estimates of exposure and response analysis. An approach to better apprehend a potential risk impact for cancer is to follow a synergistic approach, as cancer rarely occurs in response to one risk factor. The known influence of glyphosate on estrogen-regulated pathway makes it a logical target of investigation in breast cancer research. In this study, non-neoplastic MCF10A cells in a repeated glyphosate exposure pattern over 21 days were used. Glyphosate triggered a significant reduction in DNA methylation, as shown by the level of 5-methylcytosine DNA; however, in contrast to strong demethylating agent and cancer promoter UP peptide, glyphosate-treated cells did not lead to tumor development. Whereas UP acts through a DNMT1/PCNA/UHRF1 pathway, glyphosate triggered increased activity of ten-eleven translocation (TET)3. Combining glyphosate with enhanced expression of microRNA (miR) 182-5p associated with breast cancer induced tumor development in 50 % of mice. Culture of primary cells from resected tumors revealed a luminal B (ER+/PR-/HER2-) phenotype in response to glyphosate-miR182-5p exposure with sensitivity to tamoxifen and invasive and migratory potentials. Tumor development could be prevented either by specifically inhibiting miR 182-5p or by treating glyphosate-miR 182-5p-cells with dimethyloxallyl glycine, an inhibitor of TET pathway. Looking for potential epigenetic marks of TET-mediated gene regulation under glyphosate exposure, we identified MTRNR2L2 and DUX4 genes, the hypomethylation of which was sustained even after stopping glyphosate exposure for 6 weeks. The findings reveal that low pressure but sustained DNA hypomethylation occurring *via* the TET pathway primes cells for oncogenic response in the presence of another potential risk factor. These results warrant further investigation of glyphosate-mediated breast cancer risk.

Materials and methods

Cell Culture and Transfection; MCF10A cells were cultured in DMEM/F12 supplemented with 5 % horse serum (Invitrogen, Cergy Pontoise, France), 500 ng/ml hydrocortisone (Sigma-Aldrich, France), 100 ng/ml cholera toxin (Sigma-Aldrich, France), 10 µg/ml insulin (TermoFisher, France) and 20 ng/ml epidermal growth factor (EGF, SigmaAldrich, France), penicillin (100 U/ml), and 2 mmol/L L-glutamine. MCF7 and MDA-MB-231 cells were cultured in DMEM medium (Invitrogen) all supplemented with 5 % FCS and 2 mM l-glutamine. Glyphosate (CAS 1071-83-6, sc-211568) was purchased from Santa-Cruz (France), and a 10^{-8}-M stock solution was prepared in DMSO every week. Glyphosate was diluted directly in fresh cell culture medium and was fed to the cells at the time points indicated in the results section. For the transfection of RNAs, we used miRCury LNA miR mimics for the has-miR-146a, has-miR-182-5p, has-miR-27a, has-miR-500a-5p, has-miR-30a, and has-miR-495 (Qiagen, France), siRNA for siRNA-T ET3 (sc94636) and control siRNA-A (sc94636) and HIPerfect Transfection Reagent (Qiagen, France). All miRs showed similar transfection efficiency (10- to 15-fold change, as measured by RTqPCR).

DNA Extraction, 5mC ELISA, and qMSRE; A QIAcube automate and QIAmp DNA Mini QiaCube kit (Qiagen, France) were used to isolate DNA. The quantification of 5mC was performed using the 5mC DNA ELISA Kit (Zymo Research-Euromodex, France) according to the manufacturer's instructions. The 5mC DNA ELISA Kit estimates the number of 5mC on DNA without distinction of localization; therefore, the term of global DNA methylation level when referring to results obtained *via* this mode of quantification was used. Next, DNA methylation was quantified by qMSRE. Digestions were performed with adequate restriction enzymes, HpaII and AciI (NEB, France). Typically, 1 ng of genomic DNA was digested with 40 U of enzymes at 37 °C for 2 h in 50 µl of reaction. Control samples were treated in the same way but without addition of the enzyme. Five microliters of digestion mixture were used for qPCR. The QuantiFast SYBR Green PCR Kit and Rotor-Gene Q (Qiagen, France) were used to perform the qPCR. Primers were MSH3: TTTCTCCAG GGCTGGGACTTTG and CCCGACTGGATTCCCCTTTTCT; DHFR: AAACCTCAGCGCTTCACCCAAT and TGATAGG GCTGGAGGAGGAAG; DUX4: CGACACCCTCGGACAGCA and TCAAAGCAGGCTCGCAG; COL23A1: TCTCCAGG CCAGAAACAGTCTT and ATTTAGAGAGGCAGGGTC GAGA; and MTRNR2L2: ACCCCACCTGTTTACCAA and GCTACCTTTGCACGGTTAGGG.

Tumor Xenografts in Nude Mice; Cells were harvested by trypsinization, washed and resuspended in saline buffer. Cell suspensions were injected subcutaneously into the flank of 7 to 8-week-old mice (Janvier, France) in 100 µl of sterile PBS. Tumor volume based on caliper measurements was calculated using the

modifed ellipsoidal formula [Tumor volume = 1/2 (length × width^2)] according to previously published work. At the end of the observation period, the mice with xenograf tumors were euthanized, and the tumor tissues were removed for analysis. The experimental procedures with animals were in accordance with the guidelines of Institutional Animal Care and the French National Committee of Ethics. In addition, all experiments were conducted according to the Regulations for Animal Experimentation at the Plateforme Animalerie in the Institut de Recherche en Santé de l'Université de Nantes (IRS-UN) and approved by the French National Committee of Ethics. The number of mice was restricted to four per condition to limit the number of animals to the necessary minimum as in previous studies based on the fact that we anticipated to detect a highly frequent tumorigenic event (frequency superior to one to four for tumorigenesis).

Establishment of Tumor Cells; From Xenografts (PCTCdX); PCTCdX (here named Glypho-iBPCTC) were obtained after mechanical dissociation. Briefly, resected tumor tissue from mice was cut into pieces of 1–5 mm^3 and plated in a 60-mm^2 tissue culture dish with DMEM containing 10 % FBS and antibiotics. Minced pieces of tumor were incubated with 200 U/ml collagenase I (Sigma) and 500 U/ml DNaseI (Sigma) in PBS for 1 h at 37 °C with vigorous constant agitation. The single cell suspension was filtered through a 70-mm cell strainer (BD Falcon), washed with PBS, and then placed in DMEM-10 % FBS. Cell cultures were split 1:5 when confluent.

Migration Assay; Cells (3×10^5) were seeded in six-well plates, cultured until they reached 80–90 % confluence, and treated with 10 µg/ml of mitomycin C (Sigma, France) for 2 h (to prevent cell proliferation). The monolayer of cells was scratched using a two-well silicone insert (Ibidi, Germany). Cell migration was monitored by microscopy (Incellis Cell Imager, Bertin, France). The images acquired at different time points (0, 4, 8, 24, 28, 32, and 48 h) for each sample were analyzed quantitatively. For each image, distances between one side of the wound and the other side were measured with ImageJ software; the mean value of 10 measurements all along the wound was recorded. The average migration speed was obtained by calculating the ratio distance/time along the time course.

Invasion Assay; All of the procedures were performed according to the manufacturer's instructions (QCM 24-Well Collagen-Based Cell Invasion Assay, Millipore, France). In brief, 200 µl of serum-free medium containing 2×10^5 cells were added into the invasion chamber, with the bottom well of the 24-well plate containing 500 µl of complete medium. After 72 h of incubation at 37 °C, the medium was removed, and the cells were stained by placing the chamber in staining solution for 20 min at room temperature. Cells that did not invade were carefully removed from the top side of the chamber using a cotton swab. The stained chamber was inserted into a clean well containing 200 µl of extraction buffer for 15 min at room temperature. A total of 100 µl of extracted (stained) solution from the chamber was transferred into a 96-well plate, and the optical density was measured 570 nm using a spectrophotometer.

Viability Assay: MTT and XTT Tests; A cell suspension containing 105 cells was prepared, and 100 µl was distributed in sixplicates in a 96-well plate. After 24 h of incubation at 37 °C and 5 % CO_2, cells were exposed to tamoxifen for 48 h. Tamoxifen was first diluted 10 times in dimethyl sulfoxide (DMSO) and then further diluted in DMEM containing 4.5 g/L glucose, 1 % SVF, 1 % glutamine, 1 % penicillin-streptomycin at the desired concentrations. Following treatment, 10 µl of MTT (10 µg/ml) (VWR Chemicals, France) was added in each well, and the cells were incubated for 3 h. Finally, the medium containing MTT was removed, and 200 µl/well of DMSO was added to measure the optical density at 570 nm using a spectrophotometer. For the XTT test, the XTT Assay Kit was used (ab232856, Abcam, France) according to the manufacturer's instructions. Briefly, 10^5 cells were seeded in 100 µl of culture medium in each well of a 96-well plate. After 24 h of incubation at 37 °C and 5 % CO_2, cells were treated with adequate drugs. Then, 10 µl/well of XTT mixture was added for an incubation of 2 h at 37 °C and 5 % CO_2. Finally, absorbance was measured at 450 nm.

Breast Tissue and Urine Samples; Human samples were collected from the Réseau des tumorothèques du Cancéropole Grand-Ouest and Institut de Cancérologie de l'Ouest. In accordance with regulations, all subjects signed a specifc informed consent form for this biocollection approved by an Ethics Committee (CPP OUEST IV, n °18/16), the French State Department for National Education, Higher Education and

Research (Ministère de l'Education Nationale, de l'Enseignement Supérieur et de la Recherche, N ° DC-2015-2457) and the Commission Nationale de l'Informatique et des Libertés (CNIL) (compliance commitment to MR 001). The glyphosate concentration in urine samples was obtained using Glyphosate kit (Novakits, France).

mMTase and TET Activities; TET activity was determined using the Epigenase 5mC-Hydroxylase TET Activity/Inhibition Assay Kit (Colorimetric; Epigentek/Euromedex, France) according to the manufacturer's instructions. Dnmts-magnetic beads (DMB) assays were performed to estimate mMTase, such as initially described. Briefly, a typical methylation reaction required 50 µg of nuclear extract (Nuclear extract kit, Active Motif, France), 125 nM DNA oligonucleotides (probes), and 900 nM tritium-labeled AdoMet (1 mCi/ml; #NET155V001MC; PerkinElmer, France) in reaction buffer (50 mM Tris, pH 8.0, 5 mM EDTA, 10 % glycerol, 0.5 mM phenylmethylsulfonyl fluoride). After incubation at 37 °C for 1 h, reactions were quenched with an equal volume of magnetic beads suspension and incubated for 15 min at room temperature. Next, the beads were magnetically isolated from the reaction mix, and tritium incorporation was measured by scintillation counting.

In-Cell ELISA; In-cell ELISA was performed using the In-Cell ELISA Kit (Abcam, France) according to the manufacturer's instructions and after a fixation step performed with 4 % of paraformaldehyde solution (10 min at room temperature). Primary antibodies were incubated overnight at 4 °C. Adequate HRP-conjugated secondary antibodies were incubated for 1 h at room temperature. Detection was performed at 450 nm. After the washes, cells in each well were incubated with 1X Janus Green Stain for 5 min at room temperature, according to the manufacturer's instructions. Data were expressed in normalized unit, according to the following calculation: (HRPsignal 'minus' HRPsignal in absence of primary antibody)/(Janus Green signal 'minus' Janus Green signal in absence of cells). Antibodies used were anti-TET1 (sc163446, Santa Cruz, France), anti-TET2 (sc398535, Santa Cruz), anti-TET3 (sc139186, Santa Cruz), anti-ERα (sc8002, Santa Cruz), anti-PR (sc130071, Santa Cruz), and anti-HER2 (sc-393712, Santa Cruz).

ChIP Analyses; ChIP was performed using the ChIP-IT Express kit (Active Motif, France) according to the manufacturer's instructions. The cross-linking step was performed by treating the cells with 37 % formaldehyde solution for 15 min at room temperature. Sonication was performed with the Bioruptor Plus (eight cycles 30 s on/90 s off) (Diagenode, France). The QuantiFast SYBR Green PCR Kit and Rotor-Gene Q (Qiagen, France) were used to perform the qPCR. Antibodies used were Anti-IgG (Abcam, AB2410) and anti-TET3 (sc139186, Santa Cruz).

Statistical Analysis; All experiments were done at least in biological triplicates. Differences in means were assessed using Student t test, and the degree of correlation between two parameters was calculated using Pearson's test. $P < 0.05$ was considered significant.

Results

Exposure to Glyphosate Promotes TET3-Mediated Global DNA Hypomethylation in MCF10A Cells; DNA hypomethylation has been shown to play a determining role in cancer development. To verify the impact of glyphosate exposure on the global level of DNA methylation, non-neoplastic breast epithelial MCF10A cells were treated with a low dose (10-11 M) of this herbicide every three to four days over 21 days, covering three passage numbers; whereas control cultures were treated with vehicle DMSO (Figure 1A). Several articles analyzing the effect of glyphosate on human cells have reported using 10-11 M. Indeed, 90 % of MCF10A cells were viable as measured by XTT (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) assay at this concentration. Importantly, glyphosate 10-11 M is below the concentration detected in biological fluids (milk, serum, urine). As a control performed in parallel, MCF10A cells were exposed to carcinogenic UP peptide (0.5 µM) previously described to promote global DNA hypomethylation *via* the disruption of the DNMT1/PCNA/UHRF1 complex. As expected, there was a decrease in the level of 5mC-DNA in MCF10A cells treated with the UP peptide (Figure 1B). There was also a reduction in 5mC content in cells treated with glyphosate (Figure 1B), hence suggesting that

glyphosate promotes a global DNA hypomethylation as per the definition given in the introduction. The origin of glyphosate-mediated decrease in DNA methylation was assessed by measuring the levels of activity of maintenance methyltransferase (mMTase) and Ten-eleven translocation (TET), since a decrease of mMTase activity and an increase of TET activity are both causes of DNA hypomethylation. The mMTase activity remained unchanged in MCF10A cells treated with glyphosate (Figure 1C) while TET activity significantly increased in these cells (Figure 1D). Specifically, an ELISA-based assessment of the amount of the three TET family members, TET1, TET2 and TET3, revealed an overexpression of TET3 in MCF10A cells following exposure to glyphosate (Figure 1E). To confrm that glyphosate promotes TET3-mediated global DNA hypomethylation in MCF10A cells, we analysed the level of DNA methylation in MCF10A cells with siRNAmediated TET3 down-regulation. ELISA results show that the presence of siRNA-TET3 abrogates TET3 overexpression and prevents DNA hypomethylation in cells exposed to glyphosate (Figure 1F).

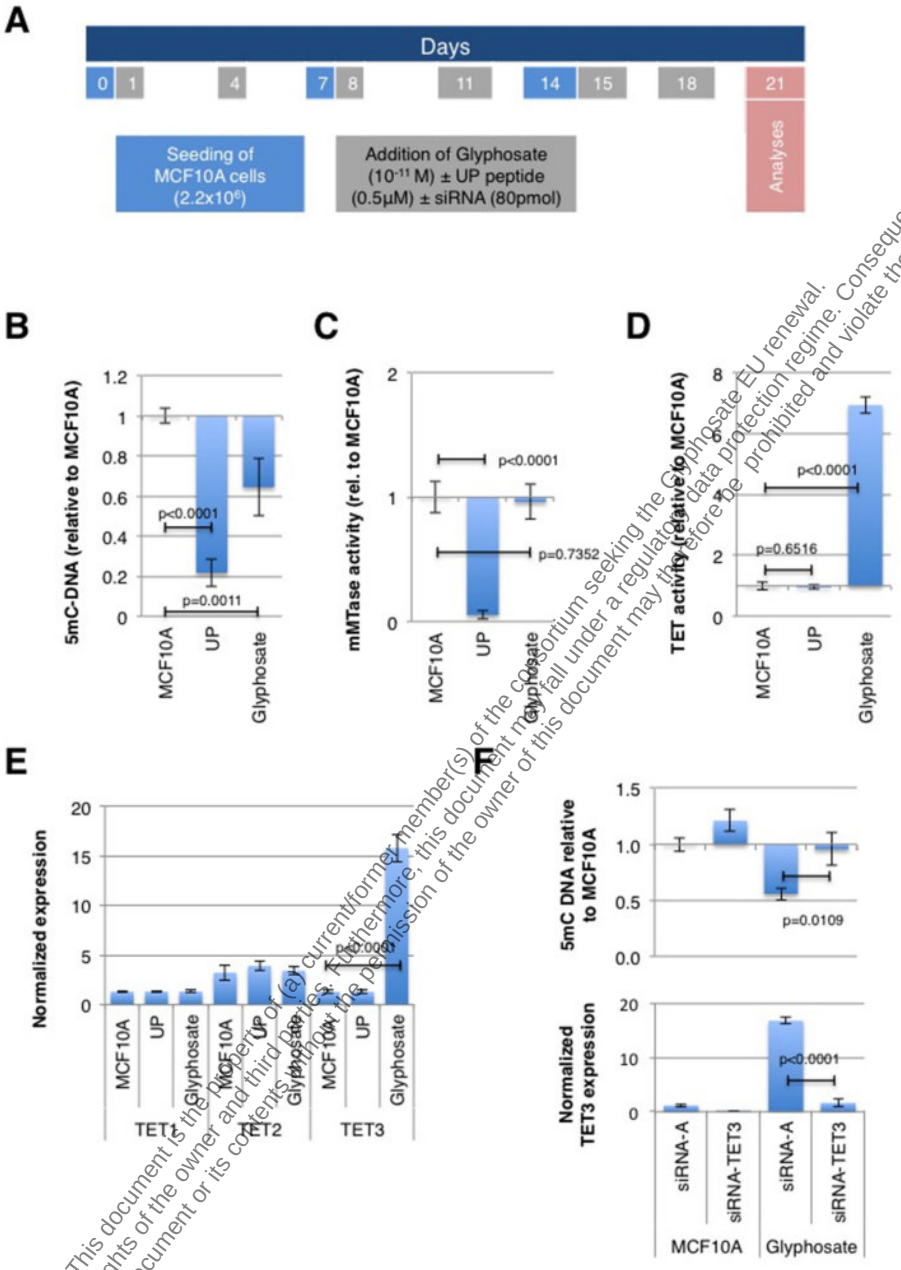


FIGURE 1 | Glyphosate exposure promotes a TET3-mediated global DNA hypomethylation. MCF10A cells were treated according to a timetable shown in **(A)** and analyzed on day 21 of culture. Explanations for color-coded days are located in corresponding color rectangles underneath the timeline. UP peptide promotes DNMT1/PCNA/UHRF1 disruption). **(B)** ELISA was used to measure the level of 5-methylcytosine (5-mC). **(C)** DMB assay was used to measure maintenance methyltransferase (mMTase). **(D)** TET assay. **(E)** In-Cell ELISA was used to quantify TET proteins. **(F)** MCF10A cells were transfected either with siRNA for TET3 or with control siRNA (siRNA-A) and treated with glyphosate (Glyphosate) or vehicle DMSO (MCF10A) according to a timetable shown in **(A)**. ELISA was used to measure the level of 5mC, and TET3 levels were determined by In-Cell ELISA and normalized to Janus Green staining intensity to account for differences in cell seeding density. For all assays, the bar graph displays the average ± standard deviation values of three independent experiments.

Glyphosate Exposure Is Tumorigenic for MCF10A Cells in a Two-Factor Hit Model; Global DNA hypomethylation is potentially tumorigenic. Therefore, MCF10A cells exposed to glyphosate were injected subcutaneously in Swiss nude mice. No tumors developed, whereas the control experiment with MCF10A cells exposed to the UP peptide led to visible tumor growth within 21 days in 100 % of the mice (Figure 2A). The Knudson's hypothesis for cancer initiation suggests that several oncogenic hits cooperate to promote cancer. This hypothesis initially based on mutations can be transposed to risk factors beyond

genetic alterations. Indeed, several microRNAs (miR) have been associated with cancer either as oncomiR (one hit) or suspected to promote cancer phenotype in light of their overexpression in cancers. To investigate the possibility of a two factor hit oncogenic impact with glyphosate, six miRs associated with poor prognosis of breast cancer [miR-182-5p, miR-27a, miR-500a-5p, miR-30a, miR-495, and miR-146a] were transfected individually in MCF10A cells. For this purpose, miRs mimics were used, and their increased expression was confirmed by RTqPCR. Tumor nodules were observed in two out of the four mice with subcutaneous injection of glyphosate-exposed MCF10A overexpressing miR-182-5p, whereas none of the other five miRs were associated with tumor formation (Figure 2B). Moreover, no tumor nodules were observed with subcutaneous injection of glyphosate/miR-182-5p/siRNA-TET3-exposed MCF10A, confirming that TET3 is implicated in glyphosate-mediated tumorigenic pathway (Figure 2C). The use of the Pan-cancer RNA-seq data available from the KM plotter database revealed that although TET3 overexpression is associated with a favorable overall survival in head and neck squamous cell carcinoma, thymoma, and thyroid carcinoma, it is associated with an unfavorable overall survival in breast cancer, as well as cervical squamous cell carcinoma, kidney renal papillary cell carcinoma, liver hepatocellular carcinoma, pheochromocytoma, paraganglioma, and uterine corpus endometrial carcinoma. We next compared several molecular signatures and phenotypic traits of primary cultures of tumor cells (PCTC) from glyphosate-induced breast tumors (Glypho-iBPCTC) with the ones of luminal A (MCF-7) and triple negative (MDA-MB-231) breast cancer cells. Only one of the two tumors led to viable Glypho-iBPCTC. In-cell ELISA confirmed that MCF7 and MDA-MB-231 cells were ERα+/PR+/HER2- (luminal A) and ERα-/PR-/HER2- (triple negative), respectively, and revealed that Glypho-iBPCTC were ERα+/PR-/HER2-, hence corresponding to a luminal B type of breast cancer with poorer outcome compared to ER+/PR+/HER2-subtype (Figure 3A). Tamoxifen/IC50 in MCF-7 and Glypho-iBPCTC were similar (Figure 3B). The QCM™ 24-Well Collagen-based cell invasion assay revealed that all cell strains had similar invasion capacity (Figure 3C), although scratch test indicated that Glypho-iBPCTC had the lowest migration ability compared to MCF-7 ($p = 0.0137$) and MDA-MB-231 cells ($p = 0.0002$) (Figure 3D). These results confirm that Glypho-iBPCTC display phenotypic traits associated with breast cancer cells *in vitro*.

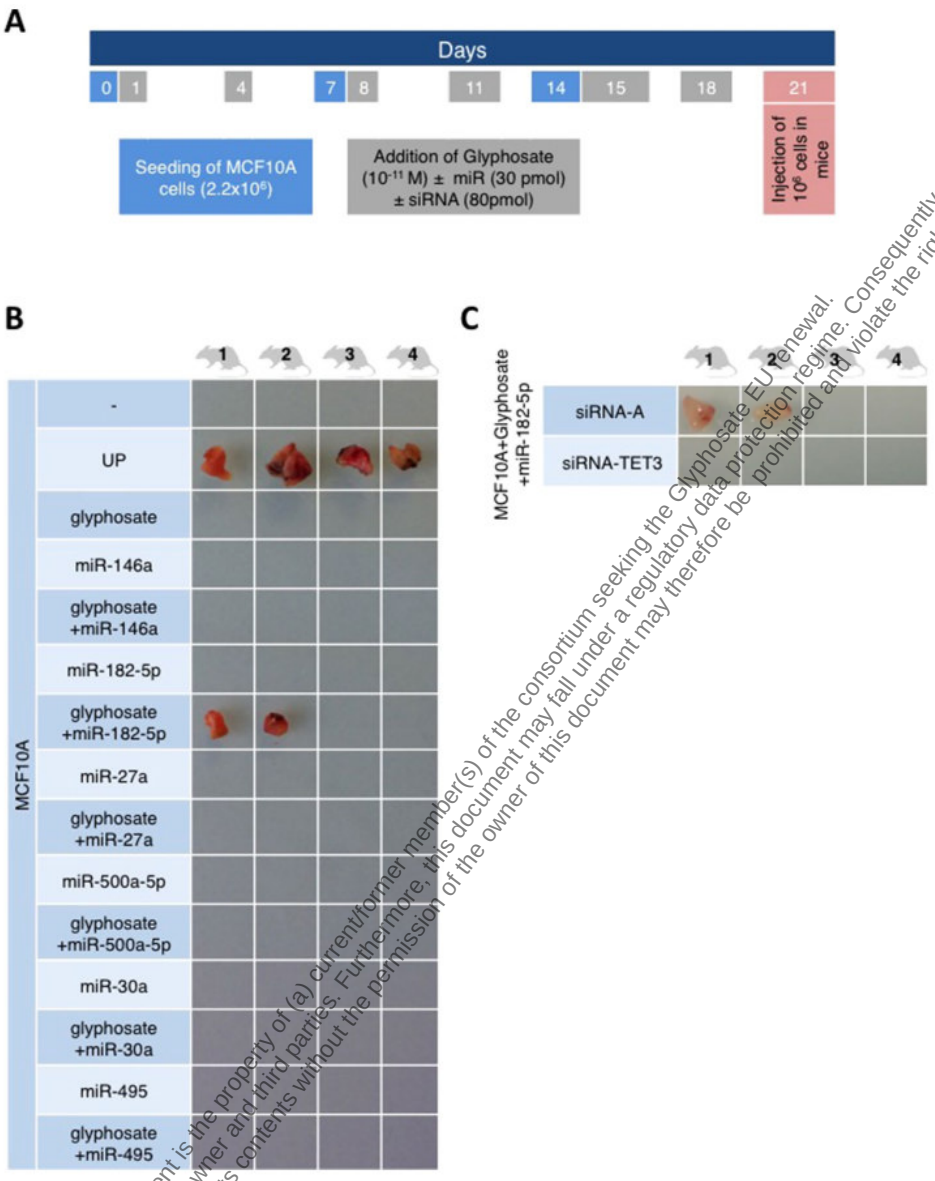


FIGURE 2 | The combination of glyphosate exposure and miR-182 overexpression is tumorigenic for MCF10A cells in a two-factor hit model. **(A)** The timetable illustrates the experiment design. Explanations for color-coded days are located in corresponding color rectangles underneath the timeline. **(B** and **C)** Four mice were injected per condition. miRCury LNA miR mimics and siRNA for TET3 were used to overexpress miRs or siRNA in MCF10A cells. Mice were euthanized 21 days after the injection of cells, and the tumors were resected. The pictures show the resected tumors.

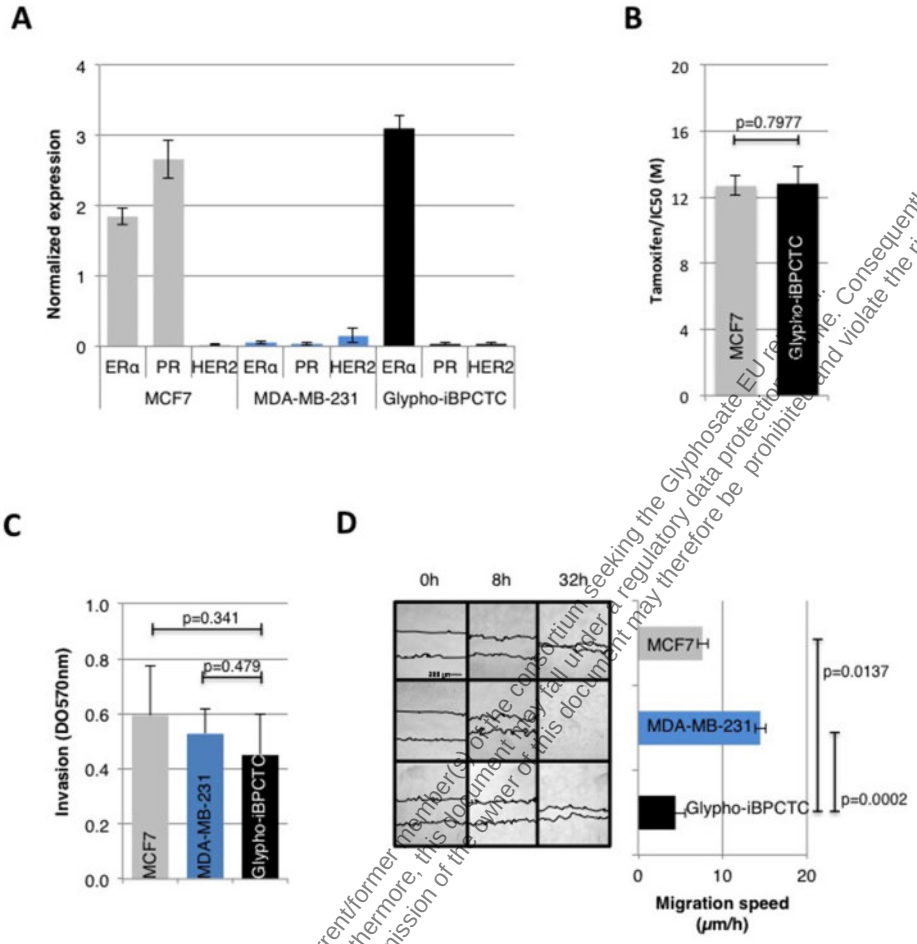


FIGURE 3 | Primary cells from glyphosate-induced breast tumor display characteristics of malignant cells. **(A)** The expression levels of ERα, PR, and HER2 were estimated in MCF7 cells, MDA-MB-231 cells, and Glypho-iBPCTC primary cells using In-Cell ELISA. Normalization to Janus Green staining intensity was performed to account for differences in cell seeding density. The bar graph displays the average ± standard deviation values of three independent experiments. **(B)** Bar graph of the viability of MCF-7 and Glypho-iBPCTC cells treated with increasing doses of tamoxifen (0, 2, 4, 6, 8, 10, 16, 19, 22 µM). Viability was measured by an MTT test, and the results represent the average ± standard deviation values of six independent experiments. The IC50 for each cell type was calculated using the IC50 Calculator (ATT Bioquest). **(C)** Bar graph showing the invasion capacity of MCF-7, MDA-MB-231, and Glypho-iBPCTC cells measured by optical density (absorbance at 570 nm). n = 3. **(D)** Confluent cultures of MCF-7, MDA-MB-231, and Glypho-iBPCTC cells were subjected to the wound healing test. The average migration speed was obtained by calculating the ratio distance/time between each acquisition time. Left: Pictures were acquired immediately after seeding (0 h) and after 8 and 32 h of culture. The bar graph represents the average ± standard deviation values of three independent experiments.

DMOG, a TET Inhibitor, Prevents Tumor Formation in Glyphosate-Challenged Cells; Some of the nutraceuticals/alicaments currently available target epigenetic pathways involved in normal homeostasis, notably those controlling DNA methylation. Like established epigenetic drugs, these sources of epigenetic modifiers offer great potentials to help determine the epigenetic path targeted by environmental factors and possibly revert the risk of tumorigenesis. MCF10A cells were transfected with miR-182-5p and exposed to 10-11 M of glyphosate (MCF10Aglyphosate/miR-182-5p) every 3 to 4 days over a 21-day period. They were also simultaneously treated with 40 µg/ml folate, a promoter of DNA methylation, or with 250 µM ascorbic acid, an activator of TET, 24 h after every glyphosate +/-miR treatment (Figure 4A). MCF10Aglyphosate/miR-182-5p cells were also treated in a similar manner with two therapeutic agents, an anti-miR-182-5p (50 nM) and dimethyloxallyl glycine (DMOG, 1 mM), a compound that blocks TET enzymatic activity (Figure 4A). For all of these conditions, the global level of DNA methylation and tumor incidence compared to untreated MCF10Aglyphosate/miR-182-5p cells (control) at the end of the 21-day treatment sequence was measured. As expected, folate and DMOG prevented glyphosate-induced DNA demethylation, whereas ascorbic acid further reduced DNA methylation in MCF10Aglyphosate/miR-182-5p cells, as shown by the level of 5mC (Figure 4B). Treatment with anti-miR-182-5p did not modify

significantly the level of 5mC compared to control. Both folate and DMOG treatments were confirmed to indeed induce hypermethylation in several cell lines. Of the two hypermethylating agents, DMOG and folate, only DMOG prevented tumor formation; there was no difference between folate and control treatments (50 % of the mice displayed tumors). Ascorbic acid and glyphosate acting synergistically on DNA hypomethylation led to a 50 % increase in tumor incidence. In contrast, although without an obvious impact on glyphosate-induced DNA hypomethylation, anti-miR-182-5p was able to prevent tumor formation (Figure 4C). These results confirm that both DNA demethylation and miR-182-5p are necessary for tumor onset. Importantly, the extent of DNA demethylation appears to set a threshold for tumor onset (i.e. the more hypomethylated, the higher the risk for tumor development).

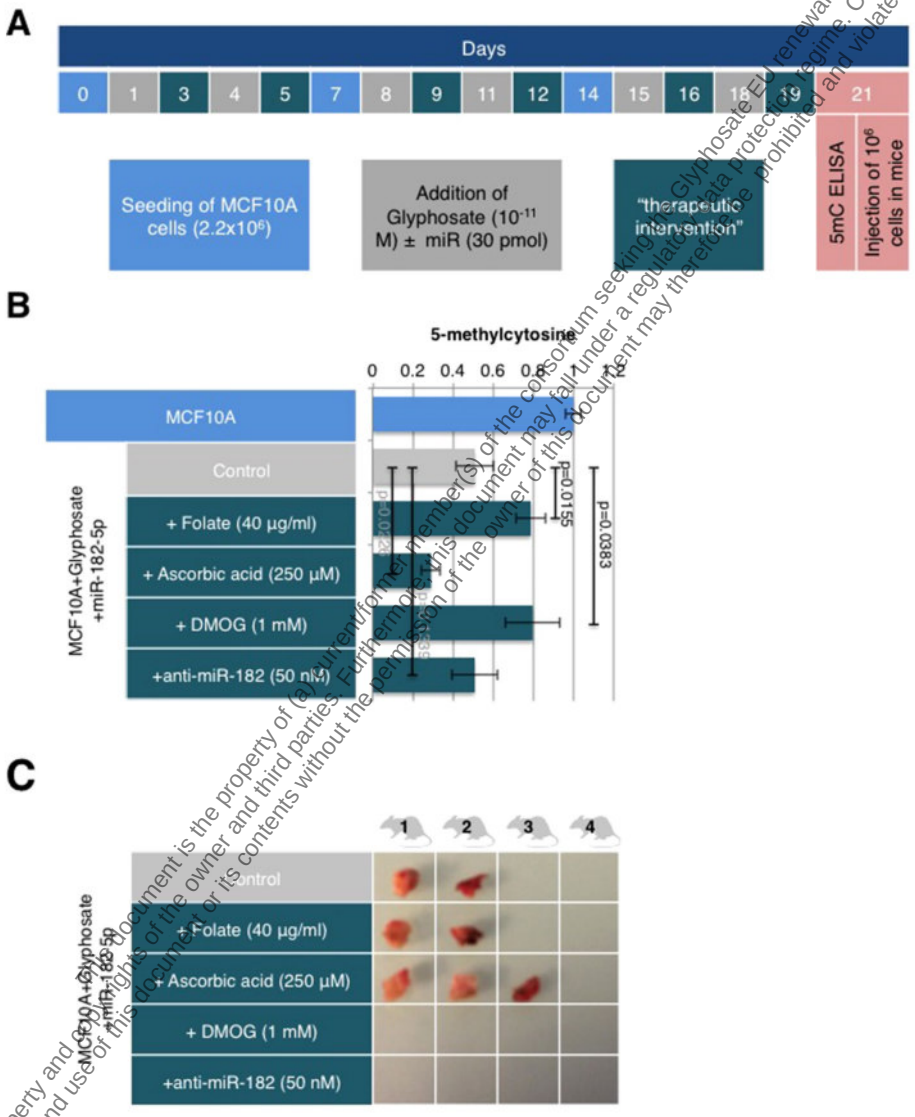


FIGURE 4 | DMOG and anti-miR-182 prevent tumor onset but differentially impact 5-meC level. **(A)** The timetable illustrating the experiment design. Explanations for color-coded days are located in corresponding color rectangles underneath the timeline. Therapeutic interventions on MCF10A cells treated with glyphosate and miR as indicated, were performed on days 3, 5, 9, 12, 16, and 19 with folate (40 µg/ml), ascorbic acid (250 µM), DMOG (1 mM), or anti-miR-182 (50 nM). **(B)** MCF10A cells were treated as shown in schedule A. DNA was extracted at day 21 and used in 5mC ELISA. The bar graph illustrates the levels of 5mC for the different conditions. **(C)** Mice were injected with the cells following the treatment schedule A and euthanized 21 days later. Shown are pictures of the resected tumors.

Glyphosate Exposure Induces Sustained TET3-Mediated Gene Demethylation; The hypomethylation induced by glyphosate treatment is sufficient for tumor onset when using a two-factor hit model with induced overexpression of miR-182-5p. Therefore, the possibility that an epimark of hypomethylation

might be imprinted in the DNA was investigated. It was postulated that the putative epimark induced by glyphosate might be the hypomethylation of TET3-targeted genes because TET3 mediates glyphosate-induced DNA hypomethylation. The chromatin immunoprecipitation (ChIP) atlas database identifies MTRNR2L2, COL23A1, MSH3, DHFR, and DUX4 as the most frequently present in TET3-ChIP hits. According to this predictive finding, ChIP experiments using anti-TET3 antibody were performed for chromatin obtained from MCF10A cells treated or not with glyphosate for 21 days, such as described in Figure 1A. Interestingly, only MTRNRL2 and DUX4 genes were immunoprecipitated by TET3 in MCF10A cells treated with glyphosate. COL23A1, MSH3, and DHFR genes were not immunoprecipitated in both untreated and treated MCF10A cells. Thus, the prediction made by the ChIP atlas database was validated for MTRNRL2 and DUX4 genes and not for the COL23A1, MSH3, and DHFR genes, suggesting a context-dependent accessibility for this set of TET3-controled genes. Accordingly, quantitative methylation-sensitive restriction enzyme (qMSRE) revealed that MTRNRL2 and DUX4 genes were strongly methylated in control cells and became hypomethylated in MCF10A cells exposed to glyphosate (Figure 5A). The involvement of TET3 in the glyphosate-induced hypomethylation of DUX4 and MTRNR2L2 was confirmed by the abrogation with siRNA-TET3 of the glyphosate-induced hypomethylation of these genes (Figure 5B). Preliminary investigation of available breast tissue from breast cancer-free women confirmed the demethylation of DUX4 and MTRNR2L2 in a woman showing glyphosate exposure based on urinary test. However, the methylation status of the five genes immunoprecipitated by TET3, MTRNR2L2, DUX4COL23A1, MSH3, and DHFR, should be kept in consideration in the future because a woman with low glyphosate exposure displayed methylation on the five genes, hence suggesting that an epimark should consider the methylation status of all these genes in future investigations (Supplementary Figure S5). The stability of epigenetic changes is an important factor for long-term risk determination. MCF10A cells were exposed to glyphosate for 21 days (as previously described; Figure 1A) and then cultured without glyphosate for 1 and 6 weeks. The DUX4 and MTRNRL2 hypomethylations remained stable, as shown by qMSRE, even after exposure to glyphosate has seized (Figure 5C). bc-GenExMiner and KM plotter indicated that a high expression of DUX4 is associated with a poor prognosis, suggesting that genes controlled by TET3 might deserve additional scrutiny in breast cancer pathogenesis.

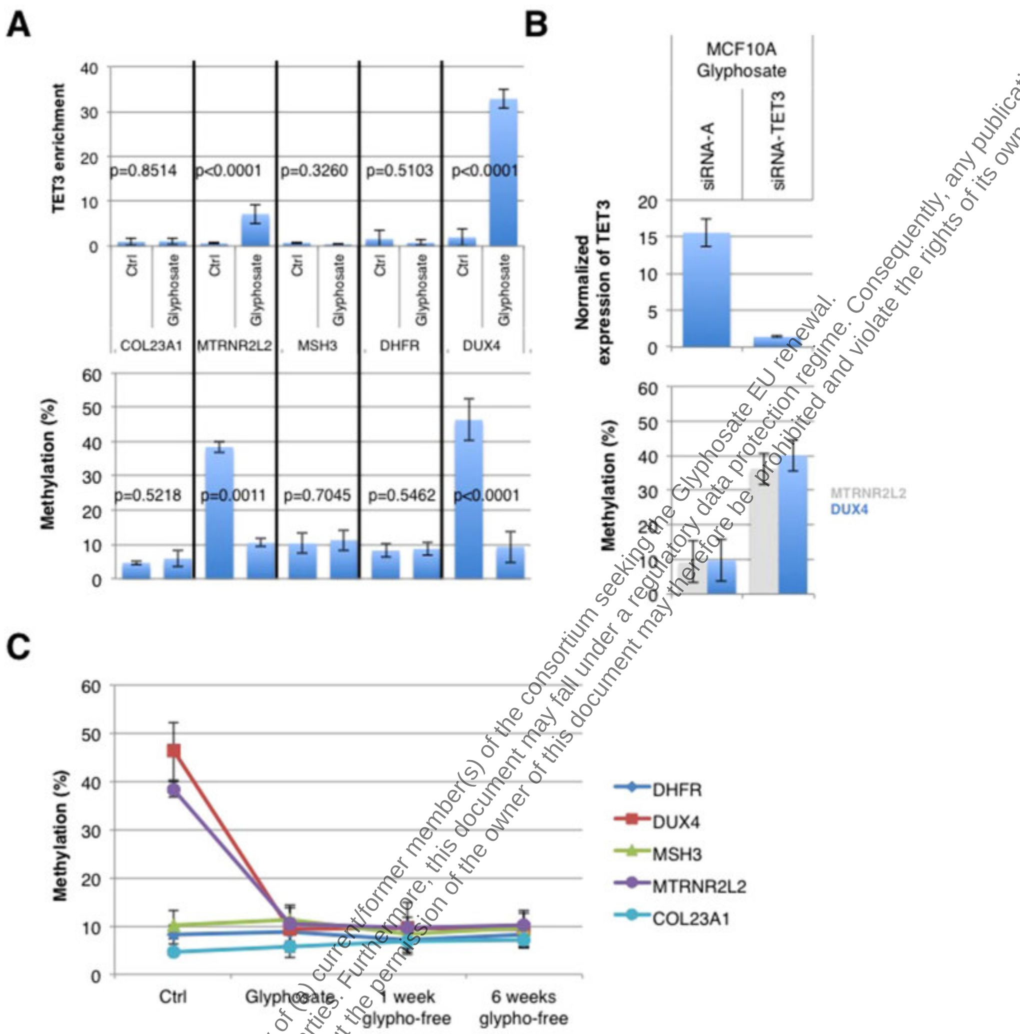


FIGURE 5 | Glyphosate-induced TET3-mediated demethylation affects *MTRNR2L2* and *DUX4* genes. **(A)** MCF10A cells were treated with glyphosate for 21 days as in the schedule shown in **Figure 2**. The graphs illustrate TET3 enrichment (top) following chromatin immunoprecipitation (ChIP) and the methylation level measured by qMSRE (bottom) of five genes defined by the ChIP atlas as being TET3-targeted genes. **(B)** MCF10A cells were treated with glyphosate for 21 days (according to the timetable of **Figure 2**), with siRNA added concomitantly to glyphosate. Bar graph (top) of TET3 expression measured with In-Cell ELISA after treatment with siRNA-TET3 (sc94636) or control siRNA-A (sc94636). Normalization to Janus Green staining intensity was performed to account for differences in cell seeding density. Bar graph (bottom) of methylation levels of *DUX4* and *MTRNR2L2* genes as measured by qMSRE. **(C)** MCF10A cells were treated with glyphosate for 21 days (glyphosate) according to the schedule shown in **Figure 1** and then cultured in glyphosate-free medium for another 1 (1 week glypho-free) or 6 (6 weeks glypho-free) weeks. Shown is the graph of the methylation level of five TET3-dependent genes. "Ctrl" represents MCF10A cells without glyphosate exposure.

Discussion

The impact of glyphosate on human health has been analysed and discussed for several years now. Recently, glyphosate exposure was correlated with shortened gestational lengths, and the level of glyphosate excretion was associated with steatohepatitis and advanced liver fibrosis in patients with fatty liver disease. However, the multiple research studies that investigated the tumorigenic effect of glyphosate as the sole risk factor had not led to convincing evidence of its implication. It is assumed that only 5–10 % of cancers are directly caused by inherited genetic abnormalities. The remaining 90 % of cancers are linked to environmental factors that directly or indirectly affect DNA, possibly triggering genetic defects or aberrations in the reading and/or expression of DNA. Environmental and lifestyle factors are pleiotropic and include diet, tobacco, infections, obesity, alcohol, radiation, stress, physical activity, exposure to heavy metals and other pollutants, such as glyphosate. This study is reporting that glyphosate exposure is not oncogenic by itself, but it acts as an oncogenic hit factor that, combined with another oncogenic hit, promotes the development of mammary tumors. At the molecular level, these findings demonstrate that glyphosate exposure can predispose breast cells to tumorigenesis *via* epigenetic reprogramming occurring

via TET3-mediated global and local DNA hypomethylation. this study and others have identified that global DNA hypomethylation promoting tumorigenesis may be caused by a deficiency of the DNMT1/PCNA/UHRF1 complex or of DNMT1 expression as shown in astrocytes, pulmonary fibroblasts, mesothelial cells, and breast cells. This study shows that glyphosate-mediated DNA hypomethylation is associated with TET3 overexpression instead of the DNMT1 pathway. The lower degree of DNA hypomethylation reached *via* the glyphosate TET3 path compared to that reached *via* UP peptide-DNMT1 path that is capable of inducing tumor onset suggests that a great intensity of global DNA hypomethylation could act as an oncogenic event, while a moderate intensity of global DNA hypomethylation might be considered a predisposing factor to cancer. The fact that active DNA demethylation orchestrated by TET can occur in resting (non-dividing) cells representing the majority of breast cells (in contrast to DNMT activity that requires cell proliferation) confers to TET-mediated mechanism a potentially higher degree of danger for cancer development. The implication of TET proteins in breast cancer growth and metastasis has been strongly documented, and the level of hypomethylation of triple-negative breast cancer has been associated with TET1 DNA demethylase activity. In the latter article, it is proposed but not shown that TET1 might act as an oncogene by leading to aberrant hypomethylation. These findings demonstrate that the hypothesis of an involvement of TET-mediated DNA hypomethylation in cancer onset was correct. Notably, siRNA-TET3 abolished the presence of glyphosate-induced global and local DUX4 and MTRNR2L2 hypomethylation, as well as tumorigenesis. The data from this study feed the ongoing debate regarding whether TET3 exerts an oncogenic role or a tumor suppressor role. For the latter role, TET3 might act by inhibiting epithelial-to-mesenchymal transition in ovarian and melanoma cancers. But the current analysis with KM plotter database revealed a potentially unfavorable outcome for breast cancers when TET3 is overexpressed. This work shows that two epigenetic events (global DNA hypomethylation and overexpression of a miR) cooperate to promote breast cancer. Other epigenetic events described to be involved in breast cancer development include the reduction of H3K9 acetylation *via* TIP60 downregulation that promotes ER-negative tumors. Histone acetyltransferase p300 activity and BIM1- mediated histone H2A ubiquitination that remodel chromatin are also two epigenetic events described as promoters for the development of aggressive breast tumors. A body of literature reports that miRs also play a crucial role in mammary tumorigenesis. In addition to oncogenic miRs, there are also miRs acting as tumor suppressors. For example, loss of miR-10b delays oncogene-induced mammary tumorigenesis overexpression of miR-489 inhibits HER2/neu-induced mammary tumorigenesis. Since the expression of miR depends on epigenetic control, it seems that either an extensive global hypomethylation of DNA (like with UP peptide) or a less extensive global hypomethylation associated with local epigenetic alterations affecting a miR might lead to tumor onset. The mechanisms associated with specific targeting of miR expression remain to be understood. Breast cancer susceptibility has been statistically linked to epigenetic age acceleration and CpG island methylation. An important question is whether exposure to pollutants that are detrimental to epigenetic homeostasis might replace or synergize with age-related epigenetic changes and thus lead to the increase in earlier onset of breast cancer that is now documented. Tis possibility is further supported by our preliminary observation that the luminal B subtype of tumor (ER+/PR-/HER2-) triggered by glyphosate exposure combined with miR-182-5p overexpression is associated with poorer outcomes than the frequent ER+/PR+/HER2-luminal A type of tumor. Indeed, luminal B type of tumors have been found to be most common in young patients. Tis phenotype obtained from one tumor produced in mice will have to be confirmed with additional means; in any case, epigenetic markers of risk would be a prime resource to help curve the incidence. There exist already DNA methylation markers that add to the prediction of tertiary and secondary outcomes over and beyond standard clinical measures. In the MCF10A model, glyphosate-induced DNA hypomethylation can be detected *via* the methylation level of only two of the five genes predicted to be controlled by TET3, MTRNR2L2 and DUX4 genes. Even if several other factors than glyphosate-induced TET3-mediated DNA hypomethylation (such as chromatin structure, other epimark, etc.) can govern the methylation status of the five genes, MTRNR2L2, DUX4, COL23A1, MSH3, and DHFR, this preliminary data with human samples support the idea that the study of the methylation status of these five genes might be important to obtain a marker of risk based on a MethylGlypho score. The current study is pursuing this direction of research by detecting and analyzing this 5-gene TET3-dependent epimark in blood samples. Possibly, glyphosate induced methylome reprogramming might be used for the detection of an increased risk for breast cancer in women living in an environment conducive to this type of pollution. Due to their concomitant expression during tumorigenesis associated with glyphosate-induced

DNA hypomethylation, DUX4 and MTRNR2L2 may appear as players in this process instead of only be considered potential biomarkers. Results with KM plotter and bc-GenExMiner indicate that DUX4 level is negatively associated with breast cancer prognosis. No data seems available on MTRN2L2 in these databases. Based on the literature, DUX4 could act as an oncogene in various sarcomas and hematological malignancies, while information could not be found in the literature revealing a putative oncogenic role for MTRNR2L2. These TET3-controlled genes are worth further investigation to establish their causal effect in mammary tumorigenesis in future work. Knowing the epigenetic pathway involved in glyphosate-mediated risk increase might lead to prevention strategies to follow detection of the epigenetic risk. The current findings suggest that TET-specific inhibitor DMOG might be a plausible therapeutic intervention since it gave a satisfactory response on both DNA methylation and tumor incidence. It would act by limiting TET3-mediated global DNA hypomethylation. In contrast, global remethylation of DNA by folate that has been considered for possible preventive effect is insufficient to prevent tumor incidence in the case of glyphosate exposure. Another interesting direction would be to limit the intake of ascorbic acid since it not only further reduced DNA methylation but also increased tumor incidence in mice. The epigenetic pathway leading to DNA hypomethylation is an important aspect to consider for further translational work on breast cancer risk.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The objective of this study was to investigate DNA hypomethylation in MCF10A cells, tumorigenic response for MCF10A Cells in a two-factor hit model, prevention of tumor formation in glyphosate-challenged cells, and TET3-Mediated Gene Demethylation following glyphosate exposure. This study was conducted *in vitro* using only one level of glyphosate. Glyphosate was not correlated to environmental exposures. In the *in vivo* portion of the study, a sufficient number of animals were not used to determine a carcinogenic response for statistical analysis. While this study is acceptable as supplemental information on the *in vitro* effects of glyphosate, it is not appropriate for endpoint derivation in human health risk assessment.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate used was insufficiently characterized and only one and extremely low concentration of glyphosate was used.

Assessment and conclusion by RMS:

Reliability criteria for *in vitro* toxicology studies

Publication: Duforestel *et al*., 2019.	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y?	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	N	Purity was not reported, source: Santa-Cruz,

		France.
Only glyphosate acid or one of its salts is the tested substance	Y	
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	Y	Non-neoplastic breast epithelial MCF10A cells.
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	NA	
Test concentrations in physiologically acceptable range (< 1 mM)	Y	One test concentration at 10^{-11}M, 10^{-5} µM (extremely low concentration) applied every 3 to 4 days over 21 days.
Cytotoxicity tests reported	Y	
Positive and negative controls	Y	
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	N	Not possible with one concentration
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate used was insufficiently characterized and only one and extremely low concentration of glyphosate was used.		

1. Information on the study

Data point:	CA 5.5/032
Report author	Hao, Y. *et al*
Report year	2019
Report title	Roundup-Induced AMPK/mTOR-Mediated Autophagy in Human A549 Cells
Document No	doi.org/10.1021/acs.jafc.9b04679 E-ISSN: 1520-5118
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary of the study according to OECD format

Glyphosate-based herbicide (GBH) is one of the most widely used pesticides based on a 5-

enolpyruvylshikimate-3-phosphate synthase target, which does not exist in vertebrates. Here, the autophagic effects of the most famous commercial GBH Roundup (RDP) on human A549 cells *in vitro* has been studied. Intracellular biochemical assay indicated opening of mitochondrial permeability transition pore, LC3-II conversion, up-regulation of beclin-1, down-regulation of p62, and the changes in the phosphorylation of AMPK and mTOR induced by RDP in A549 cells. Further experimental results indicated that all the effects induced by RDP were related to its adjuvant polyethoxylated tallow amine, not its herbicidal active substance glyphosate isopropylamine salt. All these results showed that RDP has the ability to induce AMPK/mTOR-mediated cell autophagy in human A549 cells.

Materials and methods

Chemicals - Glyphosate isopropylamine salt (≥ 95 %) was obtained from Weihai Hanfu Biochemical Pharmaceutical Co., Ltd., Weihai, China.

Cell culture - DMEM supplemented with 1 % antibiotics (streptomycin and penicillin) and 10 % fetal bovine serum was used to culture human alveolar carcinoma A549 cells (ATCC, CCL-185). The cells were placed in an incubator at 37 °C with a humidified atmosphere of 5 % CO_2.

Cell viability - The MTT assay was performed to evaluate the cytotoxicity of glyphosate in A549 cells. 1.0 × 10^5 cells in 100 µL of fresh DMEM medium were plated in 96-well plates. After incubation for 24 hours, medium was replaced with fresh medium containing glyphosate at 100 µg/mL. Fresh medium served as the control. Three replicates were used per test group. After incubation for 2 hours, the MTT reagent (20 µL/well, 5 mg/mL) was added to form formazan crystals during incubation for 4 hours at 37 °C . The medium was removed and the formazan crystals were dissolved in DMSO (150 µL/well). Optical density (OD) was measured with a microplate reader at 570 nm. Percent cell viability inhibition was calculated as follows: $cell\ inhibition(\%) = (OD_{control} - OD_{treatment})/OD_{control} \times 100$. SPSS version 17.0 was used to calculate IC_{50} values.

Monodansylcadaverine (MDC) staining – MDC staining was used to mark autophagic vacuoles. A549 cells (1 × 10^5 cells/well) were grown in 24-well plates. The cells were exposed to glyphosate at 100 µg/mL for 2 hours. Fresh medium served as the control. After rinsing twice with PBS at pH 7.4, the cells were incubated with 10 µM MDC in the dark for 20 minutes at 37 °C . After washing with PBS at pH 7.4, the treated cells were photographed and analyzed by fluorescence microscopy at 488 nm at a magnification of 200×. Three photos were taken per group and analyzed to determine the number of autophagosomes.

Visualization of double-membrane autophagosomes - Visualization of the ultrastructure of A549 cells was performed by transmission electron microscopy (TEM). A549 cells were harvested after being treated for 2 hours with glyphosate at 100 µg/mL. Fresh medium served as the control. The cells were then rinsed twice with PBS at pH 7.4 and placed in a 2.5 % glutaraldehyde solution and kept overnight at 4 °C . Subsequently, the cells were post-fixed in 1 % osmium tetroxide (OsO_4) after rinsing with PBS at pH 7.4. The cells were then dehydrated with an ascending series of ethanol solutions and embedded with Epon 812. Ultrathin sections were stained with 2 % uranyl acetate and lead citrate solutions. The images were recorded using a JEOL JEM-2100 transmission electron microscope.

Autophagic flux - Ad-mCherry-GFP-LC3B is an adenovirus that expresses the mCherry-GFP-LC3B fusion protein, which is used to analyze autophagic flux after infection of cells. A549 cells were grown in 3 cm glass bottom cell culture dishes and infected with adenovirus (40 MOI) for 24 hours. After incubation for another 24 hours, the cells were exposed to glyphosate at 100 µg/mL and incubated for 2 hours and then rinsed twice with PBS at pH 7.4. Fresh medium served as the control. The expression of mCherry and green fluorescent protein (GFP) was visualized and analyzed by a Nikon confocal microscope at 488 and 561 nm at a magnification of 100× by taking three photos per group. Diffuse yellow fluorescence indicates no occurrence of autophagy whereas yellow spots indicate autophagy. Autophagic flux was evaluated by the accumulation of mCherry-GFP-LC3B on the autophagosome membrane.

Colocalization of mitochondria and lysosomes - MitoTracker Green was used for mitochondria-specific fluorescent staining of live cells. LysoTracker Red was used for lysosomal-specific fluorescent staining of live cells. The two probes were used for mitochondrial and lysosomal colocalization imaging. A549 cells were treated with glyphosate at 100 µg/mL for 2 hours. Fresh medium served as the control. After treatment and rinsing, the cells were incubated with MitoTracker Green (0.5 µM) and LysoTracker Red (0.5 µM) for 30 minutes. Afterwards, the cells were photographed and analyzed by fluorescence microscopy at 488 and 561 nm at a magnification of 200×. Three photos were taken per group and analyzed. The counterstaining cells were photographed by a Nikon confocal microscope at a magnification of 100× and emission recorded at 488 nm for MitoTracker Green and 561 nm for LysoTracker Red. The related plugins (JACoP, colocalization threshold) of ImageJ v1.8.0 software were used to obtain a relative coefficient of colocalization between Mito and Lyso.

Opening of mitochondrial permeability transition pore (mPTP) - Opening of mPTP was measured by using calcein and $CoCl_2$. A549 cells were grown in 12-well plates and treated with glyphosate at 100 µg/mL for 2 hours. Fresh medium served as the control. After rinsing twice with PBS at pH 7.4, the cells were incubated with 1 mM calcein in the dark for 20 minutes at 37 °C and then exposed to 1 mM $CoCl_2$ for 30 minutes. The control group was not incubated with $CoCl_2$. After rinsing with PBS at pH 7.4, the treated cells were analyzed by fluorescence microscopy at 488 nm at a magnification of 200× by taking three photos per group.

Immunoblotting of proteins linked with autophagy - The relative protein expression levels of LC3, beclin-1, p62, p-AMPK, p-mTOR, and p-p70s6k in A549 cells were determined by immunoblot analysis to explore the underlying mechanisms of induced autophagy. A549 cells were treated with glyphosate at 100 µg/mL for 2 hours. Fresh medium served as the control. After treatment and rinsing the cells were lysed by a mixture of 50 µL immunoprecipitation assay lysis buffer with 0.5 µL protease inhibitor (100 mM). The total protein concentrations were determined using the Bicinchoninic Acid (BCA) Protein Assay Kit. Equal amounts of lysate proteins (50 µg) were separated by sodium dodecyl sulfate- polyacrylamide gels and transferred to polyvinylidene fluoride membranes. The membranes with the proteins were blocked in Tris-buffered saline containing 5 % nonfat dried milk and 0.05 % Tween-20 for 2 hours. Blocked membranes were incubated with rabbit polyclonal antibodies for LC3, beclin-1, p62, p-AMPK, p-mTOR, p-p70s6k, and β-actin overnight at 4 °C . After being washed three times in Tris-buffered saline containing 0.05 % Tween-20, membranes were incubated with anti-rabbit IgG secondary antibodies for 2 hours. The antibody-bound proteins were detected by Electro-Chemi-Luminescence kit and scanned by the chemiluminescent gel imaging system. The bands' grayscale values were quantified by ImageJ v1.8.0 software.

Adenosine triphosphate (ATP) content - The levels of intracellular ATP were determined by the ATP Assay Kit from Beyotime following the manufacturer's instructions. After treatment with glyphosate at 100 µg/mL or fresh medium as the control for 2 hours, A549 cells were lysed in the lysis buffer for 30 minutes at 4 °C . After centrifugation at 12,000 rpm for 15 minutes, a part of the supernatant was transferred into a light-protected 96-well plate for determination of the activity of ATP. The other part was used to determine the protein concentrations by the Pierce BCA Protein Assay Kit. The ATP concentrations (µmol/L) were converted to the protein concentrations (mg/L).

Statistical Analysis - Three separate replicates were performed for each assay. The statistical analysis process runs under SPSS version 17.0, statistical program (SPSS Inc). Data are presented as the means ± standard deviation (SD). Three independent experiments of MTT assay were analyzed by two-way ANOVA followed by Tukey post hoc testing. Different small alphabets indicate significant differences ($P \leq 0.05$). Others were subjected to one-way analysis of variance (ANOVA) followed by Dunnet's test for determining the differences with control ($^*P \leq 0.05$; $^{**}P \leq 0.01$).

Results

Viability - Inhibition of cell viability (as % of control) of A549 cells was 5.36 ± 0.82 for glyphosate at 100

µg/mL after 2 hours of treatment.

Monodansylcadaverine (MDC) staining - MDC staining in A549 cells treated with glyphosate at 100 µg/mL was not significantly different from the control.

Visualization of double-membrane autophagosomes - The visualization of autophagosomes by TEM showed an homogeneous cytoplasm with normal mitochondria in A549 cells treated with glyphosate at 100 µg/mL.

Autophagic flux - In the control and in the cells treated with glyphosate at 100 µg/mL, mCherry-GFP-LC3B is present in the form of diffuse yellow fluorescence which is indicative of the absence of autophagy.

Colocalization of mitochondria and lysosomes - The relative coefficient of colocalization between Mito and Lyso was 0.22 ± 0.01 for the control and 0.21 ± 0.02 for the cells treated with glyphosate at 100 µg/mL.

Opening of the mitochondrial permeability transition pore (mPTP) - When compared with the $CoCl_2$-treated control group, no significant increase in fluorescence was noted in the cells treated with glyphosate at 100 µg/mL which is indicative of the absence of mPTP opening.

Immunoblotting of proteins linked with autophagy - The effect of glyphosate on the autophagy related proteins beclin-1, LC3-II/I, and p62 was studied by immunoblotting. When compared with the control no difference was found in the expression ratio of LC3-II/I as well as protein expression of beclin-1 in cells treated with glyphosate at 100 µg/mL. Immunoblotting was also used to examine the phosphorylation of mTOR, AMPK, and p70s6k. No difference in phosphorylation levels of mTOR, p70s6k, and AMPK could be demonstrated in cells treated with glyphosate at 100 µg/mL when compared to controls. These results indicate that glyphosate does not contribute to the activation of the AMPK/mTOR pathway.

Adenosine triphosphate (ATP) content - When compared with the control, no difference was found in the levels of cellular ATP of cells treated with glyphosate at 100 µg/mL.

Conclusion

All commercial glyphosate formulations are more toxic than glyphosate so that the increased toxicity may be attributed to the adjuvants. The herbicidal active substance of Roundup is glyphosate isopropylamine salt and its main adjuvant is polyethoxylated tallow amine (POEA). To explore whether the herbicidal active substance or the adjuvant contributes Roundup's ability of inducing autophagy in human A549 cells, TEM, MDC, immunoblotting, and Ad-mCherry-GFP-LC3B analyses were performed on 4 concentrations of Roundup from 50 to 125 µg glyphosate acid eq./mL and one concentration of glyphosate (100 µg/mL) and POEA (35 µg/mL). This study revealed that the commercial formulation Roundup has the ability to cause autophagy in A549 cells *via* the AMPK/mTOR signaling pathway. Previous studies showed that compared with glyphosate, commercial glyphosate based herbicides are more toxic, and the toxic effect is highly correlated with the adjuvant POEA. This study indicates that the adjuvant POEA in Roundup contributes to its ability of inducing autophagy in A549 cells, and that the herbicidal active substance glyphosate has no contribution.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The effect of glyphosate, POEA and a herbicidal formulation containing POEA as a co-formulant on the AMPK/mTOR signaling pathway was studied in human alveolar carcinoma A549 cells *in vitro*. Only the results of exposure to glyphosate at 100 µg/mL are reported and discussed in this summary. The endpoints selected to study the effect of glyphosate on autophagy are inhibition of viability, monodansylcadaverine (MDC) staining to mark autophagic vacuoles, visualization of double-membrane

autophagosomes by TEM, autophagic flux, colocalization of mitochondria and lysosomes, opening of the mitochondrial permeability transition pore (mPTP), expression of proteins involved in the AMPK/mTOR signaling pathway, and ATP content. No effect could be demonstrated of glyphosate on any of these endpoints indicating that glyphosate, in contrast to POEA and Roundup, does not contribute to the activation of the AMPK/mTOR signaling pathway and has thus no role in autophagy.

This publication is relevant for the risk assessment of glyphosate but reliable with restrictions because only one glyphosate concentration was tested and no positive controls were used.

Assessment and conclusion by RMS:

Reliability criteria for *in vitro* toxicology studies

Publication: Hao, Xu *et al*., 2019	**Criteria met? Y/N?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity of ≥95 %. Source: Weihai Hanfu Biochemical Pharmaceutical Co., Ltd.,China.
Only glyphosate acid or one of its salts is the tested substance	N	Also GBH (Monsanto, St. Louis, USA) and POEA tested.
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	Y	Human alveolar carcinoma A549 cell line.
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	N	
Test concentrations in physiologically acceptable range (< 1 mM)	Y	Glyphosate only at one concentration tested: 100 µg/mL.
Cytotoxicity tests reported	Y	
Positive and negative controls	N	No positive control.
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	N	Only one concentration for glyphosate.
Overall assessment		
Reliable without restrictions		

Reliable with restrictions	Y	
Not reliable		
This publication is relevant for the risk assessment of glyphosate but reliable with restrictions because only one glyphosate concentration was tested and no positive controls were used.		

1. Information on the study

Data point:	CA 5.5/033
Report author	Pahwa, M. *et al.*
Report year	2019
Report title	Glyphosate use and associations with non-Hodgkin lymphoma major histological sub-types: findings from the North American Pooled Project
Document No	doi:10.5271/sjweh.3830 E-ISSN: 1795-990X
Guidelines followed in study	None
Deviations from current test guideline	No
GLP/Officially recognised testing facilities	Not applicable for epidemiologic studies
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary of the study according to OECD format

In this paper a pooled reanalysis of the data from 2 published non-Hodgkin's lymphoma (NHL) case control studies was conducted: McDuffie *et al*. 2001 and DeRoos *et al*. 2003. The reanalysis sought to evaluate associations for glyphosate use and NHL overall and by histological sub-type. In addition, the pooled analysis implemented more extensive control of confounding factors than in the original publications and considered the impact of excluding pesticide information provided by next-of-kin or proxy respondents. The OR for NHL overall for ever using glyphosate was 1.4 (95 % CI 1.1, 1.8). After adjustment for other pesticides, the OR was reduced to 1.1 (95 % CI 0.8, 1.5). ORs for ever using glyphosate, adjusted for other pesticides, by NHL sub-type were 0.7 (95 % CI 0.4, 1.2) for FL, 1.2 (95 % CI 0.8, 1.9) for DLBCL, 1.8 (0.9, 3.7) for SLL, and 1.5 (95 % CI 0.9, 2.6) for other NHL sub-types. Adjusted ORs excluding data from proxy respondents [supplemental table 1 in the article] were: 0.95 (95 % CI 0.7, 1.3) for NHL overall, 0.7 (95 % CI 0.4, 1.1) for FL, 1.1 (95 % CI 0.7, 1.8) for DLBCL, 1.8 (0.0, 3.8) for SLL, and 0.96 (95 % CI 0.5, 1.9) for other NHL sub-types. ORs for NHL overall were not elevated in the higher categories for years of use (> 3.5 years OR 0.9, 95 % CI 0.6, 1.4) or lifetime days of use (> 7 days OR 1.1, 95 % CI 0.7, 1.8) though there was an elevated OR for the category of more than 2 days of use/year (OR 1.7, 95 % CI 1.0, 2.9). For NHL subtypes, consistent patterns of association across exposure metrics were not seen, with the possible exception of SLL, though SLL findings were not statistically significant. In general, exclusion of proxy respondents reduced ORs to a minor extent with the exception of the analyses for other NHL subtypes. For NHL overall, ever use and analyses that considered duration of use per se or lifetime days of use did not show a relationship with glyphosate. There was a moderate association seen for glyphosate use for the metric > 2 days/year. There was some limited evidence of an association between glyphosate use and SLL across exposure metrics, but the small number of SLL cases resulted in very imprecise OR estimates as evidenced by wide 95 % CIs. The major limitation of the study is possible case recall bias in the reporting of prior pesticide use.

Materials and methods

Study population and exposure assessment
Pahwa *et al*. pooled data from case control studies in the US and Canada. For NHL specifically, this study is essentially a reanalysis of the published studies by McDuffie *et al*. (2001) in Canada and DeRoos *et al*. (2003) in the US. Case identification in the US was through cancer registries and hospitals during the 1980s in four US states (Iowa/Minnesota, Kansas, and Nebraska) and between 1991 and 1994 in six Canadian provinces (Quebec, Ontario, Manitoba, Saskatchewan, Alberta, and British Columbia). Methods for each study have been previously described. For this pooled analysis, the original histology codes were revisited and classified according to a single scheme [International Classification of Diseases for Oncology version 1 (ICD-O-1)].

Participants, or their proxies, provided information about demographic characteristics, pesticide use, agricultural exposures, and exposure to other known or suspected NHL risk factors, including lifestyle and medical and occupational history. Self-reported glyphosate use was examined using several exposure metrics: ever/never, duration (years used), frequency (days/year handled), and lifetime-days (number of years used multiplied by number of days/year handled). Categories were created for duration, frequency, and lifetime-days analyses based on the median of glyphosate used/handled among controls. Some participants had missing data for duration and frequency of glyphosate use despite reporting that they had ever used glyphosate. In duration and frequency analyses, values for missing data were assigned to cases and controls based on the median duration or frequency of reported glyphosate use among controls by state/province and 10-year age group (simple imputation) and were used for the main analyses. Ordinal analyses and associated trend tests were conducted to determine possible changes in association for increasing increments of every five years, five days/year, and ten lifetime-days of glyphosate use.

Statistical analyses
Unconditional multiple logistic regression was performed using the LOGISTIC procedure of the SAS 9.4 statistical software package (SAS Institute, Cary, NC, USA) to calculate ORs and 95 % CIs for associations between glyphosate exposure metrics (ever/never, duration, frequency, lifetime-days, and as ordinal variables) and NHL overall and by histological sub-type [diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), small lymphocytic lymphoma (SLL), and other]. Initial logistic regression models (OR) contained the following variables: age [age at diagnosis (cases); age at interview or death (controls)], state/province, sex, lymphatic or hematopoietic cancer in a first-degree relative, response by a proxy, and use of any personal protective equipment (PPE). Pesticides that were correlated with glyphosate use in the pooled data and that had previously been associated with NHL based on the individual case-control studies, specifically 2,4-dichlorophenoxyacetic acid, dicamba and malathion, were included in the more fully adjusted logistic regression models (OR). The former models will be referred to as crude and the latter models as adjusted.

Trends for duration, frequency, and lifetime-days of glyphosate use and NHL ORs were assessed by the asymptotic Cochran-Armitage trend test. Subjects who never used glyphosate were the reference group for all analyses. There was a small proportion of subjects (N=175, 2.6 % of all participants) with missing age values. These were imputed using simple imputation based on state/province- and case/control-specific means of age rounded to the nearest whole number. Sensitivity analyses were conducted by excluding proxy respondents from the main analyses.

Ethics approval and consent to participate
Ethics approval for the pooled analysis was obtained from the University of Toronto Health Sciences Research Ethics Board (#25166) and an exemption was obtained from the US National Institutes of Health Office of Human Subjects Research (#11351). Investigators of individual studies received human subjects' approval from their institutions for each study prior to collection of data.

Results

Characteristics of NHL cases and controls

A total of 1690 NHL cases and 5131 controls was available for analysis – 69.6 % of the cases and 70.6 % of the controls were from the US studies. All NHL cases and controls, including those with proxy respondents, were included in analyses of ever/never glyphosate use. For assessments involving duration of use, 1520 cases and 4183 controls were included. For frequency and lifetime-days analyses, 898 cases and 2938 controls were included. The numbers of cases and controls available for the sensitivity analysis excluding proxy respondents were smaller (Figure 1). Characteristics of NHL cases and controls, including histological sub-types, are presented in Table 1.

Figure 5.5.2-5. Subjects in main and proxy respondent analyses of glyphosate use and NHL in the North American Pooled Project (NAPP). * Duration (years) information was not collected in Kansas, ** Frequency (days/year) information was not collected in Iowa, Minnesota, and Kansas.

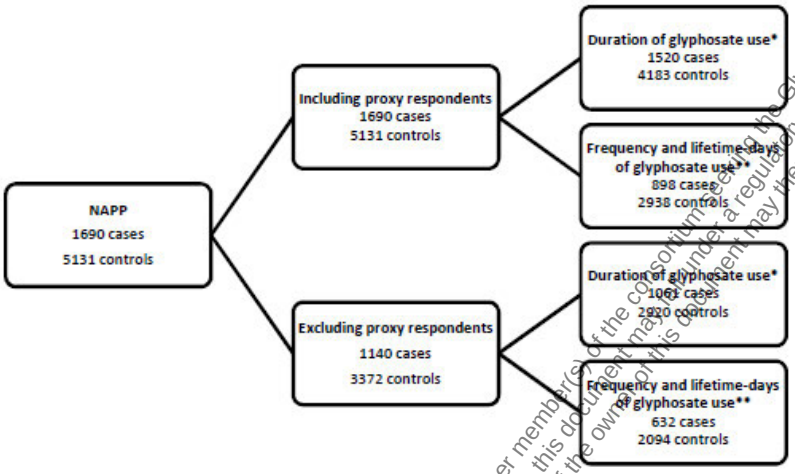


Table 5.5.2-61: Characteristics of non-Hodgkin lymphoma (NHL) cases and controls in the North American Pooled Project (NAPP). [OR=odds ratio; CI=confidence interval].

Characteristics	Cases (N=1690)		Controls (N=5131)			
	N	%	N	%	OR [a]	95% CI
Histological sub-type						
Diffuse large B-cell lymphoma (DLBCL)	647	38				
Follicular lymphoma (FL)	468	28				
Small lymphocytic lymphoma (SLL)	171	10				
Other	404	24				
State/Province U.S						
Nebraska	385	22	1432	28		
Minnesota	329	19	642	13		
Iowa	293	17	603	12		
Kansas	170	11	948	18		
Canada						
Ontario	142	8	585	11		
British Columbia	126	7	230	4		
Quebec	117	7	291	6		
Alberta	65	4	196	4		
Manitoba	34	2	113	2		
Saskatchewan	29	2	91	2		
Age (years) [b]						
≥19–≤29	26	2	277	5		
≥30–≤39	97	6	445	9		
≥40–≤49	159	9	514	10		
≥50–≤59	288	17	726	14		
≥60–≤69	564	33	1264	25		
≥70–≤79	402	24	1189	23		
≥80–≤89	137	8	610	12		
≥90	17	1	106	2		
Sex						
Male	1506	89	4424	86	1.00	ref
Female	184	11	707	14	0.84	0.75–1.17
Respondent type						
Self	1140	67	3372	66	1.00	ref
Proxy	533	32	1692	33	1.03	0.90–1.17
Unknown/missing	17	1	67	1		
Lymphatic or hematopoietic cancer in a first-degree relative						
No	1493	88	4790	93	1.00	ref
Yes	139	8	202	4	2.13	1.69–2.67
Unknown/missing	58	3	139	3		
Ever diagnosed with selected medical conditions [c]						
No	1097	65	3346	65	1.00	ref
Yes	545	32	1389	27	1.12	0.92–1.37
Unknown/missing	134	8	396	8		
Ever used any type of personal protective equipment						
No	374	22	1127	22	1.00	ref
Yes	105	6	310	6	1.12	0.86–1.45
Unknown/missing	1211	72	3694	72		

[a] Adjusted for age and state/province.
[b] Cases - mean 62.72 (SD 13.78) years; Controls - mean 61.66 (SD 17.13) years.
[c] Ever diagnosed with ≥1 of the following select medical conditions: allergies (any, food, or drug), asthma, hay fever, infectious mononucleosis, rheumatoid arthritis, tuberculosis, or received chemotherapy or radiation therapy.

Glyphosate use and associations with NHL overall and by major histological sub-type

Overall, 113/1690 cases (7 %) and 244/5131 (5 %) controls reported having used glyphosate at any point in their lifetime. In crude analyses, there was a significant association between ever use of glyphosate and NHL overall (OR1.4, 95 % CI 1.1–1.8) that was attenuated appreciably when adjusted for ever use of the pesticides 2,4-D, dicamba, and malathion (OR1.1, 95 % CI 0.8–1.5) (Table 2).[21] Adjusted ORs by for NHL subtypes were: 0.6 (95 % CI 0.4 – 1.2) for FL, 1.2 (95 % CI 0.8–1.9 for DLBCL, 1.8 (95 %CI 0.9 – 3.7) for SLL, and 1.5 (95 % CI 0.9 – 2.6) for other NHL subtypes.

When ORs for NHL and glyphosate use were examined by duration, there were lower ORs with longer use for NHL overall and for subtypes except SLL (see table 2). Conversely, there were higher ORs for those who reported use for more than 2 days per year verses those who reported use ≤ 2 days per year. Analyses by lifetime days of use – the metric used in most studies – showed near null results for all subtypes in the higher lifetime days category, except for SLL. It bears noting that the analyses by duration included 90 % of cases and controls (Kansas subjects did not have the required data), while the analyses of days/year and lifetime days included only 53 % of subjects (cases and controls from Kansas, Iowa, and Minnesota did not have the required data and were excluded). As such, these latter analyses may not be representative of results for the entire pooled study population.

[21] Adjusting for other pesticides appreciably changed the NHL OR for glyphosate. Therefore, it seems most appropriate to focus on the ORs from the adjusted analyses.

Table 5.5.2-62: Adjusted Odds Ratios (95 % confidence intervals) by Various Glyphosate Exposure Metrics

Metric	NHL overall	FL	DLBCL	SLL	Other NHL
ever use	1.1 (0.8, 1.5)	0.6 (0.4, 1.2)	1.2 (0.8, 1.9)	1.8 (0.9, 3.7)	1.5 (0.9, 2.6)
duration					
≤ 3.5 years	1.3 (0.9, 1.8)	0.7 (0.3, 1.3)	1.6 (0.97, 2.7)	1.4 (0.6, 3.7)	1.8 (0.95, 3.5)
> 3.5 years	0.9 (0.6, 1.4)	0.6 (0.3, 1.3)	0.9 (0.5, 1.7)	1.9 (0.8, 4.8)	1.1 (0.5, 2.5)
days/year					
≤ 2	0.7 (0.5, 1.2)	0.4 (0.2, 1.2)	0.7 (0.4, 1.4)	1.3 (0.4, 4.3)	1.1 (0.5. 2.7)
≥ 2	1.7 (1.0, 2.9)	1.3 (0.6, 3.2)	2.1 (1.1, 4.3)	2.3 (0.6, 8.8)	1.6 (0.6, 4.5)
lifetime days					
≤ 7	0.9 (0.5, 1.5)	0.6 (0.3, 1.6)	0.8 (0.4, 1.7)	1.0 (0.2, 4.8)	1.4 (0.6, 3.5)
> 7	1.1 (0.7, 1.8)	0.8 (0.3, 1.8)	1.1 (0.6, 2.2)	2.2 (0.7, 6.9)	1.3 (0.5, 3.3)

Sensitivity analyses excluding proxy respondents

A sensitivity analysis was performed by excluding cases and controls whose data were provided by proxy respondents. The overall pattern of OR estimates were generally similar to the main analyses. However, for SLL, ORs were slightly higher in the > 2 days/year subgroup OR 2.3 (95 % CI 0.6, 8.8) with proxies compared to OR 2.6 (95 % CI 0.7, 10.1)) without proxies. For other NHL subtypes, ORs were slightly to appreciably lower for ever use with proxies OR 1.5 (95 % CI 0.9, 2.6) compared to OR without proxies of 1.0 (95 % CI 0.5, 1.9); OR for > 3.5 years of use 1.1 (95 % CI 0.5, 2.5) compared to OR without proxies 0.5 (95 % CI 0.2, 1.6)); and OR for > 7 lifetime days with proxies 1.4 (95 % CI 0.6, 3.5) compared to OR without proxies of 1.1 (95 % CI 0.4, 3.3)).

Discussion & Conclusion

The objective of this study was to evaluate potential associations between glyphosate use and NHL in the NAPP, a pooled dataset that allowed for a more comprehensive analysis than previously possible in the individual studies. For NHL overall, the OR for ever use of glyphosate and NHL was near null when adjusted for reported use of 2,4-D, dicamba, and malathion (OR1.1, 95 % CI 0.8, 1.5). Analyses by years of use and lifetime days of use showed near null results for NHL overall (OR 0.9 and 1.1, respectively), while the OR for > 2 days/year was elevated (OR 1.7, 95 % CI 1.0, 2.9). It bears noting, however, that the analyses by days per year and lifetime days of use included only 50 % of the pooled population – essentially the Canadian subjects and 1 of the 4 US case-control studies. It is uncertain, therefore, how representative these results are for the entire pooled population. The results of those analyses should be interpreted accordingly.

In analyses of NHL sub-types, there tended to be moderate positive associations between the various glyphosate exposure metrics and SLL. However, as there were only 15 SLL cases overall who ever used glyphosate and 14, 7 and 7 exposed SLL cases in the analyses by years of use, days per year, and lifetime days of use, respectively, the results were not statistically significant and the 95 % CIs were imprecise. For other cell types, there were moderate positive relationships for glyphosate use of more than 2 days per year, though ORs were near null for these cell types for the higher category of years of use and lifetime days of use.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The main advantage of this pooled analysis compared with the previously published individual studies was to enable a more comprehensive analysis for glyphosate with regard to confounding factors and proxy respondents. In general, adjusting for use of 2,4-D, dicamba, and malathion reduced ORs for glyphosate. Analyses that excluded proxy respondents were generally similar to analyses that included

them, though there were some instances, specifically for other NHL subtypes, where excluding proxies appreciably reduced the adjusted OR.

Left unaddressed in this pooled analysis is the intractable issue of case-recall bias in case control studies. Crump has shown in an analysis of all the case control studies that have reported ORs for glyphosate, including the studies in this pooled analysis, that results for all pesticides were markedly skewed toward positive associations (Crump K, Risk Analysis DOI: 10.1111/risa.13440). Crump noted particularly that the ORs for individual pesticides in the McDuffie *et al*. study (and 2 other studies not included in this pooled analysis) were nearly all greater than 1.0. He considered this evidence of case recall bias. Fundamentally, using self-reported exposure recollections from cases and controls violates the basic principle that data should be collected under equivalent circumstances for the groups to be compared (viz., cases and controls). That is impossible when pesticide recall is likely to be affected by their grievous illness for cases and not for controls. Accordingly, while this pooled analysis is an advance in understanding confounding by other pesticides and in assessing the impact of reporting by proxies (except in analysis where 50 % of the subjects were excluded due to data limitations) in the 2 included studies, systematic error related to case recall bias remains an outstanding issue for interpreting the results for glyphosate.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because it concerns a pooled case control study which is subject to recall and selection bias. Notably, potential case-recall bias remains an unresolved issue in this pooled reanalysis.

Assessment and conclusion by RMS:

Reliability Criteria: Epidemiology studies

Publication: Pahwa M. *et al.,* 2019	**Criteria met? Y/N/?**	**Comments**
Study Design		
Adequate study design given study objectives	Yes	Pooled reanalysis of 2 previously published studies.
Appropriate study population to address potential glyphosate-related health outcomes	Uncertain	Populations had very limited glyphosate exposure frequency.
Exposure studied		
Exposure to formulations with glyphosate as a.s.	Yes	
Exposure to formulations with other a.s.	Yes	
Exposure to other farm exposures	Uncertain	
Study Conduct/analysis		
Adequate description of study population	Yes	
Adequate description of exposure circumstances	Yes	
Comparable participation by groups being compared	No	Much less participation by controls: example: McDuffie study –

Publication: Pahwa M. *et al.,* 2019	**Criteria met? Y/N/?**	**Comments**
		participation 67 % for cases, 48 % for controls.
Information provided by proxy respondents	Yes, substantial	31 % for cases, 40 % for controls in DeRoos study; 21 % for cases, 15 % for controls McDuffie study (per Chang & Delzell 2016)
Adequate statistical analysis	Yes	More comprehensive than the original publications regarding confounding & proxy responses. Data for 47 % of subjects were missing for analyses by days of use per year and lifetime cumulative days of use.
Adequate consideration of personal confounding factors	Yes	Better than original studies.
Adequate consideration of potentially confounding exposures	Yes	Better than original studies
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Yes	
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because illustrates bias toward positive findings for glyphosate in the original publications due to confounding and, in part, due to proxy responses. Recall bias unresolved. Missing data for 47 % of subjects for analyses by days/year of use and lifetime cumulative days of use hinders interpretation of related results.		

1. Information on the study

Data point:	CA 5.5/034
Report author	Wang, L. *et al.*
Report year	2019
Report title	Glyphosate induces benign monoclonal gammopathy and promotes multiple myeloma progression in mice
Document No	doi.org/10.1186/s13045-019-0767-9 E-ISSN: 1756-8722
Guidelines followed in study	None

Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary of the study according to OECD format

Glyphosate is the most widely used herbicide in the USA and worldwide. There has been considerable debate about its carcinogenicity. Epidemiological studies suggest that multiple myeloma (MM) and non-odgkin lymphoma (NHL) have a positive and statistically significant association with glyphosate exposure. As a B cell genome mutator, activation-induced cytidine deaminase (AID) is a key pathogenic player in both MM and B cell NHL. Vk*MYC is a mouse line with sporadic MYC activation in germinal center B cells and considered as the best available MM animal model. Vk*MYC mice and wild-type mice were treated with drinking water containing 1000 mg/L of glyphosate and examined animals after 72 weeks. Vk*MYC mice under glyphosate exposure developed progressive hematological abnormalities and plasma cell neoplasms such as splenomegaly, anaemia, and high serum IgG. Moreover, glyphosate caused multiple organ dysfunction, including lytic bone lesions and renal damage in Vk*MYC mice. Glyphosate-treated wild-type mice developed benign monoclonal gammopathy with increased serum IgG, anaemia, and plasma cell presence in the spleen and bone marrow. Finally, glyphosate upregulated AID in the spleen and bone marrow of both wild-type and Vk*MYC mice. Conclusions: These data support glyphosate as an environmental risk factor for MM and potentially NHL and implicate a mechanism underlying the B cell-specificity of glyphosate-induced carcinogenesis observed epidemiologically.

Materials and methods

Mouse model and treatments; All chronic and acute animal experiments were performed in accordance with NIH guidelines and under protocols approved by the Cleveland Clinic Institutional Animal Care and Use Committee. Wild-type (WT) C57Bl/6 mice were purchased from the Jackson Laboratory (Bar Harbor, ME). Vk*MYC mice in the C57Bl/6 genetic background were obtained from Dr. Leif Bergsagel (Mayo Clinic, Scottsdale, AZ). Vk*MYC and WT mice were intercrossed to obtain WT and Vk*MYC littermates. Sex-matched WT and Vk*MYC mice (8 weeks old) were assigned to treatment or control groups based on body weight. For chronic study of glyphosate effects, treatment groups were provided 1.0 g/L glyphosate (Sigma-Aldrich, St. Louis, MO) in their drinking water for 72 weeks. Regular drinking water was provided for the control groups (Fig. 1a). Every 6 weeks, blood was collected from the tail vein of mice, and the serum IgG level was measured. At week 72, the remaining 3 surviving Vk*MYC mice reached humane endpoints. These 3 treated Vk*MYC mice were used for M-spike detection and pathologic analyses, along with mice from other groups. Other Vk*MYC mice that were sacrificed before week 72 were analysed for total serum IgG levels, complete blood cell count, and total serum creatinine. For comparison, mice from other groups were euthanized at week 72 and their tissues and blood analyzed. For acute treatment, 8-week old mice (n = 5 per group) were given 0, 1.0, 5.0, 10.0, or 30.0 g/L of glyphosate for 7 days before sacrifice. The same variables were analyzed in the acute study.

Blood and post-mortem assays; Whole-blood complete blood count (CBC), IgG enzymelinked immunosorbent assay (ELISA), serum protein electrophoresis, flow cytometry, and histological examinations of relevant tissues were performed as described previously. Serum creatinine was measured by ELISA using a creatinine assay kit (#ab65340, Abcam, Cambridge, MA) according to the manufacturer's protocol.

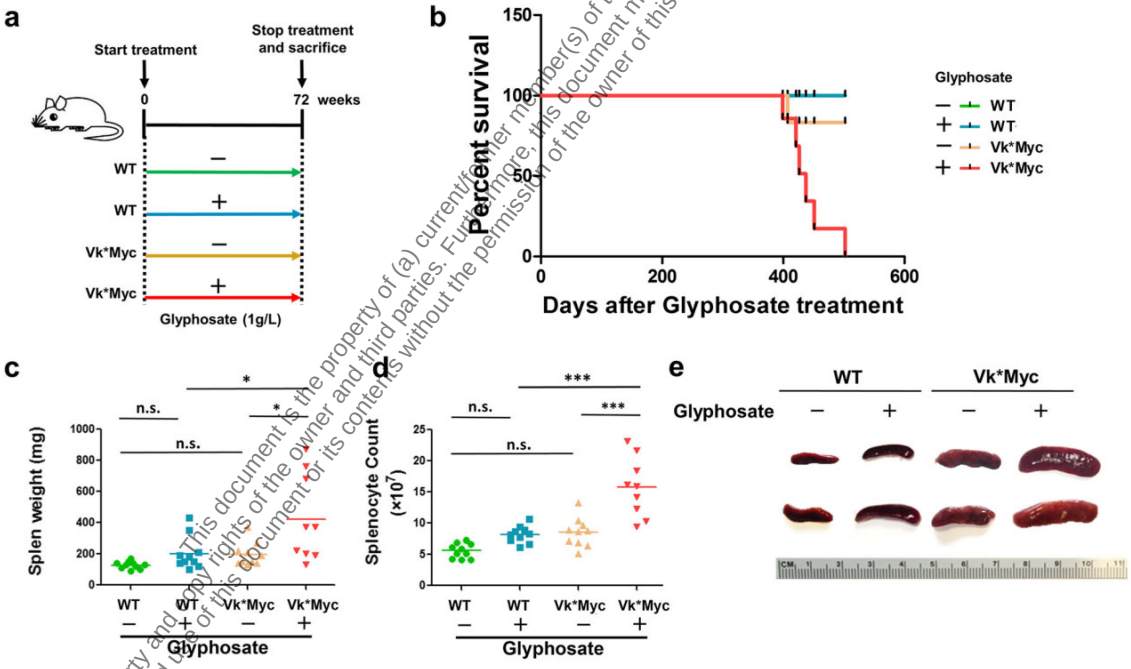
Western blotting analyses; Mouse tissues were processed for Western blotting. The antibodies were from Cell Signaling Technology (Danvers, MA, USA): AID (L7E7) (#4975) and β-actin (#3700). Blotting was run with 3 technical replicates. Horseradish peroxidase- conjugated anti-rabbit or anti-mouse IgG was used as the secondary antibody.

Statistics; Statistical analysis was carried out using GraphPad InStat 3 software (GraphPad Software, Inc., San Diego, CA, USA). The statistical significance between the groups was determined by one-way or two-way analysis of variance (ANOVA) with the appropriate post hoc testing using Tukey's test. Statistical significance was accepted at $P \leq 0.05$. All data are shown as mean ± SEM unless otherwise indicated.

Results

*Chronic glyphosate exposure reduces survival and induces splenomegaly in Vk*MYC mice*; Eight-week-old Vk*MYC mice and their WT littermates were provided 1.0 g/L glyphosate in drinking water for 72 weeks, and animals were monitored at regular intervals before sacrifice (Figure 5.5.2-6a). Glyphosate significantly affected the health of Vk*MYC mice, all of which had to be euthanized by week 72 (Figure 5.5.2-6b). Surviving mice in other groups were sacrificed at week 72 (at age 80 weeks) for necropsy. Inspection of organs revealed a marked increase in spleen weight and size in Vk*MYC mice treated with glyphosate compared to the other 3 groups (Figure 5.5.2-6c, e). Glyphosate significantly augmented the splenocyte number in Vk*MYC mice (Figure 5.5.2-6d). Findings indicate that glyphosate induces splenomegaly in both WT and Vk*MYC mice.

Figure 5.5.2-6: Glyphosate reduced survival and induced splenomegaly in Vk*MYC mice. a Schematic diagram of the chronic glyphosate exposure regimen in 4 groups of mice. b The percentage of mice surviving under glyphosate exposure. The line (blue) to indicate untreated WT mice aligned directly with that for WT treated mice and so was not visible. c Mouse spleen weight at sacrifice. d The total number of splenocytes per spleen from mice at sacrifice. e Representative images of spleens from 4 groups (2 per group).



*Hematological abnormalities occur in Vk*MYC mice with chronic glyphosate exposure*; As illustrated in Fig. 2a, untreated Vk*MYC mice exhibited higher IgG levels than untreated WT mice. Upon glyphosate exposure, WT mice showed moderate yet steady increasing in IgG levels, suggesting that glyphosate induces benign monoclonal gammopathy, a mouse equivalent to human MGUS. Vk*MYC mice receiving glyphosate had greater IgG elevation, and by week 30, IgG levels jumped to 11.78 g/L, more than 5-fold the 2.07 g/L observed in untreated Vk*MYC mice. From week 36 to week 72, the mean IgG level was significantly higher in treated WT and Vk*MYC mice compared to the untreated control groups, and

Vk*MYC mice, treated or untreated, had higher IgG levels than their WT counterparts. Overt MM diagnosis was determined by serum protein electrophoresis (SPEP) analysis to detect the M-spike, which is a significant IgG monoclonal peak. SPEP results showed that Vk*MYC mice treated with glyphosate had a clear M-spike, whereas weaker M-spike was observed in glyphosate- treated WT mice. No clear M-spike was present in the untreated WT mice or Vk*MYC mice (Fig. 2b). This is the direct *in vivo* evidence that glyphosate exposure leads to M-spike, a cardinal hematological abnormality consistent with MM. Hematological abnormalities were present in glyphosate treated mice as compared to untreated control mice (Fig. 2c–i). Data support the notion that glyphosate induces multiple hematological abnormalities characteristic of MM in mice.

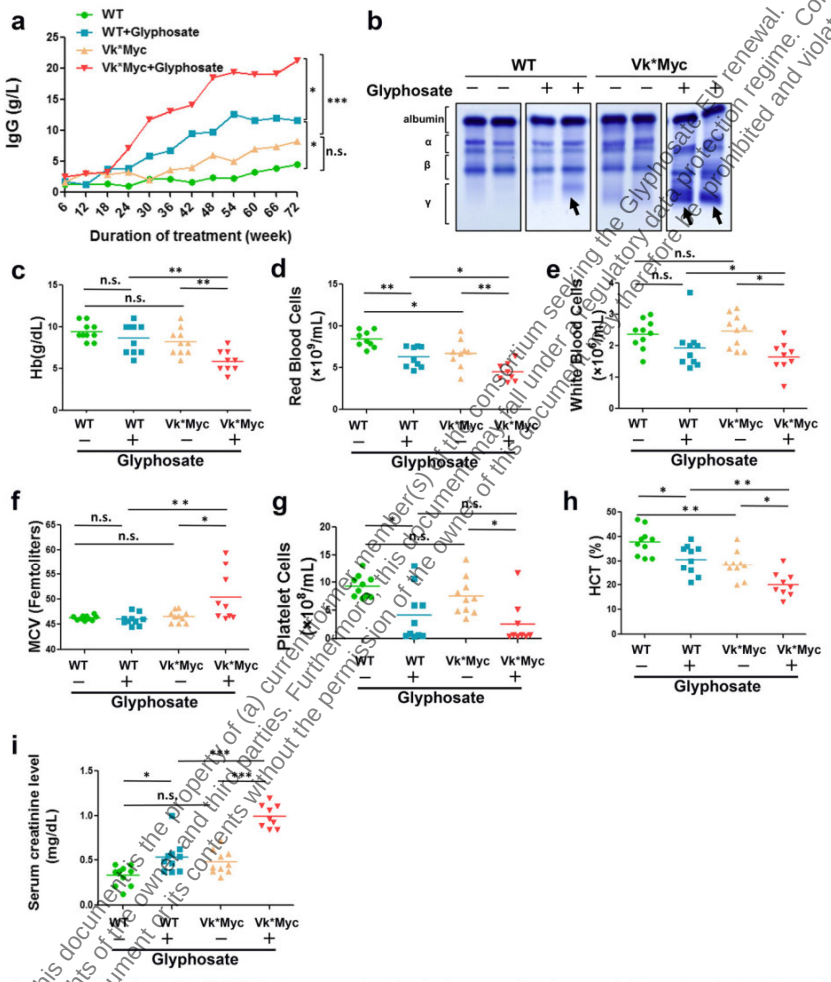


Fig. 2. Hematological abnormalities found in Vk*MYC mice treated with glyphosate. **a** Total serum IgG in mice during 72 weeks of glyphosate treatment. Mouse blood samples were collected and assayed for IgG every 6 weeks. **b** Immunoglobins from mice as determined by SPEP at week 72. Arrows indicate IgG clonal peaks (M-spike; γ-globulin peak). SPEP was performed for all mice in each group, and representative results of 2 mice per group are shown. **c**–**h** Complete blood cell counts in mice. Hemoglobin concentration (Hb, **c**), red blood cell count (**d**), white blood cell count (**e**), mean red cell volume (MCV, **f**), platelet cell count (**g**), and hematocrit (HCT, **h**) are shown. **i** Total serum creatinine in mice at week 72. The horizontal lines indicated the mean value. Data were analyzed by two-way ANOVA (**b**) or one-way ANOVA (**a**, **d**, **e**). $n = 10$ mice per group

*Vk*MYC mice chronically exposed to glyphosate develop progressive plasma cell neoplasms;* Plasma cells exhibit CD138hi B220–(high CD138 expression without B220 expression). Flow cytometric analyses of cells harvested from the spleens and bone marrow showed expansion of plasma cells in mice under glyphosate exposure. A marked increase in the numbers of CD138hi B220– cells was detected in both WT and Vk*MYC mice treated with glyphosate (Fig. 3a). These data demonstrate that glyphosate treatment expands the plasma cell population in the spleen and bone marrow in both WT and Vk*MYC mice.

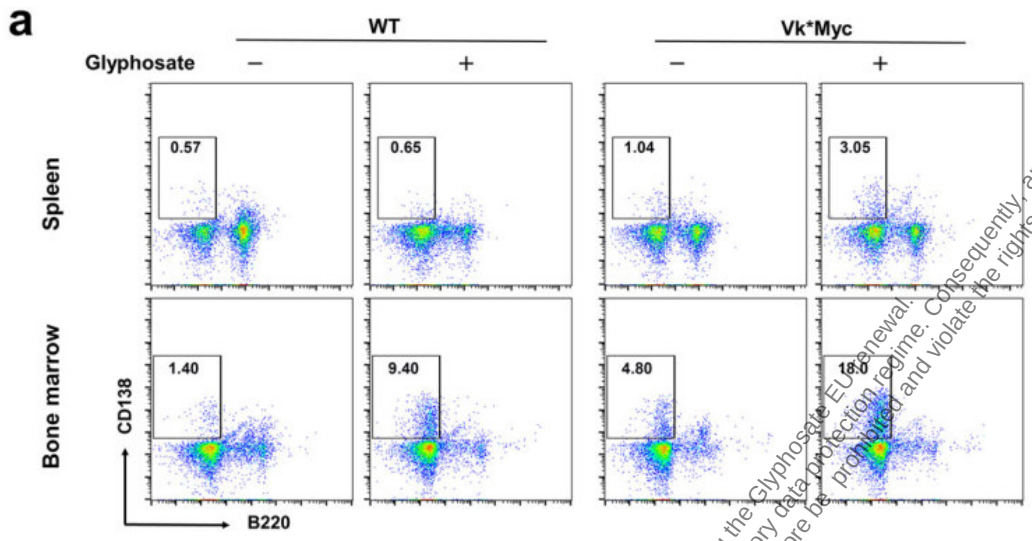


Fig. 3 Glyphosate-treated Vk*MYC mice developed progressive plasma cell neoplasms. **a** Representative flow cytometry plots detecting cell surface markers CD138 (*Y*-axis) and B220 (*X*-axis) in splenocytes (upper panel) and bone marrow cells (lower panel). The numbers on the axes denoted the $\log_{10}$ values of fluorescence. The numbers in the inserts show the percentage of $CD138^{high}B220^{-}$ cells in the entire cell population.

Chronic glyphosate exposure triggers multiple organ dysfunction; To determine whether target organ damage occurred in glyphosate-treated mice, the femoral shaft, spleen, liver, lung, and kidney were sectioned and stained with hematoxylin and eosin (H&E). Severe destructive osteolytic bone lesions in the femoral shaft were readily detectable in glyphosate-treated Vk*MYC mice. Treated WT mice showed smaller bone lesions. No lesions were observed in the control groups (Fig. 4a). Plasma cells with a perinuclear clear zone and eccentric round nucleus were observed in glyphosate-treated WT and Vk*MYC mice (Fig. 4b, c). Next, the histopathologic changes in the liver, lung, and kidney were analyzed. In glyphosate-treated mice, hepatic fibrosis and collagen deposition were observed in Vk*MYC mice, whereas WT mice showed less severe liver damage; the 2 control groups had normal hepatic tissue architectures (Fig. 4d). The lungs in treated Vk*MYC mice were severely damaged, with large distal air spaces filled by lymphocytes, neutrophils, cell debris, and hyperplastic pneumocytes; those from untreated WT mice had normal alveolar spaces and alveolar septa lined with normal pneumocytes. The lungs from treated WT mice and untreated Vk*MYC mice showed an intermediate phenotype (Fig. 4e). Renal tubular obstruction by large casts, indicative of necrotic tubular cells, were detected in glyphosate-treated WT and Vk*MYC mice, but not in the untreated groups; there were more and larger casts in treated Vk*MYC kidneys than in WT kidneys (Fig. 4f). Taken together, these data indicate that glyphosate treatment damages multiple organs in both WT and Vk*MYC mice with more severe damage occurring in Vk*MYC mice.

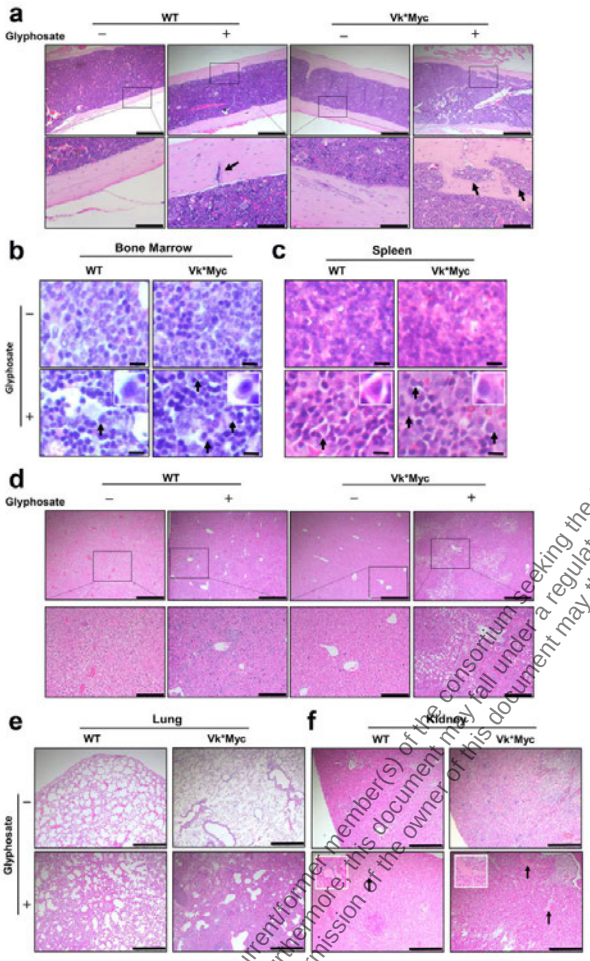


Fig. 4 Glyphosate led to multiple organ dysfunction. **a** Histological evaluation of bone morphology from 4 groups of mice. Bone lytic lesions (indicated by arrows) were detected in the femoral shaft of Vk*MYC mice treated with glyphosate. Scale bar = 500 μm (top) or 100 μm (bottom). **b** Infiltrating plasma cells in the bone marrow of glyphosate-treated mice. Scale bar = 20 μm. Arrows pointed to plasma cells. **c** Infiltrating plasma cells in the spleen of glyphosate-treated mice. Scale bar = 20 μm. Arrows point to plasma cells. **d** Collagen deposition in the liver was observed in glyphosate-treated Vk*MYC mice. $n = 10$ mice per group. Scale bar = 500 μm (top) or 200 μm (bottom). **e** Destruction of lung morphology was observed in glyphosate-treated Vk*MYC mice. $n = 10$ mice per group. Scale bar = 500 μm. **f** Protein deposition (indicated by arrows) in the kidney was observed in glyphosate-treated Vk*MYC mice. $n = 10$ mice per group. Scale bar = 500 μm. All panels show 1 representative image each from 4 groups of mice unless otherwise indicated

Chronic glyphosate exposure induces AID upregulation; To investigate the underlying mechanisms of glyphosate-mediated MGUS induction and MM progression, expression of activation-induced cytidine deaminase (AICDA, also known as AID) in mice treated with 1.0 g/L glyphosate for 72 weeks was examined. It was found that AID was upregulated in both the bone marrow and the spleen of WT and Vk*MYC mice (Fig. 5a).

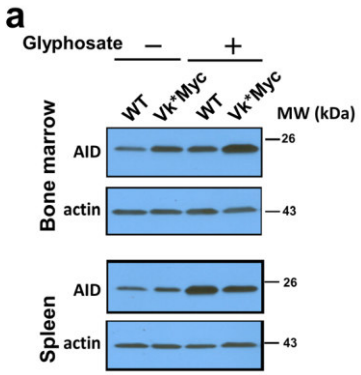


Fig. 5 Glyphosate-induced AID upregulation. **a** Western blotting analysis of mice treated with 1.0 g/L of glyphosate for 72 weeks.

Acute glyphosate exposure induces AID upregulation; To determine the acute effect of glyphosate, 8-week-old WT and Vk*MYC mice were treated with increasing doses of glyphosate (1, 5, 10, and 30 g/L) in drinking water for 7 days. This acute treatment neither increased spleen weight nor affected body weight significantly. Next, expression of AID in the spleen, bone marrow, and lymph nodes were analysed. It was found that AID was upregulated in a glyphosate dose-dependent manner in the spleen and bone marrow of WT and Vk*MYC mice treated with 10 and 30 g/L of glyphosate (Fig. 5c). Given the role of AID in MM pathogenesis in the context of its capacity to induce mutations and chromosome translocations, these results from mice with chronic and acute glyphosate treatment support an AID mediated mutational mechanism in the etiology of MGUS and MM under glyphosate exposure.

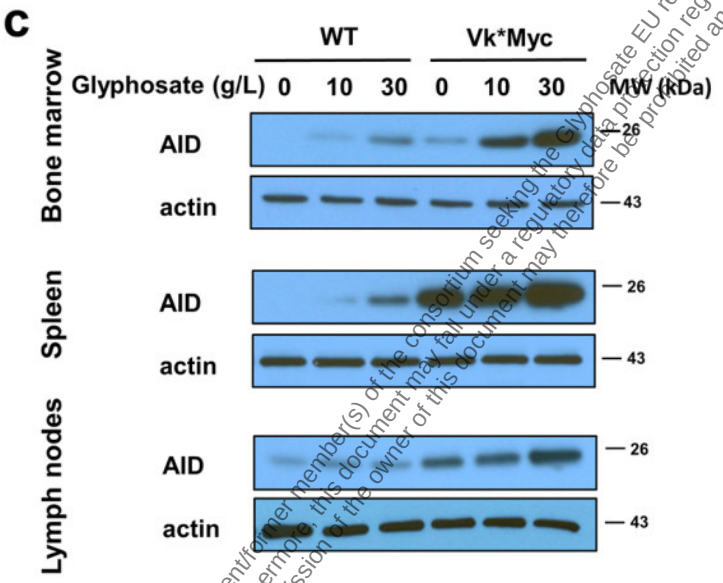


Fig. 5 c Western blotting analysis of mice treated with glyphosate for 7 days. One representative mouse

Discussion

In this study, it was demonstrated that glyphosate induces benign monoclonal gammopathy (mouse equivalent to MGUS in human) in WT mice and promotes MM progression in Vk*MYC mice. In Vk*MYC mice, glyphosate causes hematological abnormalities like anemia and multiple organ dysfunction like lytic bone lesions and renal damage, which are hallmarks of human MM. Beyond epidemiology and animal models, the mechanism of action is the third pillar required to define a compound as a carcinogen. Numerous studies have revealed that glyphosate may induce DNA damage, oxidative stress, inflammation, and immunosuppression, as well as modulate cell proliferation and death and disrupt sex hormone pathways. However, these mechanistic studies have failed to explain why glyphosate exposure is only positively associated with MM and NHL. Our results demonstrate that glyphosate treatment, either at a chronic low dose or acute high doses, upregulates the expression of AID in the bone marrow and spleen of both WT and Vk*MYC mice. The data disclose, for the first time, that glyphosate elicits a B cell-specific mutational mechanism of action in promoting carcinogenesis, as well as offering experimental evidence to support the epidemiologic finding regarding its tissue specificity in carcinogenesis (i.e. only increasing the risk for MM and NHL). The "acceptable daily intake (ADI)" of glyphosate currently allowed in the USA, defined as the chronic reference dose as determined by EPA, is 1.75 mg/kg body weight/day; an average adult male or female in the USA who weighs 88.8 or 76.4 kg and drinks 2 L (8 glasses) water daily containing 77.7 (for male) or 66.9 (for female) mg/L glyphosate would reach the ADI. A dose of 1,000 mg/L glyphosate in drinking water (~ 15-fold the ADI) was chosen in this study, which caused significant adverse effects and accelerated MM progression in Vk*MYC mice, i.e. animals predisposed to MM.

Conclusion
The data provide *in vivo* evidence to support that glyphosate induces MGUS and promotes disease progression to MM. A B cell-specific mutational mechanism for glyphosate exposure that increases MM and NHL risk was uncovered, providing a molecular basis for human epidemiological findings.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The objective of this study was to investigate the pathogenic role of glyphosate in multiple myeloma using Vk*MYC mice. The study did demonstrate the ability of glyphosate to impact measured parameters in the tested models. However, this study is not appropriate for human health risk assessment. The number or animals per group was below the recommended number for guideline toxicity studies and to perform sufficient statistical analysis. Only one dose level was used in the chronic study. It was not possible to correlate effects with a glyphosate dose-response as the water consumption (and therefore test substance intake) of animals was not provided and it is therefore impossible to calculate a dose on a mg/kg bw basis for risk assessment.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate used was not characterized, only one dose was considered for the chronic study and the number of animals used per group was either too low (acute study) or not reported (chronic study).

Assessment and conclusion by RMS:

Reliability criteria for *in vivo* toxicology studies

Publication: Wang *et al*., 2019	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y?	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	N	Purity not reported. Source: Sigma-Aldrich, St Louis, USA.
Only glyphosate acid or one of its salts is the tested substance		
AMPA is the tested substance		
Study		
Test species clearly and completely described	Y	Wild-type (WT) C57Bl/6 mice and Vk*MYC mice.
Test conditions clearly and completely described		
Route and mode of administration described	Y	Oral *via* drinking water.
Dose levels reported	Y	For the chronic study: 1 g/L in drinking water for 72 weeks.

		For the acute study: 1, 5, 10, 30 g/L for 7 days.
Number of animals used per dose level reported	Y	5/group for the acute study, no/group not reported for the chronic study.
Method of analysis described for analysis test media	N	
Validation of the analytical method	N	
Analytical verifications of test media	N	
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical control data of the laboratory reported	N	
Dose-effect relationship reported	N	Not possible. Only one dose level used.
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate used was not characterized, only one dose was considered for the chronic study and the number of animals used per group was either too low (acute study) or not reported (chronic study).		

1. Information on the study

Data point:	CA 5.5/035
Report author	Andreotti, G. *et al*.
Report year	2018
Report title	Glyphosate Use and Cancer Incidence in the Agricultural Health Study
Document No	doi: 10.1093/jnci/djx233 ISSN: 0027-8874
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Yes/Reliable/Reliable with restrictions

2. Full summary of the study according to OECD format

In 2005, an evaluation of glyphosate and cancer risk was conducted in the Agricultural Health Study (AHS) (DeRoos *et al*. 2005). This evaluation considered glyphosate use reported at enrollment (1993–1997) and included 2,088 cancers diagnosed between enrollment and 2001. No statistically significant associations were found for any cancer site. For NHL, the relative risk (RR) adjusted for age, personal factors and other pesticides was 1.1 (95 % CI 0.7-1.9) and there was no trend of increasing RRs with increasing frequency of glyphosate. For multiple myeloma, the overall adjusted RR was 2.6 (95 % CI 0.7 – 9.4) and the assessment of RR by frequency of glyphosate use did not evidence a significant trend. Andreotti *et al*. 2018 updated the 2005 AHS publication by DeRoos *et al*. (2005), extending cancer incidence follow-up through

2012 in North Carolina and 2013 in Iowa and incorporating additional exposure information from a follow-up questionnaire. The authors also dealt with missing information through imputation and conducted sensitivity analyses to address the potential for various types of bias in their primary analyses. This 2018 publication includes a total of 7,290 cancers, 3.6 times as many as in the earlier publication. The median lifetime days of glyphosate use for cohort members who reported glyphosate use (83 % of the cohort) was 48 days (interquartile range (IQR) 20 to 166 days). The authors found no evidence of an association between glyphosate use and risk of any solid tumour, NHL (RR 0.87 (95 % CI 0.64, 1.20 in the highest intensity weighted exposure quartile, p_{trend} 0.95), or multiple myeloma (RR 0.87, 95 % CI 0.45, 1.69 highest quartile, p_{trend} 0.84). They found a moderately elevated RR for acute myelogenous leukaemia that was not statistically significant (RR 2.44, 95 % CI 0.94, 6.32, p_{trend} = 011). The findings for cancer types were consistent across different exposure metrics, in various sensitivity analyses, and for lagged exposure analyses meant to address cancer induction-latency.

Materials and methods

Study design

Briefly, 57,310 individuals seeking licenses to apply restricted-use pesticides were enrolled between 1993 and 1997. Of the enrolled participants, 63 % completed a follow-up phone interview approximately five years after enrollment (1999–2005). Incident cancer diagnoses were ascertained *via* linkage to cancer registries in Iowa (through 2013) and North Carolina (through 2012). Cancer diagnoses were classified according the International Classification of Disease– Oncology, 3rd Revision. Subtypes of lymphoid malignancies were defined according to the Surveillance, Epidemiology, and End Results Program Lymphoma Subtype Recodes. According to this updated classification of lymphoid malignancies, multiple myeloma was included in the analyses as a subtype of non-Hodgkin's lymphoma. Vital status was ascertained *via* state mortality registries and the National Death Index, and state of residence was regularly updated using various government databases.

Exposure assessment

Lifetime use of glyphosate and 49 other pesticides was ascertained at enrollment and in the follow-up questionnaire. At enrollment, applicators reported the number of years and days per year each pesticide was used, while at follow-up applicators reported the number of days each pesticide was used in the most recent year farmed. Using this information, three metrics of cumulative lifetime exposure were created for each pesticide: ever/never use, lifetime days of use (days per year multiplied by the number of years), and intensity-weighted lifetime days (lifetime days multiplied by an intensity score). The intensity score was derived from an algorithm based on literature-based measurements and information provided by the applicator, specifically whether the participant mixed or applied pesticides, repaired pesticide-related equipment, used personal protective equipment, and application method used. For participants who did not complete the follow-up questionnaire (37 %), multiple imputation was used to impute pesticide use since enrollment. Factors used to impute pesticide use included demographic data and medical history, as well as factors related to farm characteristics and reported pesticide use at enrollment.

Statistical analysis

For this analysis, individuals who had a history of cancer at enrollment (n=1096), did not live in North Carolina or Iowa (n=343), or did not report whether they had used glyphosate or not at enrollment (n=1620) were excluded, resulting in an analytic sample of 54,251 licensed farmers and applicators. Individuals accumulated person-time from enrollment until the earliest of the following events: movement out of state, diagnosis of cancer, death, or end of the follow-up period (December 31, 2012 in NC, December 31, 2013 in IA). Poisson regression was used to calculate incidence rate ratios (RRs) and 95 % confidence intervals (CIs), and PROC MIANALYZE was used to obtain the appropriate variance for the imputed data. All statistical significance tests were two-sided and considered to be statistically significant when $p \leq 0.05$. RRs for total cancer and for cancer sites with at least 20 exposed cases were evaluated. For analyses by exposure level, based on the distribution among all cancer cases, cumulative lifetime days and intensity-weighted lifetime days of glyphosate exposure into quartiles, tertiles, or the median were categorized, such that there were at least five exposed cases in each category. Linear trend was evaluated according to the

Wald test using the median of each exposure category as a continuous variable. Risk estimates were adjusted for attained age (continuous), cigarette smoking status (never, former, current), alcohol drinks per month (none, ≤ 6 per month, ≥ 7 per month), family history of any cancer (yes, no), state of recruitment (North Carolina, Iowa), and the five pesticides most highly correlated with glyphosate based on lifetime days and intensity-weighted lifetime days (r > 0.4: atrazine, alachlor, metolachlor, trifluralin, 2,4-D). Lagged exposure was also evaluated allowing for 5, 10, 15, or 20 years to address the induction-latency period for specific cancers. Other potential confounding factors were calculated, including body mass index (BMI; <25, 25–<30, _30 kg/m^2) and pack-years of cigarettes smoked (tertiles of use among former and current smokers). The numbers of women and nonwhites were small, precluding adjustment for sex and race for most cancer sites; in sensitivity analyses, the risks in men and whites alone were assessed. For lymphohematopoietic cancers, RRs were additionally adjusted for occupational exposure to solvents, gasoline, x-ray radiation, and engine exhaust, and pesticides linked to lymphohematopoietic malignancies in previous AHS analyses (lindane, DDT, diazinon, terbufos, and permethrin). The risk of NHL excluding multiple myeloma was calculated for comparison with previously published studies. Lastly, sensitivity analyses were conducted to evaluate the impact of including additional exposure information.

Results

Among 54,251 participants, 44,932 (82.8 %) reported ever using glyphosate at enrollment or during follow-up. Among the participants who used glyphosate, the median lifetime days of use was 48 (interquartile range [IQR] = 20–166 days), and the median lifetime years of use was 8.5 years (IQR = 5–14 years). A total of 7,290 incident cancers were diagnosed during the follow-up period. Among the participants who used glyphosate and were diagnosed with cancer during follow-up (n=5,779), the median lifetime days of use was 38.75 (IQR = 13.75–108.5 days), and the median lifetime years of use was 8.0 (IQR = 3.5–13.0). Selected characteristics of the study participants by glyphosate use are presented in Table 1. Risk ratios for intensity-weighted lifetime days of glyphosate use and cancer risk are shown in Table 2. Glyphosate use was not associated with total cancer or with lymphohematopoietic malignancies. There also was no evidence for positive associations with NHL (RR in the highest intensity weighted days of glyphosate use quartile = 0.9 (95 % CI 0.6 – 1.2)), multiple myeloma ($RR_{quartile\ 4}$ = 0.9, 95 % CI 0.5 – 1.7) or for any NHL subtype. Although not statistically significant, the authors observed an elevated RR for acute myeloid leukemia (AML; n = 57 exposed cases) among applicators in the highest quartile of intensity weighted glyphosate use compared with never users (n = 18 cases, RR.2.4, 95 % CI = 0.9 to 6.3, P_{trend} =.11). The results based on intensity weighted days of use were very similar to results based on unweighted days of use.

The impact of lagging exposure on risk estimates for lymphohematopoietic cancers was evaluated for intervals of 5, 10, 15 and 20 years. The patterns of risk for lagged exposures were similar to those for unlagged exposures.

Conclusion

In conclusion, the authors found no evidence of an association between glyphosate use and risk of any solid tumor, NHL, or multiple myeloma. They found an elevated RR for AML that was not statistically significant, but that merits evaluation in AHS updates or other studies. This findings across cancer types were consistent across different exposure metrics, in various sensitivity analyses, and for lagged exposure analyses meant to address cancer induction-latency.

Table 1. Selected characteristics of the Agricultural Health Study population by glyphosate use

Characteristics*	Never-used glyphosate No. (%)	Lifetime days of glyphosate use† < Median No. (%)	Lifetime days of glyphosate use† ≥ Median No. (%)
Total	9319 (100.0)	19 714 (100.0)	24 727 (100.0)
Age at enrollment, y			
<30	814 (8.7)	1726 (8.8)	2372 (9.6)
30–39	1730 (18.6)	4293 (21.8)	6612 (26.7)
40–49	2217 (23.8)	5304 (26.9)	7437 (30.1)
50–59	2051 (22.0)	4261 (21.6)	4759 (19.2)
60–69	1797 (19.3)	3043 (15.4)	2738 (11.1)
70+	710 (7.6)	1087 (5.5)	809 (3.3)
Sex			
Male	8887 (95.4)	19 220 (97.5)	24 203 (97.9)
Female	432 (4.6)	494 (2.5)	524 (2.1)
Race			
White	8838 (94.8)	19 128 (97.0)	24 267 (98.1)
Black and other	441 (4.7)	538 (2.7)	404 (1.6)
Missing	40 (0.4)	48 (0.2)	56 (0.2)
State of recruitment			
Iowa	6692 (71.8)	12 668 (64.3)	15 756 (63.7)
North Carolina	2627 (28.2)	7046 (35.7)	8971 (36.3)
Applicator type			
Private (farmer)	8476 (91.0)	18 717 (94.9)	21 932 (88.7)
Commercial	843 (9.0)	997 (5.1)	2795 (11.3)
Highest level of education			
High school or less	6528 (70.1)	11 409 (57.9)	12 005 (48.6)
Beyond high school	2569 (27.6)	7884 (40.0)	12 213 (49.2)
Missing	222 (2.4)	421 (2.1)	509 (2.1)
Body mass index, kg/m²			
<25	1656 (17.8)	3779 (19.2)	4168 (16.9)
25–<30	3044 (32.7)	7123 (36.1)	8492 (34.3)
30+	1435 (15.4)	3175 (16.1)	3985 (16.1)
Missing	3184 (34.2)	5637 (28.6)	8082 (32.7)
Cigarette smoking status			
Never	4987 (53.5)	10 371 (52.6)	12 876 (52.1)
Former	2621 (28.1)	6004 (30.5)	7295 (29.5)
Current	1526 (16.4)	3147 (16.0)	4355 (17.6)
Missing	185 (2.0)	192 (1.0)	201 (0.8)
Cigarette smoking pack-years			
Never	4987 (53.5)	10 371 (52.6)	12 876 (52.1)
Former, tertile 1	896 (9.6)	2004 (10.2)	2471 (10.0)
Former, tertile 2	791 (8.5)	1865 (9.5)	2198 (8.9)
Former, tertile 3	741 (8.0)	1748 (8.9)	2109 (8.5)
Current, tertile 1	548 (5.9)	1037 (5.3)	1513 (6.1)
Current, tertile 2	453 (4.9)	975 (4.9)	1399 (5.7)
Current, tertile 3	461 (4.9)	1076 (5.5)	1376 (5.6)
Missing	442 (4.7)	638 (3.2)	785 (3.2)
Usual number of alcohol drinks per month in year prior enrollment			
Never	3150 (33.8)	6406 (32.5)	6946 (28.1)
≤6/mo	3036 (32.6)	6646 (33.7)	8240 (33.3)
≥7/mo	2492 (26.7)	5631 (28.6)	8646 (35.0)
Missing	641 (6.9)	1030 (5.2)	895 (3.6)

(continued)

Table 1. (continued)

Characteristics*	Never-used glyphosate No. (%)	Lifetime days of glyphosate use† < Median No. (%)	Lifetime days of glyphosate use† ≥ Median No. (%)
Family history of cancer			
No	5452 (58.5)	10 846 (55.0)	13 866 (56.1)
Yes	3226 (34.6)	7700 (39.1)	9876 (39.9)
Missing	641 (6.9)	1168 (5.9)	985 (4.0)

*Data from the enrollment questionnaire.

†Based on median cumulative lifetime days of glyphosate use among all cancer cases (38.75 days)

Table 2. Cancer incidence in relation to intensity-weighted lifetime days of glyphosate use in the Agricultural Health Study

Cancer site*	Glyphosate use†	No.	RR (95% CI)‡	P_{trend}‡
All cancers				
	None	1511	1.00 (reference)	
	Q1	1445	0.99 (0.91 to 1.07)	
	Q2	1443	0.99 (0.91 to 1.07)	
	Q3	1440	1.04 (0.96 to 1.13)	
	Q4	1451	0.99 (0.91 to 1.08)	.91
Oral cavity				
	None	33	1.00 (reference)	
	Q1	36	0.95 (0.56 to 1.60)	
	Q2	35	0.92 (0.54 to 1.57)	
	Q3	35	0.96 (0.56 to 1.65)	
	Q4	35	0.84 (0.48 to 1.46)	.54
Colon				
	None	116	1.00 (reference)	
	Q1	104	1.00 (0.74 to 1.35)	
	Q2	102	1.03 (0.76 to 1.39)	
	Q3	102	1.06 (0.78 to 1.44)	
	Q4	96	1.01 (0.74 to 1.38)	1.00
Rectum				
	None	50	1.00 (reference)	
	Q1	43	0.81 (0.51 to 1.28)	
	Q2	55	1.16 (0.76 to 1.76)	
	Q3	39	0.80 (0.50 to 1.29)	
	Q4	46	0.84 (0.52 to 1.34)	.43
Pancreas				
	None	25	1.00 (reference)	
	Q1	42	1.80 (1.05 to 3.08)	
	Q2	42	1.69 (0.98 to 2.94)	
	Q3	24	1.09 (0.59 to 2.02)	
	Q4	23	1.06 (0.57 to 1.97)	.14
Lung				
	None	144	1.00 (reference)	
	Q1	117	0.92 (0.70 to 1.22)	
	Q2	138	1.19 (0.91 to 1.56)	
	Q3	159	1.39 (1.07 to 1.82)	
	Q4	131	1.00 (0.76 to 1.33)	.78
Melanoma				
	None	56	1.00 (reference)	
	Q1	59	1.00 (0.67 to 1.50)	
	Q2	67	1.18 (0.80 to 1.74)	
	Q3	69	1.12 (0.75 to 1.67)	
	Q4	78	1.17 (0.78 to 1.74)	.53
Prostate				
	None	579	1.00 (reference)	
	Q1	571	0.99 (0.87 to 1.12)	
	Q2	564	0.95 (0.83 to 1.08)	
	Q3	559	1.03 (0.91 to 1.18)	
	Q4	571	0.99 (0.86 to 1.13)	.89
Testicular				
	None	7	1.00 (reference)	
	T1	17	1.28 (0.49 to 3.34)	
	T2	12	0.74 (0.26 to 2.09)	
	T3	11	0.57 (0.20 to 1.67)	.07
Bladder				
	None	66	1.00 (reference)	
	Q1	86	1.29 (0.91 to 1.82)	
	Q2	68	1.04 (0.72 to 1.51)	
	Q3	66	1.09 (0.75 to 1.59)	
	Q4	79	1.26 (0.87 to 1.82)	.42

(continued)

Table 2. (continued)

Cancer site*	Glyphosate use†	No.	RR (95% CI)‡	P_{trend}‡
Kidney				
	None	54	1.00 (reference)	
	Q1	54	1.13 (0.74 to 1.71)	
	Q2	50	0.91 (0.59 to 1.41)	
	Q3	45	0.87 (0.55 to 1.38)	
	Q4	53	1.03 (0.66 to 1.61)	.95
Lymphohematopoietic				
	None	161	1.00 (reference)	
	Q1	136	0.87 (0.64 to 1.19)	
	Q2	126	0.88 (0.66 to 1.17)	
	Q3	137	0.93 (0.71 to 1.23)	
	Q4	144	1.00 (0.74 to 1.34)	.43
Hodgkin lymphoma				
	None	7	1.00 (reference)	
	M1	7	0.59 (0.17 to 2.11)	
	M2	11	0.90 (0.25 to 3.24)	.94
Non-Hodgkin lymphoma				
	None	135	1.00 (reference)	
	Q1	113	0.83 (0.59 to 1.18)	
	Q2	104	0.83 (0.61 to 1.12)	
	Q3	112	0.88 (0.65 to 1.19)	
	Q4	111	0.87 (0.64 to 1.20)	.95
Non-Hodgkin lymphoma B cell				
	None	128	1.00 (reference)	
	Q1	102	0.79 (0.55 to 1.13)	
	Q2	93	0.76 (0.56 to 1.05)	
	Q3	106	0.88 (0.64 to 1.21)	
	Q4	103	0.86 (0.62 to 1.19)	.86
Chronic lymphocytic lymphoma/small lymphocytic leukemia				
	None	36	1.00 (reference)	
	Q1	28	0.75 (0.40 to 1.41)	
	Q2	26	0.76 (0.41 to 1.41)	
	Q3	26	0.90 (0.50 to 1.62)	
	Q4	27	0.87 (0.48 to 1.58)	.71
Diffuse large B cell lymphoma				
	None	27	1.00 (reference)	
	Q1	28	1.11 (0.60 to 2.07)	
	Q2	23	0.94 (0.49 to 1.80)	
	Q3	30	1.13 (0.59 to 2.17)	
	Q4	22	0.97 (0.51 to 1.85)	.83
Marginal-zone lymphoma				
	None	4	1.00 (reference)	
	M1	6	0.39 (0.06 to 2.45)	
	M2	5	0.44 (0.09 to 2.17)	.67
Follicular lymphoma				
	None	16	1.00 (reference)	
	T1	21	0.89 (0.37 to 2.15)	
	T2	11	0.61 (0.23 to 1.60)	
	T3	20	0.85 (0.36 to 2.03)	.95
Multiple myeloma				
	None	30	1.00 (reference)	
	Q1	19	0.70 (0.36 to 1.36)	
	Q2	26	0.94 (0.50 to 1.76)	
	Q3	19	0.78 (0.39 to 1.56)	
	Q4	24	0.87 (0.45 to 1.69)	.84
Non-Hodgkin lymphoma T cell				
	None	2	1.00 (reference)	
	M1	14	4.25 (0.73 to 24.64)	
	M2	6	1.53 (0.23 to 10.38)	.31

(continued)

Table 2. (continued)

Cancer site*	Glyphosate use†	No.	RR (95% CI)‡	P_{trend}‡
Acute myeloid leukemia				
	None	9	1.00 (reference)	
	Q1	13	1.62 (0.60 to 4.38)	
	Q2	14	1.70 (0.61 to 4.73)	
	Q3	12	1.46 (0.49 to 4.37)	
	Q4	18	2.44 (0.94 to 6.32)	.11
Chronic myeloid leukemia				
	None	7	1.00 (reference)	
	M1	5	0.36 (0.09 to 1.43)	
	M2	11	0.82 (0.23 to 2.98)	.36

*Cancer sites are based and presented in order of Surveillance, Epidemiology, and End Results Site Recode ICD-O-3. CI = confidence interval; RR = rate ratio.
†Quartiles: Q1: 1–598.9; Q2: 599–1649.9; Q3: 1650–4339.9; Q4: ≥4340.0. Tertiles: T1: 1–866.24; T2: 866.25–2963.9; T3: ≥2964.0. Median: M1: 1–1649.9; M2: ≥1650.0.
‡Poisson regression was used to model rate ratios and confidence intervals, and P values were calculated using a two-sided Wald test. All models adjusted for age, state of recruitment, education, cigarette smoking status, alcohol per month, family history of cancer, atrazine, alachlor, metolachlor, trifluralin, 2,4-D

3. Assessment and conclusion

Assessment and conclusion by applicant:

The AHS is an ongoing prospective cohort study of glyphosate and other pesticides. It was initiated in 1993 and has been ongoing for more than 25 years. Researchers from the US National Cancer Institute and other government agencies initiated the AHS as a prospective cohort study to eliminate the possibility of case-recall bias – an intractable potential bias in case control studies that rely on self-reported exposure information. Crump (Risk Analysis DOI:10.1111/risa.13440) has recently illustrated that the results from the glyphosate case-control studies align closely with what would be expected from case recall bias.

In addition to obviating concerns about case-recall bias, the Andreotti *et al*. publication is noteworthy on several counts. First, the frequency of glyphosate use by participants (median = 48 days, IQR 20 to 166 days) vastly exceeds that in the glyphosate case-control studies. In those studies the most frequent days of use category is > 10 days (Eriksson M, *et al*. Int J Cancer. 2008; 123:1657-1663), while most of the case control studies' primary analyses were based on 1 day or more of use in a lifetime. Second, the participants in the AHS were licensed pesticide applicators who were considered by the authors to be very capable to report pesticide use accurately compared with other study populations. Third, the analyses by Andreotti *et al*. controlled for a multitude of personal factors and for other pesticides in addition to incorporating a wide range of sensitivity and lagged analyses (allowing for up to 20+ years induction-latency). No other study has evaluated the relationship between glyphosate use and cancers as extensively. The AHS is, by far, the most informative and relevant study epidemiologic study for glyphosate to date. The authors found no evidence of an association between glyphosate use and risk of any solid tumour, NHL, or multiple myeloma.

Accordingly, given the AHS results for NHL among those with extensive glyphosate use (n = 111 exposed cases, RR = 0.9, 95 % CI 0.6 – 1.2), it is unlikely that the positive associations for glyphosate and NHL in some case control studies are valid. As follow-up of the AHS cohort continues, it remains to be seen whether subsequent results will identify relationships between individual cancers and glyphosate use that are relevant for risk evaluations.

Assessment and conclusion by RMS:

Reliability Criteria: Epidemiology studies

Publication: Andreotti G. *et al.,* 2018	**Criteria met? Y/N/?**	**Comments**
Study Design		
Adequate study design given study objectives	Yes	
Appropriate study population to address potential glyphosate-related health outcomes	Yes	Most appropriate population studied to date. Highest frequency of glyphosate use by far.
Exposure studied		
Exposure to formulations with glyphosate as a.s.	Yes	
Exposure to formulations with other a.s.	Yes	
Exposure to other farm exposures	Yes	
Study Conduct/analysis		
Adequate description of study population	Yes	
Adequate description of exposure circumstances	Yes	
Comparable participation by groups being compared	Yes	
Information provided by proxy respondents	No	
Adequate statistical analysis	Yes	Very comprehensive
Adequate consideration of personal confounding factors	Yes	Very comprehensive
Adequate consideration of potentially confounding exposures	Yes	Very comprehensive
Overall assessment		
Reliable without restrictions	Yes	Most reliable epidemiology study for glyphosate users versus non-users.
Reliable with restrictions	Yes	Certain analyses are limited: dose is not known, only frequency of use. So, "dose response" analyses must be interpreted cautiously.
Not reliable	No	

1. Information on the study

Data point:	CA 5.5/036
Report author	Presutti, R. *et al*.
Report year	2016
Report title	Pesticide exposures and the risk of multiple myeloma in men: An analysis of the North American Pooled Project
Document No	DOI: 10.1002/ijc.30218 ISSN: 0020-7136

Guidelines followed in study	None
Deviations from current test guideline	No
GLP/Officially recognised testing facilities	Non-GLP
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary of the study according to OECD format

Three case-control studies in the United States and Canada were pooled to create the North American Pooled Project (NAPP) to investigate associations between pesticide use and haematological cancer risk. This analysis used data from 547 MM cases and 2700 controls. Pesticide use was evaluated as follows: ever/never use; duration of use (years); and cumulative lifetime- days (LD) (days/year handled 3 years of use). Odds ratios (ORs) and 95 % confidence intervals (CIs) were estimated using logistic regression adjusted for age, province/state of residence, use of proxy respondents and selected medical conditions.

Materials and Methods

Study population - The North American Pooled Project (NAPP) is comprised of three population-based incident case-control studies conducted by the U.S. National Cancer Institute in Kansas, Iowa/Minnesota and Nebraska in the 1980s, and the Cross Canada Study of Pesticides and Health (CCSPH), a population-based incident case-control study that was conducted in Quebec, Ontario, Manitoba, Saskatchewan, Alberta and British Columbia in the early 1990s. All 4 studies aimed to investigate the effects of pesticides and other agricultural exposures on the risk of lymphatic and hematopoietic cancers. The present analysis is restricted to a subset of three NAPP studies conducted in Iowa, Nebraska and Canada (CCSPH) where multiple myeloma (MM) cases were recruited. The study design and data collection in the CCSPH were modeled after the U.S. studies, making the data amenable to pooling. Eligible participants included white men aged 30 years or older in Iowa, white men and women aged 21 years or older in Nebraska, and men aged 19 years or older in Canada. Deceased participants were considered eligible in Iowa and Nebraska, but not in the Canadian study. Proxy respondents were used on behalf of deceased subjects in Iowa and Nebraska, and were permitted in Iowa, Nebraska, and Canada for participants requiring assistance due to illness or disability. Incident MM cases were identified using state and provincial cancer registry records, with the exception of Nebraska and Quebec, where cases were recruited from hospitals. Population-based controls were identified using random digit dialing (all studies), Medicare records and state mortality files (Iowa and Nebraska), health insurance records (Alberta, Saskatchewan, Manitoba and Quebec), telephone listings (Ontario) and voter lists (British Columbia). Cases and controls were frequency-matched to the overall case distribution by age (62 years in Nebraska and Canada, 65 years in Iowa), vital status and year of death (if applicable), sex (Nebraska) and province of residence (Canada). The participation rates in the MM subset of NAPP studies were modest for Canadian controls (48 %) and higher in Iowa (78 %) and Nebraska (85 %). Participation rates were higher for cases in Canada (58 %), Iowa (84 %) and Nebraska (88 %).

Exposure assessment - A set of *a priori* pesticides to be investigated in this analysis included agents that showed positive (significant or nonsignificant) associations in the earlier U.S. and Canadian studies. Pesticides that met these criteria were 2,4-D, captan, carbaryl, chlordane, DDT, glyphosate, lindane, malathion, methoxychlor, permethrin and the pyrethrins. Self-reported information on pesticide use, farming activities and demographic characteristics were obtained through standardized interviews with participants. Individuals who provided an affirmative answer to general questions about pesticide use or exposure to substances within broad groups (i.e. insecticides, herbicides, fungicides) were subsequently asked more detailed follow-up questions regarding specific agents, including the frequency and duration of exposure. Participants who did not report any pesticide use were classified as unexposed. Among individuals reporting pesticide use, missing information for duration or frequency of exposure was treated

as missing or unknown. Information on duration of pesticide use (years) was collected in all studies, whereas frequency information (days per year) was only collected in Canada and Nebraska. The data from Nebraska were excluded from the analysis since the number of exposed cases for pesticides of interest was low and the proportion of missing data was prohibitively high (>40 %).

Exposure metrics - Associations were examined for dichotomous exposure (ever/never pesticide use) and by major chemical classes i.e. phenoxy herbicides, and organochlorine, organophosphate and carbamate insecticides. The duration of exposure was evaluated for each individual pesticide using years of self-reported use. Cumulative exposure was investigated using a composite lifetime days (LD) metric, defined as: LD = years of pesticide use x days/year of pesticide use. Analyses of cumulative exposure were restricted to the Canadian subset of the NAPP data, where sufficient information about both years and days/year was available. For subjects with missing information for the duration of pesticide use, simple conditional imputation was carried out. Age- and state/province-specific median values for years and days/year were assigned to participants classified as exposed based on the ever/never metric. Imputed values were only assigned if <35 % of exposure duration data were missing among cases, and if the proportions of missing data differed by <20 % between cases and controls.

Statistical analysis - Descriptive analyses were performed on potential confounders identified from the literature including age, province/state of residence, use of a proxy respondent, farming history (ever lived or worked on a farm) and personal medical history. Covariates that were significantly ($p < 0.05$) associated with MM or those that produced meaningful changes (≥5 %) in the OR estimates were retained in the final models. Unconditional logistic regression was used to calculate odds ratios (OR) and 95 % confidence intervals (CI) for pesticide exposure variables with adjustment for age, province/state of residence, use of proxy respondent, and ever being diagnosed with any allergy, hay fever, or rheumatoid arthritis. For all analyses of individual pesticides, the referent population consisted of subjects who did not report any pesticide use, or those who indicated that they had not used that specific agent. Duration of pesticide exposure and cumulative LD were modeled as ordinal variables and linear trends were examined (*p*-trend). Cut-offs for categories were based on the median duration and LD among cases and controls for each pesticide. The use of proxy respondents was also considered as an effect modifier and sensitivity analyses were conducted excluding information provided by proxy respondents All analyses were performed using SAS version 9.3.

Results

The analysis included a total of 587 MM cases and 3,588 controls from Iowa, Nebraska and six Canadian provinces. Female cases ($N = 40$) and female matched controls ($N = 707$) contributed by the Nebraska study were excluded due to the very low prevalence of pesticide use among females. The youngest MM case in the NAPP dataset was 31 years old and, therefore, the controls of an age of 30 years and younger were excluded ($N = 181$) to maintain a comparable distribution of age between cases and controls. The final analysis included 547 male cases and 2,700 male controls. Among the participants, cases were older than controls. This was expected since a common age-matched control group was used for all cancer sites in the NAPP and the majority of MM cases are typically diagnosed at slightly older ages (>65 years) than non-Hodgkin lymphoma and Hodgkin lymphoma cases. Proxy respondents were used for 35 % of the cases and 28 % of the controls overall. Associations of demographic characteristics and medical history covariates with MM were modeled using logistic regression, with adjustment for age (in years) and province or state of residence. A history of any type of cancer among first-degree relatives was significantly associated with MM risk. However, having a first-degree relative with any lymphatic or haematopoietic cancer, including MM, was not associated with MM. A number of conditions associated with stimulation of the immune system showed statistically significant inverse associations with MM risk. In this summary only data on glyphosate are reported.

Pesticide exposure (ever/never in the NAPP) - For the "never" use of glyphosate 502 (91.8 %) cases and 2,504 (92.7 %) controls and for the "ever" use of glyphosate 45 (8.2 %) cases and 196 (7.3 %) controls were identified. The adjusted OR was 1.29 (95 % CI 0.9-1.85). When proxy respondents were excluded then the adjusted OR was 1.07 (95 % CI 0.69-1.66).

Pesticide exposure relative to the years of exposure (Iowa and Canada subset of the NAPP) – For no exposure to glyphosate 471 (91.4 %) cases and 1,832 (91.7 %) controls, for up to 3 years of exposure to glyphosate 22 (4.3 %) cases and 87 (4.3 %) controls and for more than 3 years 22 (4.3 %) cases and 80 (4.0 %) controls were identified. The adjusted OR was 1.30 (95 % CI 0.79-2.16) for up to 3 years of exposure and 1.34 (95 % CI 0.80-2.23) for more than 3 years of exposure. The trend was not statistically significant (p = 0.16). When the adjusted OR was taken with exclusion of proxy respondents it was 1.19 (95 % CI 0.67-2.11) for up to 3 years of exposure and 0.95 (95 % CI 0.50-1.83) for more than 3 years exposure. The trend was not statistically significant (p = 0.90).

Pesticide exposure relative to the number of lifetime days (LD) (Canadian subset of the NAPP) - For no exposure to glyphosate 310 (90.6 %) cases and 1,228 (91.0 %) controls, for up to 6 LD of exposure to glyphosate 18 (5.3 %) cases and 65 (4.8 %) controls and for more than 6 LD 14 (4.1 %) cases and 56 (4.2 %) controls were identified. The adjusted OR was 1.35 (95 % CI 0.76-2.40) for up to 6 LD of exposure and 1.11 (95 % CI 0.59-2.07) for more than 6 LD of exposure. The trend was not statistically significant (p = 0.48). When the adjusted OR was taken with exclusion of proxy respondents it was 1.43 (95 % CI 0.76-2.70) for up to 6 LD of exposure and 0.94 (95 % CI 0.44-1.99) for more than 6 LD of exposure. The trend was not statistically significant (p = 0.74).

Discussion and Conclusions

Despite the increase in the overall sample size resulting from pooling data from the CCSPH and U.S. NCI studies, the numbers of exposed participants were still low for some pesticides, and information for duration or frequency was sparse and not collected in all MM studies. Our ability to investigate the effects of high levels of exposure was further limited, since few participants reported frequent and long- term pesticide use. Exposure misclassification due to the use of proxy respondents may also influence results. Studies have shown that farmers may be able to recall pesticide use better compared to nonfarmers, and certain types of proxy respondents, such as friends and family members who also work in agriculture, may be more likely to recall the use of certain specific pesticides. It should be recognized that a large number of comparisons were made and some of the effect estimates were based on small numbers of exposed cases and controls. Therefore, it cannot be excluded that some of the observed associations may represent chance findings. Despite these limitations, this study has several important strengths. The NAPP is one of the largest pooled case-control studies of agricultural exposures and haematopoietic cancers. This analysis was the first to investigate the association between pesticide exposure and MM risk in a pooled sample of Canadian and American participants. Since similar pesticides were used in both Canada and the United States, it was possible to investigate the effects of these exposures in the NAPP overall. Furthermore, similarities in the design of the case-control studies facilitated successful pooling of these datasets, which afforded a larger sample size for more comprehensive and powerful analyses. Specifically, the investigation of different aspects of pesticide exposure, such as duration of use and cumulative lifetime exposure provided an informative and novel addition to this analysis of pesticide use alone. A further advantage of this study was the extensive medical history information that was collected in the Canadian and U.S. studies. This allowed us to take into account the influence of several conditions that result in sustained stimulation of the immune system, such as rheumatoid arthritis, systemic lupus erythematosus, certain viral infections and allergies, which were inversely associated with MM in our data.

No statistically significant increases in risk of multiple myeloma (MM) associated with self-reported exposure to glyphosate were observed.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Four population-based incident case-control studies (3 US studies and one Canadian study) pooled in the North American Pooled Project (NAPP) aimed to investigate the effects of pesticides and other agricultural exposures on the risk of lymphatic and hematopoietic cancers. The present analysis is restricted to a subset of three NAPP studies (Iowa, Nebraska and Canada) where multiple myeloma

(MM) cases were recruited. Self-reported information on pesticide use, farming activities and demographic characteristics was collected and the odds ratios (OR) were calculated for "ever/never" exposure, years of exposure and cumulated lifetime days of exposure to glyphosate with and without exclusion of proxy respondents. The result is that no statistically significant increases in risk of multiple myeloma (MM) associated with self-reported exposure to glyphosate were observed.

This publication is considered relevant for glyphosate risk assessment but reliable with restrictions because it concerns pooled case control studies which are subject to recall bias and in which confounding factors could not be ruled out.

Assessment and conclusion by RMS:

Reliability criteria for epidemiology studies

Publication: Presutti *et al*., 2016	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines/practices.	Y	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Exposure to formulations with only glyphosate as a.s.		
Exposure to formulations with glyphosate combined with other a.s.		
Exposure to various formulations of pesticides	Y	
Study		
Study design – epidemiological method followed	Y	Pooled case control studies
Description of population investigated	Y	
Description of exposure circumstances	Y	May be subject to recall bias
Description of results	Y	
Have confounding factors been considered	N	Confounding factors cannot be ruled out
Statistical analysis	Y	
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Not reliable		
This publication is considered relevant for glyphosate risk assessment but reliable with restrictions because it concerns pooled case control studies which are subject to recall bias and in which confounding factors could not be ruled out.		

1. Information on the study

Data point:	CA 5.5/037
Report author	Sorahan T.
Report year	2015

Report title	Multiple Myeloma and Glyphosate Use: A Re-Analysis of US Agricultural Health Study (AHS) Data
Document No	doi:10.3390/ijerph120201548 E-ISSN: 1660-4601
Guidelines followed in study	None
Deviations from current test guideline	NA
GLP/Officially recognised testing facilities	Non-GLP
Acceptability/Reliability:	Yes/Reliable without restrictions

2. Full summary of the study according to OECD format

The Relative risks (RRs) for exposed and non-exposed subjects were calculated using Poisson regression; subjects with missing data were not excluded from the main analyses. Using the full dataset adjusted for age and gender the analysis produced a RR of 1.12 (95 % CI 0.50 to 2.49) for ever-use of glyphosate. Additional adjustment for lifestyle factors and use of ten other pesticides had little effect (RR 1.24, 95 % CI 0.52 to 2.94). There were no statistically significant trends for multiple myeloma risks in relation to reported cumulative days (or intensity weighted days) of glyphosate use. The doubling of risk reported previously arose from the use of an unrepresentative, restricted dataset and analyses of the full dataset provides no convincing evidence in the AHS for a link between multiple myeloma risk and glyphosate use.

Materials and Methods

The secondary data file which served as a basis for this study was provided by researchers of the Agricultural Health Study (AHS) taking care of the privacy of the participants. AHS researchers supplied an informative description of the file and the file was found to be internally consistent as well as consistent with data descriptions supplied earlier. All subjects gave their informed consent for inclusion before they participated in the AHS study and ethics approval for the original data collection by AHS researchers was obtained from the Institutional Review Board of the National Institutes of Health. This secondary analysis was conducted in accordance with the Declaration of Helsinki and the protocol was approved by the University of Birmingham Science, Technology, Engineering and Mathematics Ethical Review Committee.

Data on lifestyle factors and use of pesticides were collected from 57,311 private and commercial pesticide applicators from Iowa and North Carolina. Previous analyses were carried out on three subsets of data: Set 1 comprised 54,315 applicators excluding those with any cancers diagnosed before enrolment, applicators lost to follow-up, who had missing data for age at enrolment, or who provided no information on whether they had ever used glyphosate. Set 2 comprised 49,211 applicators and further excludes applicators with missing data on level of education, smoking history, or use of alcohol. Set 3 comprised 40,719 applicators and further excludes applicators with missing data on either use or estimated cumulative exposure days for 2,4-D (2,4-dichlorophenoxy acetic acid), alachlor, atrazine, metolachlor, or trifluralin, and missing data on ever-use of benomyl, maneb, paraquat, carbaryl, or diazinon. The objective of this analysis was to examine findings in an as full a dataset as possible and some analyses have also been carried out on a larger fourth set of 55,934 applicators, a category that does not exclude applicators with missing data on ever-use of glyphosate but only applicators with cancers diagnosed before enrolment, applicators lost to follow-up, or who had missing data for age at enrolment.

Poisson regression was used to estimate RRs and 95 % CIs associated with glyphosate exposure metrics, with and without adjustment for other variables. Each variable under analysis was classified into levels or categories. The analytical approach for the full dataset was to have a "not known/missing" category for each variable so that analyses of all available cases could be maintained. However, it was necessary to ensure that there was at least one case of multiple myeloma in each level of each variable for the regression

to successfully calculate RRs. There were no cases of multiple myeloma in those applicators with "unknown use of 2,4-D", such applicators were combined with those reporting "no use" to create a new category of "no claim of use". There were no cases of multiple myeloma in applicators with an "unknown level of education". These applicators were combined with those reporting no education beyond high school. All significance tests were two-tailed and tests for trend (where applicable) were calculated by scoring the levels of a variable and treating the variable as unfactored. All analyses were performed with the EPICURE statistical software, using the double precision DOS version 2.12 of DATAB and AMFIT, dated March 2002.

Results

There were 32 cases of multiple myeloma in Set 1, 26 cases in Set 2, and 22 in Set 3. For the calculation of the RRs there was no adjustment for gender. None of the RRs calculated (RR including statistical adjustment for age at enrolment and RR with additional adjustments) for the three sets is statistically significant and in Set 1, the largest data set, the RR for ever-use of glyphosate is close to unity (RR 1.08, 95 % CI 0.48 to 2.41). The point estimates of risk for the smaller datasets show an approximate doubling of risk irrespective of whether adjustment for other variables is carried out. The largest RR is shown for the fully adjusted model of the smallest dataset (RR 2.79, 95 % CI 0.78 to 9.96).

Estimated risks of multiple myeloma for reported ever-use for the 54,315 applicators in Set 1 (total of 32 cases) were calculated in relation to use of pesticides and other variables (smoking, alcohol consumption, family history of cancer and education). The RR for ever-use of glyphosate, with adjustment for age at enrolment and gender only, is close to unity (RR 1.12, 95 % CI 0.50 to 2.49). The RR for ever-use of glyphosate is little changed with additional adjustment for all 14 other variables (RR 1.24, 95 % CI 0.52 to 2.94).

RR estimates were calculated for multiple myeloma in terms of levels of reported cumulative days of glyphosate use and levels of estimated intensity-weighted exposure days for the 54,315 applicators in Set 1. For each exposure metric three sets of RRs have been calculated: firstly, adjusting for age at enrolment and gender; secondly, with additional adjustment for cigarette smoking, use of alcohol, family history of cancer, and level of education; and thirdly, with additional adjustment for use of ten other pesticides. Two tests for trend were applied to each of these analyses, the first scored the levels of cumulative exposure as 1-4, the second scored the level by mean values. There were no statistically significant trends, but a not statistically significantly elevated RR was shown for the highest category of intensity-weighted exposure days in the fully adjusted model (RR 1.87, 95 % CI 0.67 to 5.27). There were no cases of multiple myeloma in glyphosate users with unknown extent of use.

RR estimates were calculated for multiple myeloma in terms of levels of ever-use of glyphosate, reported cumulative days of glyphosate use, and estimated intensity-weighted exposure days for the 55,934 applicators in Set 4 with a total of 34 cases of multiple myeloma. The risk of multiple myeloma in ever-users of glyphosate was close to unity (RR 1.18, 95 % CI 0.36 to 8.20) and there were no significant trends with either of the two cumulative exposure metrics.

Discussion

This study found no significant trends of multiple myeloma risk with reported cumulative days of glyphosate use and unexceptional point estimates of risk for ever-use of glyphosate. This was irrespective of whether the analyses had adjustment for a few basic variables (age and gender) or adjustment for many other lifestyle factors or pesticide exposures, as long as data on all available pesticide applicators were used. The suspiciously elevated RRs reported previously arose from the use of restricted data sets that, probably by chance, turned out to be unrepresentative. These restrictions would seem to be unnecessary because there is no technical problem in dealing with missing data in Poisson regression. The practice of restricting analyses to subjects with complete data for all variables is, perhaps, a procedure to be carried out with caution as it is clear from this example that such restrictions can lead to misleading findings. It also ignores the fact that findings for missing categories can often be interesting in their own right.

Conclusion
This secondary analysis of AHS data does not support the hypothesis that glyphosate use is a risk factor for multiple myeloma, and suggests that the practice of restricting analyses to subjects with complete data for all variables is perhaps not to be recommended.

3. Assessment and conclusion

Assessment and conclusion by applicant:
In this study the relative risk estimates for exposed and non-exposed applicators were calculated using Poisson regression and subjects with missing data were not excluded from the main analyses. When using the full dataset adjusted for age and gender the analysis produced a RR close to unity for ever-use of glyphosate. Additional adjustment for lifestyle factors and use of ten other pesticides had little effect. This study found no statistically significant trends of multiple myeloma risk with reported cumulative days of glyphosate use and unexceptional point estimates of risk for ever-use of glyphosate. This was irrespective of whether the analyses had adjustment for a few basic variables (age and gender) or adjustment for many other lifestyle factors or pesticide exposures, as long as data on all available pesticide applicators were used. The suspiciously elevated RRs reported previously arose from the use of restricted data sets that, probably by chance, turned out to be unrepresentative.

This publication concerns a secondary analysis of the data from the Agricultural Health Study (AHS) and is considered relevant for the risk assessment of glyphosate and reliable without restrictions because it complies with the reliability criteria of a well conducted epidemiology study.

Assessment and conclusion by RMS:

Reliability criteria for epidemiology studies

Publication: Sorahan T., 2015	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines/practices.		
Study completely described and conducted following scientifically acceptable standards	Y	Secondary analysis of the AHS data
Test substance		
Exposure to formulations with only glyphosate as a.s.		
Exposure to formulations with glyphosate combined with other a.s.		
Exposure to various formulations of pesticides	Y	
Study		
Study design – epidemiological method followed	Y	
Description of population investigated	Y	
Description of exposure circumstances	Y	
Description of results	Y	
Statistical analysis	Y	
Overall assessment		
Reliable without restrictions	Y	
Reliable with restrictions		

Publication: Sorahan T., 2015	**Criteria met? Y/N/?**	**Comments**
Not reliable		
This publication concerns a secondary analysis of the data from the agricultural health study and is considered relevant for the risk assessment of glyphosate and reliable without restrictions because it complies with the reliability criteria of a well conducted epidemiology study.		

CA 5.6 Reproductive toxicity

The potential of glyphosate to cause effects on **reproduction** (reproductive performance, fertility, development) was examined in several multi-generation studies. Further, two one-generation studies are available which were considered as supportive data to assess reproductive toxicity. Reliable and relevant publications on reproductive toxicity were also taken into account for hazard assessment.
No specific reproductive toxicity potential was concluded for glyphosate in the previous evaluation (RAR, 2015). A significant decrease in homogenisation-resistant spermatid count and equivocal effects on litter size was observed in individual studies at high doses which caused parental toxicity. Further, weak effects on the offspring consisting of reduced pup weight and delayed preputial separation were seen at high dose levels and were associated with signs of paternal toxicity. Treatment-related effects in parent animals were similar to those seen in subchronic and chronic toxicity studies and occurred at comparable dose levels.

Developmental toxicity studies were performed in rats and rabbits.
Glyphosate did not cause teratogenicity in any of the conducted studies. Adverse effects on the number of viable foetuses and the foetal weight were noted in rats and rabbits at higher dose levels also causing maternal toxicity. A reduced ossification and a higher incidence of skeletal and/or visceral anomalies at these dosages were also indicative of foetotoxicity. Overall, there is an inconsistent pattern of the most commonly occurring cardiac defects at maternally toxic doses without a clear dose effect. The overall ~~lowest~~ NOAEL for developmental effects was 300 mg/kg bw/day in rats (CA 5.6.2/003) and 175 mg/kg bw/day in rabbits ([redacted], 1991; CA 5.6.2/014 and CA 5.6.2/015).

CA 5.6.1 Generational studies

In the previous glyphosate evaluation (RAR, 2015) a number of multi-generation studies were reviewed. The available data are summarised below. In addition, results of the conducted one-generation studies are considered as supportive data for the current assessment. Relevant and reliable literature was also assessed to finally evaluate intrinsic properties of glyphosate on reproductive tissues and organs. A summary of the considered literature is shown in the below table.

Table Error! Use the Home tab to apply Überschrift 2 to the text that you want to appear here.**-1-1: Summary of generational studies on glyphosate**

Annex Point	Study	Species Study type	Substance(s)	Reference list- related category§	Result	
					NO(A)EL [mg/kg bw/day (ppm)] ♂/♀	LO(A)EL Targets / Main effects
CA 5.6.1/001 CA 5.6.1/002 CA 5.6.1/003	[redacted], 2007	Crl:CD (SD) IGS BR: rat (2-generation,	Glyphosate technical (Purity:	Valid, Category 2a	**Parental:** 351 (5000); **Offspring:**	**1000-1600** (15000 ppm)

Table Error! Use the Home tab to apply Überschrift 2 to the text that you want to appear here.**.1-1: Summary of generational studies on glyphosate**

Annex Point	Study	Species Study type	Substance(s)	Reference list- related category§	Result	
					NO(A)EL [mg/kg bw/day (ppm)] ♂/♀	LO(A)EL Targets / Main effects
		diet, 0, 1500, 5000, 15000 ppm)	95.7 %)		351 (5000); **Reproduction:** 351 (5000);	**Parental:** increased liver and kidney weights (effect on liver non-adverse) **Offspring**: delay in preputial separation in F1 males **Reproduction:** decreased homogenisation resistant spermatid count
CA 5.6.1/004	[redacted] 2000	Alpk:AP$_f$SD: rat (2-generation, diet, 0, 1000, 3000, 10000 ppm)	Glyphosate acid technical (Purity: 97.6 %)	Valid, Category 2a	**Parental:** 322 / 459 (3000); **Offspring:** 322 / 459 (3000) **Reproduction:** 1072 / 911 – 2425 (10000)	1063 / 1634 ♂/♀ (10000 ppm) **Parental:** body weight of F1 males ↓ during pre-mating **Offspring:** reduced body weight of F1A pups during lactation **Reproduction:** no effects
CA 5.6.1/005	[redacted], 1997	Crj:CD^{-} (SD): rat (2-generation, diet, 0, 1200, 6000, 30000 ppm)	Glyphosate technical, HR-001 (Purity: 94.6 %)	Valid, Category 2a	**Parental:** 417-458 / 485-530 (6000); **Offspring:** 417-458 / 485-530 (6000)**;** **Reproduction:** 2150-2411 / 2532-2760 (30000)	2150-2411 / 2532-2760 ♂/♀ (30000 ppm) **Parental:** loose stool, slight decrease in mean body weight in F1 ♂ at 2nd generation selection, caecum distension **Offspring:** reduced body weight (F0 ♂ and F1 ♀ during lactation), caecum distension **Reproduction:** no effects
CA 5.6.1/006	[redacted]	Wistar: rat (2-	Glyphosate	Supportive,	**Parental:**	**Parental:**

Table Error! Use the Home tab to apply Überschrift 2 to the text that you want to appear here.**.1-1: Summary of generational studies on glyphosate**

Annex Point	Study	Species Study type	Substance(s)	Reference list- related category§	Result	
					NO(A)EL [mg/kg bw/day (ppm)] ♂/♀	LO(A)EL Targets / Main effects
	1993	generation, diet, 0, 100, 1000, 10000 ppm)	technical (Purity: 96.8 %)	Category 2a	700-800 / 700-800 (10000); **Offspring:** 700-800 / 700-800 (10000); **Reproduction:** 700-800 / 700-800 (10000)	no effects **Offsping:** no effects **Reproduction:** no effects
CA 5.6.1/007 CA 5.6.1/008	[redacted], 1992	Crl:CD (SD) BR VAF/Plus: rat (2-generation, diet, 0, 1000, 3000; 10000 ppm)	Glyphosate technical (Purity: 99.2 %)	Valid, Category 2a	**Parental:** 668-771 / 752-841 (10000); **Offspring:** 668-771 / 752-841 (10000); **Reproduction:** 668-771 / 752-841 (10000)	**Parental:** no adverse effects **Offspring:** no effects **Reproduction:** no effects
CA 5.6.1/009	[redacted] 1991	Crl:CD (SD) BR VAF/Plus strain: rat (1-generation dose range-finder, diet, 0, 3000, 10000, 30000 ppm)	Glyphosate technical (Purity: 98.6 %)	Valid, Category 2a	NO(A)EL not relevant in this dose range-finding study	**Parental:** slightly lower body weight gain, macroscopic salivary gland changes (non-adverse), macroscopic gastro-intestinal changes, minimal granular basophilic cytoplasm of acinar cells in 2/10 females with minimal hypertrophy of acinar cells in females at 3000 ppm **Offspring:** reduced litter and pup weight at 30000 ppm, macroscopic salivary gland changes starting at 3000 ppm in sacrificed pups (non-adverse) **Reproduction:** no effects
CA 5.6.1/010	[redacted] 1990	SD: rat (2-generation, diet, 0, 2000, 10000, 30000 ppm)	Glyphosate (Purity: 97.7 %)	Valid, Category 2a	**Parental:** 666-711 / 777-804 (10000); **Offspring:** 666-711 / 777-804 (10000)	1983-2320 / 2322-2536 mg/kg bw/day (30000 ppm): **Parental:** reduced body weight,

Table Error! Use the Home tab to apply Überschrift 2 to the text that you want to appear here.**.1-1: Summary of generational studies on glyphosate**

Annex Point	Study	Species Study type	Substance(s)	Reference list- related category§	Result	
					NO(A)EL [mg/kg bw/day (ppm)] ♂/♀	LO(A)EL Targets / Main effects
					Reproduction: 1983-2230 / 2322-2536 (30000)	soft faeces, **Offspring:** reduced body weight, equivocal effect on litter size **Reproduction:** no effects
CA 5.6.1/011	[redacted], 1988	Wistar: rat (3-generation, diet, 0, 75, 150, 300 ppm)	Glyphosate technical (Purity: Not reported)	Supportive, Category 2a	**Parental:** 15 (300); **Offspring:** 15 (300) **Reproduction:** 15 (300);	**Parental:** no effects **Offspring:** no effects **Reproduction:** no effects
CA 5.6.1/012	[redacted] 1988	Wistar: rat (1-generation, gavage, 0, 5, 10 mg/kg bw/day)	Glyphosate technical (Purity: Not reported)	Supportive, Category 2a	**Parental:** 10; **Offspring:** 10 **Reproduction:** 10	**Parental:** no effects **Offspring:** no effects **Reproduction:** no effects
CA 5.6.1/013	[redacted] 1985	Wistar: rat (3-generation, diet, 0, 200, 1000, 5000)	Glyphosate (Purity: not reported)	Invalid, Category 4b	**Parental:** (5000); **Offspring:** (5000) **Reproduction:** (5000)	**Parental:** no adverse effects **Offspring:** no adverse effects **Reproduction:** no adverse effects
CA 5.6.1/014	[redacted], 1981	CD (SD): rat (3-generation, diet, 0, 3, 10, 30 mg/kg bw/day)	Glyphosate (Purity: 100 % (considered 100 % active ingredient for dosing preparations)	Supportive, Category 2a	**Parental:** 30 **Offspring:** 30 **Reproduction:** 30	**Parental**: no effects **Offspring**: no effects **Reproduction:** no effects

§ The category describes the acceptability/reliability of the study within the AIR 5 submission. This category does not describe hazard classification.

Publications on reproductive toxicity

A literature search for the active substance glyphosate was performed in accordance to the provisions of the EFSA Guidance "Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) 1107/2009" and updated Appendix to this Guidance document. The following publications were found relevant and reliable for this section and the summaries are thus presented below and are part of the general discussion at the beginning of the section.

Table Error! Use the Home tab to apply Überschrift 2 to the text that you want to appear here.**.1-2: Publications on reproductive toxicity on glyphosate**

Annex Point	Study	Study type	Substance(s)	Results (given for glyphosate exposure only)	Applicant assessment
CA 5.6.1/015	Gorga *et al.*, 2020	*in vitro* effects on Sertoli cell cultures, 10-100 ppm	Glyphosate and Roundup Full II	Altered Sertoli cell junction barrier permeability and decreased testosterone-stimulated TER; redistribution of claudin11	This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate used was not sufficiently characterised, there was no positive control and most of the endpoints were tested at only 2 concentrations preventing any dose-response evaluations, with the highest concentration exceeding a physiologically relevant dose.
CA 5.6.1/016	Manservisi *et al.*, 2019	SD rat (13 weeks, dose range-finder OECD 443), drinking water, 1.75 mg/kg bw/day	Glyphosate (Purity: 99.5 %) and Roundup (Bioflow)	Offspring: increased anogenital distance at PND 4 in males; increase in plasma TSH concentration in males	This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because of the limited number of animals used per dose level and only one dose level tested.
CA 5.6.1/017	Pham *et al.*, 2019	Swiss mouse (GD 0 p.c. PND 2), drinking water 0, 0.5, 5, 50 mg/kg bw/day	Glyphosate (Purity: 99.2 %) and Roundup 3 Plus	No adverse effects observed on male offspring and endocrine endpoints including testicular function	This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the test conditions were not clearly described and the number of animals tested per dose level is too limited.
CA 5.6.1/018	Ren *et al.*, 2019	ICR Mouse (GD 0 up to PND 21) drinking water, 0.5%	Glyphosate (Purity: 99.2 %) and Roundup 3 Plus	Offspring: hepatic steatosis, increased serum and liver concentrations of lipids	This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate used is not sufficiently characterised, only one dose level was tested, there was large inter animal variability observed and too few animals per dose level were analysed.
CA 5.6.1/019	Zhang *et al.*, 2019	*in vitro* effects on mice oocyte cultures, 500 µM	Glyphosate (Purity: not reported)	Effects on oocyte development and apoptotic fate	This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because of the poor characterisation of the

Table Error! Use the Home tab to apply Überschrift 2 to the text that you want to appear here.**.1-2: Publications on reproductive toxicity on glyphosate**

Annex Point	Study	Study type	Substance(s)	Results (given for glyphosate exposure only)	Applicant assessment
					glyphosate tested, no cytotoxicity testing, the lack of a positive control and insufficient dose-response characterisation at biologically relevant doses.
CA 5.6.1/020	Johansson *et al.*, 2018	SD rat (14 days), gavage, 0, 2.5, 25 mg/kg bw/day	Glyphosate (Purity: ≥96 %) and in herbicide formulation (Glyfonova)	No effects observed on either of the various testicular parameters examined	This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because only two dose levels were used to explore the dose-effect relationship for the endpoints assessed.
CA 5.6.1/021	Panzacchi *et al.*, 2018	SD rat (treatment from weaning to PND 73 or 125), drinking water, 1.75 mg/kg bw/day	Glyphosate (Purity: >99.5 %) and Roundup Bioflow	No effects on examined endpoints. Similar toxicity profile of pure glyphosate and reference formulation.	This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because only one dose level for glyphosate and MON 52276 was considered, only 8 animals were used per dose and per sex and the method of analysis of glyphosate and AMPA in urine and its validation were not fully reported.
CA 5.6.1/022	Perego *et al.*, 2017	in vitro effects on bovine granulosa and theca cells, 0, 0.01, 0.03, 0.5, 1.7, 5.0 µg/mL	Glyphosate (Purity: >99.5 %)	Impaired granulosa cells proliferation and estradiol production at 5 µg/mL, but no effects at lower, physiologically relevant, concentrations	This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate tested was not sufficiently characterised, no positive controls were used and the tests were conducted with only one or 2 test concentrations of glyphosate.
CA 5.6.1/023	Dai *et al.*, 2016	SD rat (5 weeks), gavage, 0, 5, 50, 500 mg/kg bw/day	Glyphosate (Purity 90 %)	Reduced food consumption at 50 mg/kg bw/day, decreased weight of seminal vesicle gland, coagulating gland as well as the total sperm count at 500 mg/kg bw/day	This publication is considered relevant but reliable with restrictions because there are deviations from regulatory guidelines for reproductive toxicicity studies and the reproductive effects seen are not corroborated by the results from guideline studies at similar dose

Table Error! Use the Home tab to apply Überschrift 2 to the text that you want to appear here.**.1-2: Publications on reproductive toxicity on glyphosate**

Annex Point	Study	Study type	Substance(s)	Results (given for glyphosate exposure only)	Applicant assessment
					levels.
CA 5.6.1/024	Forgacs *et al.*, 2012	*in vitro*, murine Leydig cell culture, 1, 3, 10, 30, 100, 300, 600 µM	Glyphosate (Purity: not reported)	No effect on induction of testosterone production or alteration of rhCG induction of testosterone	This publication is considered relevant for glyphosate risk assessment but reliable with restrictions because the test substance was not characterised and the results of only one concentration level were reported.

GD gestational day
PND post-natal day

In the most recent two-generation study by [redacted]. (CA 5.6.1/001; CA 5.6.1/002 and CA 5.6.1/003) possible treatment-related effects on the parental and offspring generations were observed. Briefly, increased liver (F0 and F1 females) and kidney weights were observed in females of both generations at 15000 ppm. No similar findings were detected in males. No toxicological concern regarding the significantly increased liver weights is considered due to the absence of any histopathological changes in the liver. Further, at 15000 ppm a significant decrease in homogenisation resistant spermatids (HRS, cauda epididymis) was counted in F0 males (control: 400 million/gram; 15000 ppm: 309 million/gram**). No similar effects were seen at lower dose levels or at any dose levels in F1 males. In F1 male offspring preputial separation was delayed at 15000 ppm without any additional developmental retardation indicating a delay in male sexual maturation. Sperm changes and histopathological examinations did not reveal any changes in the testis or epididymes. Although, the later onset of preputial separation in male offspring at 15000 ppm had obviously no impact on reproductive performance in week 29, a treatment related effect on sexual maturation at parental toxic dose cannot be excluded. However, it is important to note that this finding occurred at the limit dose at which parental toxicity was also apparent. Based on the above mentioned effects, a NOAEL of 5000 ppm (ca. 351 mg/kg bw/day) is considered for parental, reproductive and offspring toxicity.

In the [redacted] (2000) (CA 5.6.1/004) study, fertility and reproductive performance was not adversely affected by treatment. No impact on sexual maturation was observed up to the highest dose level of 10000 ppm. A reduction in body weight of F1A pups was observed at the highest applied dose of 10000 ppm resulting in a subsequent reduction in body weight of the selected F1 parent males for the duration of the mating period. A NOAEL of 3000 ppm (equivalent to 322 and 459 mg/kg bw/day for males and females, respectively) was considered for the parental generations and offspring for both generations. A NOAEL of 10000 ppm is derived for reproductive toxicity.

In a further two-generation study performed by [redacted] (1997) (CA 5.6.1/005), no treatment-related signs of toxicity were noted in parental animals up to 6000 ppm. At 30000 ppm, treatment-related adverse effects were observed that consisted of defecation of loose stool in males and females, and lower body weights in males compared to control group males in both generations. Further, distension of the caecum and increased liver and kidney weights were evident at this dose level in males. Decreased prostrate weights were detected in F1 males at necropsy. Reproductive performance was not affected by the treatment in any dose group. Slightly lower gestation indices were observed in F1 females at 6000 and 30000 ppm which were considered unrelated to treatment, as shown by the results of the reciprocal crosses of F1 animals with untreated rats. Pups of both sexes of the F1 and F2 generation showed significantly decreased body weight

at 30000 ppm. Based on the results the NOAEL for parental and offspring toxicity was considered to be 6000 ppm (417 mg/kg bw/day). A NOAEL of 30000 ppm is considered for reproductive toxicity.

Supportive data indicating no hazard on reproduction are obtained by the two-generation study performed by [redacted] (1993) (CA 5.6.1/006). Parameters like general health, growth of parents, gestation/lactation period, body weight and food consumption, gross necropsy findings of pups and parents were unaffected by treatment up to the highest tested dose of 10000 ppm. Further, glyphosate did not affect mortality incidence, parturition performance, mean litter size, pup weight and male and female fertility index. Thus, the highest dose of 10000 ppm is considered to be the NOAEL for parental, reproductive and offspring toxicity.

In the study by [redacted] [redacted] (1992) (CA 5.6.1/007 and CA 5.6.1/008) no effects on reproduction (reproductive performance, fertility, parturition, lactation, sperm parameters and oestrus cycle) were noted up to the maximum dose of 10000 ppm. Oral administration of glyphosate to rats by dietary admixture at a maximum dose level of 10000 ppm for two successive generations resulted in minimal effects consisting of increased food and water consumption of F1 females and possibly reduced body weights of F1 males. Minimal histopathological changes on the salivary glands were observed in parental and offspring animals at 10000 ppm and to a lower extent at 3000 ppm. This observation was also noted in other repeated-dose studies with glyphosate but is considered an adaptive response to high dietary doses of glyphosate, which is a strong organic acid, and can therefore cause irritation of the oral cavity leading to increased salivary excretion. Thus, the parental reproductive and offspring NOAELs are considered to be 10000 ppm, corresponding to 668 and 752 mg/kg bw/day in males and females, respectively.

In the respective one-generation dose-range finding study performed by [redacted] [redacted] (1991) (CA 5.6.1/009) 3000, 10000 and 30000 ppm were applied from day 3 of pregnancy through to termination of the study. No effects on reproductive parameters were noted at any dose. Maternal toxicity was dose-dependently evident starting already at the lowest dose tested determined by slightly lower body weight gain, macroscopic salivary gland changes and macroscopic gastro-intestinal changes, minimal granular basophilic cytoplasm of acinar cells with minimal hypertrophy of acinar cells. Findings in the offspring were observed at 30000 ppm and included a reduction in litter and mean pup weight, loose faeces, reduced food intake and food conversion ratio and, in males only, a slight continuation of the reduced weight gain evident from birth to weaning was observed in the offspring. Findings at post mortem examination included macroscopic changes to the salivary gland and gastro-intestinal disturbances in males and females.

The two-generation study by [redacted] (1990) (CA 5.6.1/010) confirmed that glyphosate does not affect reproduction capacity in rats exposed over two consecutive generations to 30000 ppm. Maternal toxicity was evident in the high dose group at 30000 ppm indicated by soft stool and reduced body weights. At the same dose level, pups showed decreased body weights (about 10 %) when compared to controls. In the 10000 ppm dose group, decreased pup body weights were observed, but the effect was less pronounced and occurred not in both sexes of all generations. The NOAEL for reproductive toxicity was considered to be 30000 ppm. The NOAEL for parental and developmental toxicity was considered to be 10000 ppm.

Two studies are available from [redacted] (1988) (CA 5.6.1/011 and CA 5.6.1/012) including a three-generation and a one-generation study in rats. Both studies are considered as supportive data due to the very low dietary concentrations applied. In both studies, reproduction was not affected up to the maximum concentration of 300 ppm (calculated to be similar to 15 mg/kg bw/day) or 10 mg/kg bw/day in the three-generation and one-generation study, respectively. Likewise, no effects indicating parental, offspring or developmental toxicity were observed.

Based on the submitted reproductive toxicity studies, an overall parental NOAEL is 700 mg/kg bw/day, well below the lowest parental LOAEL of 1000 mg/kg bw/day ([redacted] 1997, CA 5.6.1/005; [redacted], 2000, CA 5.6.1/004; [redacted] [redacted] [redacted], 2007, CA 5.6.1/001 – CA 5.6.1/003; Figure 5.6.1-1), which is somewhat consistent with the subchronic studies. Parental toxicity was observed in those, independent studies mainly indicated by gastro-intestinal disturbances (soft stool, distension of caecum), reduced body weight and

organ weight changes. Furthermore, increased food and water consumption and an increased incidence of hypertrophy and basophilic staining of the parotid (males and females) and submaxillary (females) salivary glands were observed. The minimal histological alterations in the salivary gland are considered as an adaptive response to high dietary doses of glyphosate, which is a strong organic acid, due to local irritation of the oral cavity leading to increased and prolonged salivary excretion. The lowest NOAEL for parental toxicity was defined as 3000 ppm (322 and 459 mg/kg bw/day in males and females, respectively ([redacted], 2000, CA 5.6.1/004).

In offspring, body weight gain was reduced at parentally toxic dose levels and, in one study, preputial separation was delayed, and considered secondary to parental toxicity ([redacted], 2007, CA 5.6.1/001 – CA 5.6.1/003). The overall NOAEL/LOAEL values were the same as for parental toxicity, 700 mg/kg bw/day and 1000 mg/kg bw/day respectively (Figure 5.6.1-2).

Regarding reproductive toxicity, equivocal effects on litter size was observed in one study ([redacted] 1990, CA 5.6.1/010) at the extremely high dose of 2000 mg/kg bw/day. In another study a significant decrease in homogenisation-resistant spermatid count in F0 males was observed at ca. 1000 mg/kg bw/day, and considered secondary to general toxicity ([redacted], 2007, CA 5.6.1/001 – CA 5.6.1/003). The overall NOAEL for reproductive toxicity was 700 mg/kg bw/day (Figure 5.6.1-3).

Published studies rated as reliable and relevant for hazard assessment of reproductive toxicity include *in vitro* and *in vivo* investigations. In some studies, highly specific endpoints on cellular level including molecular investigations on protein and RNA level were investigated. An interpretation of those molecular and cell type-specific observations in regard to reproductive toxicity in intact organisms is challenging. The biological significance of effects observed in these specific studies in intact organs within their physiological environment is not yet validated and hence, concluding on reproductive toxicity is currently not considered as appropriate.

In the following, the most relevant reported findings after glyphosate treatment are discussed briefly.

In some studies the effects of glyphosate and Round-up formulations have been investigated. In the following, the focus is on effects after glyphosate treatment. The results obtained after treatment with the Round-up formulations are described in the respective summaries (please refer to CA 5.6.1/015 - CA 5.6.1/024).

Effects on the male reproductive system were investigated in two *in vitro* and four *in vivo* studies following different treatment protocols. Briefly, *in vitro* exposure of Sertolli cells to glyphosate was reported to alter Sertolli cell junction barrier permeability and to decrease testosterone-stimulated TER. Further, a redistribution of claudin11 was observed ([redacted] (2020) (CA 5.6.1/015). No effects on the induction of testosterone production or alteration of recombinant human CG induction of testosterone was reported in Leydig cells up to 600 µM (Forgacs (2012) (CA 5.6.1/024).

Under *in vivo* conditions, decreased weight of seminal vesicle gland, coagulating gland as well as the total sperm count was reported in rats after subacute exposure to 500 mg/kg bw glyphosate/day (Dai *et al.* (2016) (CA 5.6.1/023). Further, sperm-depleted seminiferous tubuli were reported in mice exposed to 5 mg glyphosate/kg bw/day (Pham *et al.* (2019) (CA 5.6.1/017). No similar effect was observed in the same study in mice sacrificed at later time points. For both test groups, exposure to glyphosate was stopped at PND 20. The biological significance of the observed findings on seminiferous tubules depletion remains highly questionable as the overall quality of the obtained data is not sufficiently conclusive to indicate test substance-specific adverse effects on testicular function and development especially as the effect was only seen in the mid-dose group.

No effects on testes have been observed after *in vivo* exposure up to 25 mg/kg bw/day over 14 weeks in a NTP study (Johansson *et al.* (2018) (CA 5.6.1/020). Likewise, sperm parameters (number of mature spermatids in the testis, daily sperm production, number and sperm transit time through caput/corpus and

cauda epididymis and morphology) remained to be unaffected by glyphosate exposure at the US ADI of 1.75 mg/kg bw/day for over 13 weeks (Manservisi *et al.* (2019) (CA 5.6.1/016).

Overall, the published data might suggest that the male reproduction system was adversely affected by glyphosate exposure leading to reduced sperm counts at doses of at least 500 mg/kg bw/day. Likewise, a significant decrease in homogenisation-resistant spermatid count in F0 males was observed at ca. 1000 mg/kg bw/day in the regulatory study performed by [redacted] (2007), and considered secondary to overt toxicity (CA 5.6.1/001 – 003). Based on the latter study, a NOAEL of 351 mg/kg bw/day was derived for reproductive toxicity, which covers the effects described in the literature by Dai *et al.* (2016) (CA 5.6.1/023).

Effects on maturation of oocytes was investigated in two studies under *in vitro* conditions. Zhang *et al.* (2019) (CA 5.6.1/019) reported that 500 µM glyphosate treatment negatively affected development of mouse oocytes as indicated by reduced rates of germinal vesicle breakdown and first polar body extrusion. Further, molecular analysis indicated increased reactive oxygen species and an influence of glyphosate on DNA stability and intracellular signaling pathways relevant for apoptosis. As only one concentration was applied in this study, no distinction between spontaneous or treatment-related causes is possible and hence, biological significance of these observations is rather difficult to evaluate. Further, Perego *et al.* (2017) (CA 5.6.1/022) investigated ovarian function in bovine granulosa and theca cells after glyphosate stimulation. A slight, non-dose-related alteration in bovine granulosa cell proliferation and estradiol production was observed. Due to the isolated occurrence of the observed effects without any dose-response relationship, the biological significance of those findings is rather limited and not considered to indicate hazardous properties of glyphosate on overall ovarian function.

In general, effects observed on isolated oocytes which have been taken out from their physiological, cellular environment (which can be considered as protective environment towards e.g. xenobiotics and also physical stressors), can only be considered to be of limited *in vivo*-relevance. Thus, when interpreting the reported findings on isolated oocytes together with the results of the performed multi-generation studies, in which fertility in females was unaffected by glyphosate treatment in any study, the effects hypothesised in the publications are not considered to conclusively indicate a hazard to female fertility.

In addition to these rather specific investigations, three further *in vivo* studies are published, in which reproductive and developmental toxicity were investigated in a manner which can be considered as similar to OECD guideline studies with limitations and deviations (For details please refer to CA 5.6.1/016; CA 5.6.1/018 and CA 5.6.1/022). Manservisi *et al.* (2019) (CA 5.6.1/016) performed a pilot study for an extended-one generation study (OECD 443). Reproductive parameters remained to be unaffected by glyphosate exposure at 1.75 mg/kg bw/day (representing the US ADI).

Overall, a delay in male sexual development indicated by increased anogenital distance observed on PND 4 and an increased TSH level in plasma was reported in male rats. Due to relevant methodological limitations, including the low number of test animals and timing of blood sample collection, interpretation of the reported findings should be considered with high uncertainty. However, a significant delay in sexual maturation in male offspring (F1) indicated by delayed preputial separation (occurring after 45.9 days in the mean versus 43.0 days in the control group) was also observed in the regulatory study performed by [redacted] (2007) (CA 5.6.1/001 – 003), which became apparent at the top dose level of 15000 ppm (higher than 1000 mg/kg bw/day), and considered secondary to parental toxicity. Thus, following a weight-of-evidence approach a negative effect on male sexual development cannot be fully excluded.

No clear evidence for glyphosate-induced foetal or developmental toxicity was found in the study reported by Ren *et al.* (2019) (CA 5.6.1/018). Hepatic steatosis and increased serum and liver concentrations of lipids were announced by the authors to occur in the offspring. Although the authors concluded that there were treatment related effects on foetal and offspring body weight after treatment with 0.5 % glyphosate solution (w/v, 0.5 g/100 mL) during pregnancy, there is no evidence from this study to suggest that glyphosate exposure has had any impact on foetal or pup development (Please refer to CA 5.6.1/018 for

further discussion).

Further, pregnancy and early life stages were reported to be unaffected by glyphosate exposure after subchronic exposure to 1.75 mg/kg bw/day (Panzacchi *et al.* (2018) (CA 5.6.1/021).

Overall, a comprehensive review of recently published literature did not provide conclusive evidence that glyphosate exposure negatively affects reproduction.

Figure 5.6.1-01. Rat multigenerational study parental NOAEL and LOAEL values, showing "overall" parental NOAEL of 700 mg/kg bw/day [1. [redacted] (2007) (CA 5.6.1/001 – 003); 2. [redacted] (2000) (CA 5.6.1/004); 3. [redacted] (1997) (CA 5.6.1/005); 4. [redacted] (1993) (CA 5.6.1/006); 5. [redacted] (1992) (CA 5.6.1/007 – 008); 6. [redacted] (1990) (CA 5.6.1/010); 7. [redacted] (1988) (CA 5.6.1/0011); 8. [redacted], (1988) (CA 5.6.1/012); 9. [redacted] (1981) (CA 5.6.1/14)]

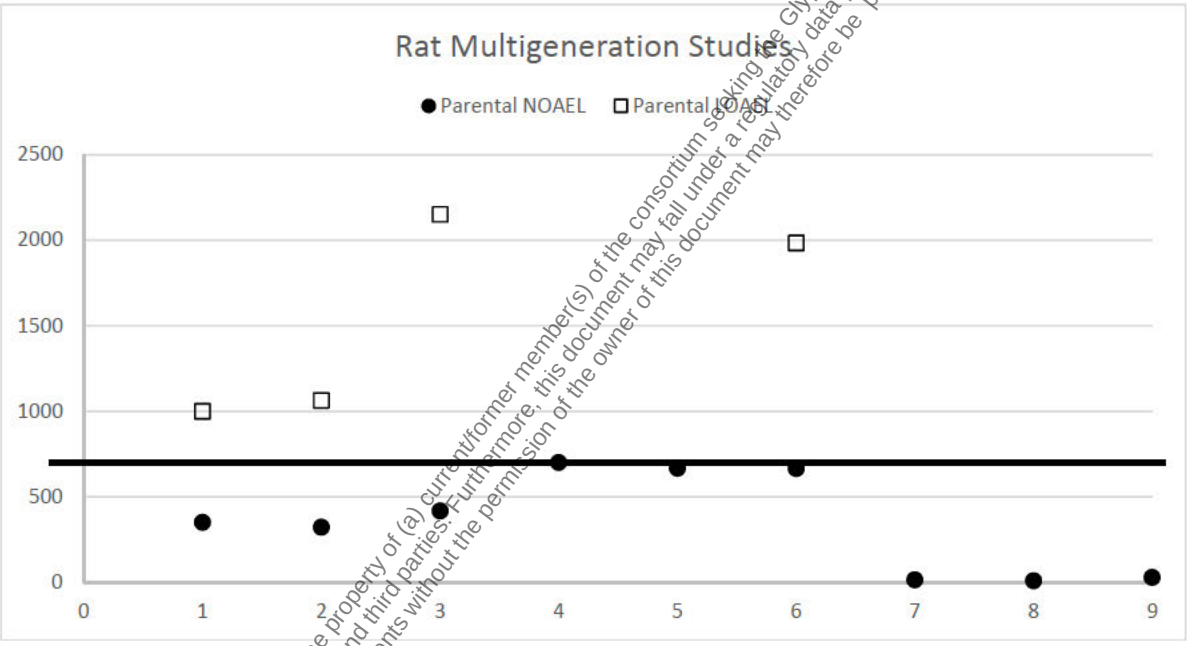


Figure 5.6.1-02. Rat multigenerational study offspring NOAEL and LOAEL values, showing "overall" offspring NOAEL of 700 mg/kg bw/day [1. [redacted] (2007) (CA 5.6.1/001 – 003); 2. [redacted] (2000) (CA 5.6.1/004); 3. [redacted] (1997) (CA 5.6.1/005); 4. [redacted] (1993) (CA 5.6.1/006); 5. [redacted] (1992) (CA 5.6.1/007 – 008); 6. [redacted] (1990) (CA 5.6.1/010); 7. [redacted] (1988) (CA 5.6.1/0011); 8. [redacted] (1988) (CA 5.6.1/0012); 9. [redacted] (1981 (CA 5.6.1/0014))]

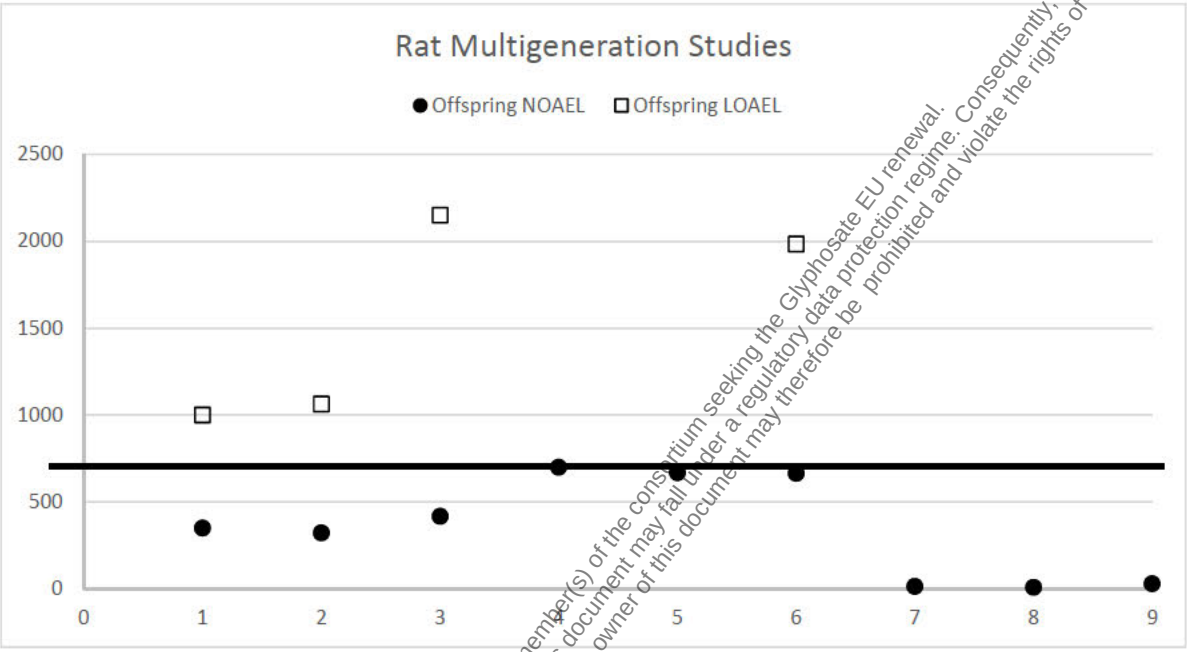
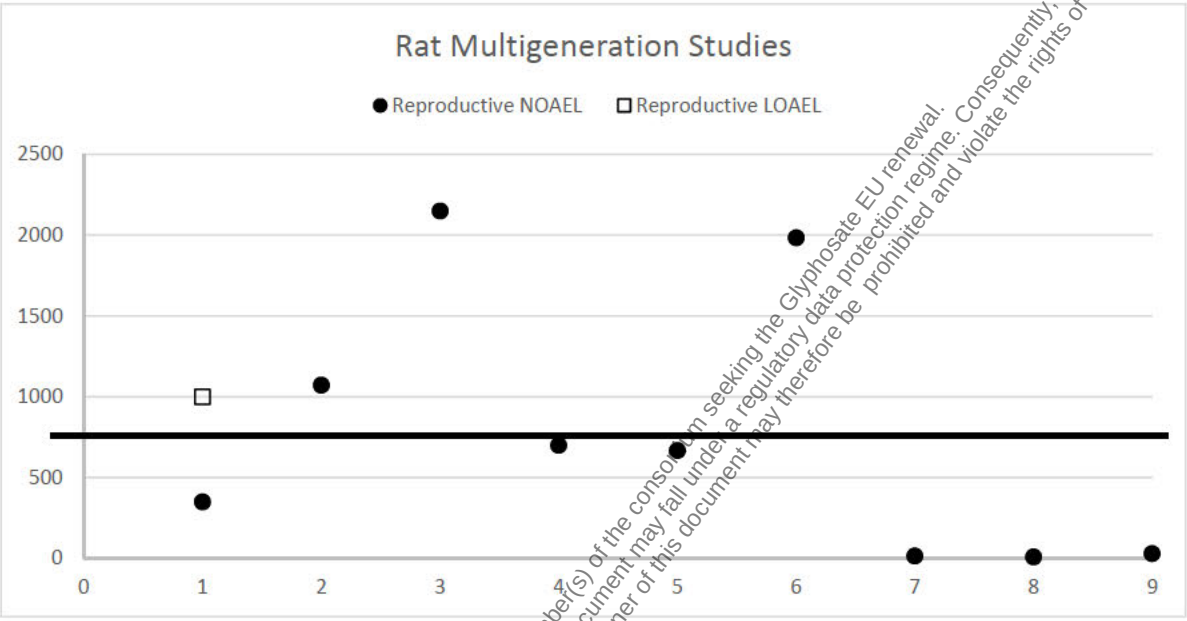


Figure 5.6.1-03. Rat multigenerational study reproductive NOAEL and LOAEL values, showing overall reproductive NOAEL of 700 mg/kg bw/day [1. [redacted] (2007) (CA 5.6.1/001 - 003); 2. [redacted] (2000) (CA 5.6.1/004); 3. [redacted] (1997) (CA 5.6.1/005); 4. [redacted] (1993) (CA 5.6.1/006); 5. [redacted] (1992) (CA 5.6.1/007 - 008); 6. [redacted] (1990) (CA 5.6.1/0010); 7. [redacted] (1988) (CA 5.6.1/0011); 8. [redacted] (1988) (CA 5.6.1/0012); 9. [redacted] (1981) (CA 5.6.1/0014)]



Taking into account all available generational studies on glyphosate, the overall multigenerational study NOAEL in rats for parental, offspring and reproductive toxicities is 700 mg/kg bw/day.

1. Information on the study

Data point:	**CA 5.6.1/001**
Report author	[redacted]
Report year	2007
Report title	Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat
Report No	2060/0013
Document No	Not reported
Guidelines followed in study	OECD 416 (2001), JMAFF 2-1-17 (2001), US-EPA OPPTS 870.3800 (1998)
Deviations from current test guideline (OECD 416, 2001)	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

1. Information on the study

Data point:	**CA 5.6.1/002**
Report author	[redacted]
Report year	2008
Report title	Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat (First amendment to report)
Report No	2060/0013
Document No	Not reported
Guidelines followed in study	OECD 416 (2001), JMAFF 2-1-17 (2001), US-EPA OPPTS 870.3800 (1998)
Deviations from current test guideline (OECD 416, 2001)	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

1. Information on the study

Data point:	**CA 5.6.1/003**
Report author	[redacted]
Report year	2008
Report title	Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat (Second amendment to report)
Report No	2060/0013
Document No	Not reported
Guidelines followed in study	OECD 416 (2001), JMAFF 2-1-17 (2001), US-EPA OPPTS 870.3800 (1998)
Deviations from current test guideline (OECD 416, 2001)	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Glyphosate technical was administered by dietary admixture to three groups of 28 male and female F0 generation Sprague-Dawley rats each, at dietary concentrations of 1500, 5000 and 15000 ppm (equivalent to a mean achieved dosage of 104, 351 and 1063 mg/kg bw/day for males and 162, 530 and 1634 mg/kg

bw/day for females respectively). A further group of 28 male and 28 female F0 animals was exposed to basal laboratory diet to serve as a control.

Clinical signs, body weight development, food and water consumption were monitored during the study. After 10 weeks of treatment, pairing of animals within each dose group was undertaken on a 1:1 basis. At weaning of offspring from the F0 mating phase, groups of 24 male and 24 female offspring from each dose group were selected to form the F1 generation. The remaining surviving F0 females and unselected offspring were terminated at Day 21 post partum, followed by the termination of all F0 male dose groups. The offspring selected for the F1 generation were dosed for at least 10 weeks and then paired within each dose group to produce the F2 litters. At weaning of the F2 litters all surviving adults and their offspring were killed, followed by the termination of all F1 male dose groups.

Oestrous cycle assessment was performed daily for three weeks prior to mating for both the F0 and F1 generations. Observations for positive evidence of mating were recorded together with the start and completion of parturition. During the maturation phase of the F1 generation offspring, males and females were evaluated for sexual maturation. The ano-genital distance was recorded for all F2 generation offspring on Day 1 post partum. During the lactation phases daily clinical observations were performed on all surviving offspring, together with litter size. Litter weight, individual offspring weights and landmark developmental signs were also recorded on specific days post partum.

All animals at termination were subjected to a gross necropsy examination and histopathological evaluation of selected tissues was performed.

The following treatment-related effects were observed:
During the end of the lactation phases, females showed less body weight loss when compared to controls for the F0 and F1 generations. There was no adverse effect on body weight change for males throughout the treatment period, or for females during the pre-pairing and gestation phases of the study.
An increase in liver weights was noted for females treated with 15000 ppm from both generations. No such effect was noted for males treated with 15000 ppm or for animals of either sex treated with 5000 or 1500 ppm. However, this finding was considered as an adaptive response to treatment and not as an adverse health effect.

There were no treatment-related histopathological changes for F0 generation animals. Treatment-related changes in the F1 generation were confined to the presence of lower incidences and severities of cortical vacuolation of the adrenal glands for treated males when compared to controls.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material:

Identification:	Glyphosate technical
Description:	White crystalline solid
Lot/Batch #:	H05H016A
Purity:	95.7 % (w/w)
Stability of test compound:	Not reported
Vehicle:	Plain diet
Test Animals:	
Species:	Rat
Strain:	Sprague-Dawley Crl:CD (SD) IGS BR
Source:	[redacted]

Age:	Approximately 8 weeks
Sex:	Males and females
Weight at dosing:	Males: 138 – 257 g; females: 140 – 195 g
Acclimation period:	At least 14 days
Diet/Food:	Rodent PMI 5002 (certified) diet (BCM IPS Limited, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Initially in groups of up to four in polypropylene cages with stainless steel grid floors and tops, suspended over polypropylene trays lined with absorbent paper. During mating animals were house one male : one female. Mated females were housed individually during gestation and lactation in polypropylene cages with solid floors and stainless steel lids, furnished with softwood flakes.
Environmental conditions:	Temperature: 21 ± 2 °C Humidity: 55 ± 15 % Air changes: at least 15/hour 12 hours light/dark cycle

B. STUDY DESIGN

In life dates
2005-11-18 to 2006-11-06

Animal assignment and treatment
In a two-generation reproduction study groups of 28 Sprague-Dawley rats per sex of the F0 generation received daily dietary doses of 0, 1500, 5000 and 15000 ppm (equivalent to mean achieved dose levels of 0, 104, 351 and 1063 mg/kg bw/day for males, and 0, 162, 530 and 1634 mg/kg bw/day for females) glyphosate technical in diet. The dose levels were chosen based on results of a previously conducted study and aimed to achieve the maximum regulatory limit of 1000 mg/kg bw/day. After 10 weeks of treatment pairing of animals within each dose group was undertaken on a one male: one female basis, to produce the F1 litters. At weaning of offspring from the F0 mating phase, groups of twenty-four male and twenty-four female offspring from each dose group were selected to form the F1 generation. The remaining surviving F0 females and unselected offspring were terminated at Day 21 post partum, followed by the termination of all F0 male dose groups. The offspring selected for the F1 generation were dosed for at least 10 weeks and then paired within each dose group to produce the F2 litters. At weaning of the F2 litters all surviving adults and their offspring were killed, followed by the termination of all F1 male dose groups.

Diet preparation and analyses
For preparation of diet mixtures a known amount of the test substance was mixed with a small amount of basal diet at a constant speed for 19 minutes in a Hobart QE200 mixer. This pre-mix was then added to larger amount of basal diet and blended for further 30 minutes in a Hobart H800 mixer.
The stability and homogeneity of the test material in diet were determined. Dietary admixtures were analysed for achieved concentration weekly for the first four weeks of the study and monthly thereafter.

Clinical observations
A check for clinical signs of toxicity, ill-health or behavioural changes was made once daily.

Body weight
Individual body weights were recorded for F0 males on day 1 (prior to treatment) and at weekly intervals for F0 and F1 males until termination. F0 and F1 females were weighed daily until mating was evident.

Body weights for females showing evidence of mating were recorded on days 0, 7, 14 and 21 post coitum. Females with live litters were weighed on days 1, 4, 7, 14 and 21 post partum.

Food consumption and compound intake
During the maturation period, weekly food consumption was recorded for each cage of adults. For females showing evidence of mating, food consumption was recorded for the periods covering days 0 - 7, 7 - 14 and 14 – 21 post coitum. For females with live litters, food consumption was recorded for the period covering days 1 - 4, 4 - 7, 7 - 14, 14 - 21 post partum.
Food conversion efficiency (the ratio of body weight change / dietary intake) was calculated retrospectively for males for both the pre-mating and post-mating phases of the study. For females, food conversion efficiency was only calculated for the pre-mating phases of the study. Due to offspring growth, milk production and weaning, food efficiency could not be accurately calculated for the gestation and lactation phases of the study.

Water consumption
Water intake was observed daily by visual inspection of water bottles for any overt change.

Reproduction parameters
Oestrus cycle
Prior to pairing of females for the F0 and F1 mating phases, a vaginal smear was taken daily for twenty-one days and examined microscopically to determine the stage of oestrous.

Pregnancy and parturition
Pregnant females were observed at approximately 0830, 1230 and 1630 hours daily, and at approximately 0830 and 1230 hours on weekends and public holidays. In addition, the females were observed around the period of expected parturition. The date of mating, date and time of start and end of parturition and duration of gestation was recorded.

Litter data
The following litter data were recorded:
The number of offspring born, the number of offspring alive recorded daily and reported on day 1, 4, 7, 14, 21 post partum. On days 1, 4 and 21, the sex of individual offspring was recorded. The clinical condition of offspring during lactation, as well as individual offspring and total litter weights were recorded after birth on day 1, 4, 7, 14.

Physical and sexual development
All live offspring were observed for the detachment and unfolding of pinna, incisor eruption and eyelid separation and assessed for reflexological response to stimuli by assessing surface righting reflex on day 1 post partum and air righting reflex on day 17 post partum. Pupillary reflex and auditory startle response were performed on day 21 post partum.

All selected F1 offspring were observed for sexual development and the body weight for each individual animal at the time of sexual maturation was recorded. In addition, the ano-genital distance was recorded for all F2 generation offspring on day 1 post partum.

Sacrifice and pathology
All surviving adult females and surviving offspring, except offspring selected to form the F1 generation, as well as surviving males were sacrificed on day 21 post partum.
All adult animals and offspring, including those dying during the study, were subjected to a full external and internal examination, and any macroscopic abnormalities were recorded. For females the uterine implantation sites were counted. In addition, the corpora lutea of all ovaries from pregnant females were counted at necropsy.

The following organs of F0 males and females from each dose group that were sacrificed at the end of the

study sampled, weighed and preserved, except for the thyroids, which were weight after fixation:
adrenals, brain, left cauda epididymis, epididymides, kidneys, liver, ovaries, prostrate, pituitary, seminal vesicles (with coagulating gland and fluids), spleen, testes, thymus, thyroid glands, and uterus (with cervix and oviducts).

The following organs from one male and one female offspring from the F0 and F1 pairings were weighed: brain, spleen, thymus, and uterus.

The following tissues were preserved from all F0 males and females from each dose group in 10 % buffered formalin, except for the right epididymis, right testis, which were fixed in Bouins fluid and 70 % IMS: adrenals, coagulating gland, right epididymis, ovaries, right testis, pituitary, prostrate, seminal vesicles, Uterus (with oviducts) and cervix, vagina and all gross lesions.

A detailed histopathological examination was performed on all sampled tissues from all F0 and F1 control and high-dose animals, and on animals that died or were killed *in extremis*.
During the histopathological examination there were indications of treatment-related changes in the adrenal glands for the F1 animals. Thus, the microscopic examination was subsequently extended to include similarly prepared sections of adrenals from the F1 animals from the 5000 and 1500 ppm dose groups.

Semen assessment
At necropsy of adult F0 and F1 males at least 200 individual sperms were evaluated for motility, motility characteristics, and morphology. In addition, samples of the testis and cauda epididymis of the control and high dose animals were homogenised and examined for homogenisation resistant spermatids.

Evaluation of the oocyte number
From ten control and ten high dose females of the F1 generation slides of the ovaries were prepared and analysed for visible oocytes. The identified oocytes were classified as small, medium or large follicles.

Statistics
Organ weight (absolute and relative to terminal body weight), weekly body weight gain, litter weights and offspring body weights were assessed for dose response relationships by linear regression analysis, followed by one way analysis of variance (ANOVA) incorporating Levene's test for homogeneity of variance. Where variances were shown to be homogenous, pair wise comparisons were conducted using Dunnett's test. Where Levene's test showed unequal variances the data were analysed using non-parametric methods: Kruskal-Wallis ANOVA and Mann-Whitney 'U' test.
The non-parametric methods were also used to analyse implantation loss, offspring sex ratio and developmental landmarks and reflexological responses.
Probability values (p) are presented as follows:
$p<0.001$ ***
$p<0.01$ **
$p<0.05$ *
$p\geq0.05$ (not significant)

Histopathology data were analysed using the following methods to determine significant differences between control and treatment groups for the individual sexes:
1. Chi-squared analysis for differences in the incidence of lesions occurring with an overall frequency of one or greater.
2. Kruskal-Wallis one-way non-parametric analysis of variance for the comparison of severity grades for the more frequently observed graded conditions.
Probability values (p) were calculated as follows:
$p<0.001$ +++ --- ***
$p<0.01$ ++ -- **
$p<0.05$ + - *
$p<0.1$ (+) (-) (*)

p≥0.1 N.S. (not significant)
(+)-signs indicate positive differences from the control group, and (-)-signs indicate negative differences.
* refer to overall differences between group variation which is non-directional.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS

Stability analyses indicated that the dose preparations at nominal concentrations of 1500, 5000 and 15000 ppm were stable for at least six weeks at ambient temperature.
Analyses for homogeneity at the start of treatment indicated that the dose preparations were homogeneous.
Analyses for achieved concentration performed on ten separate occasions demonstrated that the prepared dietary admixture concentrations given to the animals were in the range of 83 to 102 % of the nominal concentration.

B. TEST COMPOUND INTAKE

The group mean achieved dosages are summarised in the table below.

Table 5.6.1-03-: Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat ([redacted], 2007): Group mean achieved dose levels

Group	Dietary concentration (ppm)	Estimated dose level (mg/kg bw/day)	Mean achieved dose level (mg/kg bw/day)			
			Males	Females		
				Maturation	Gestation	Lactation
Control	0	0	0	0	0	0
Low	1500	75	104	126	108	252
Intermediate	5000	250	351	423	358	808
High	15000	750	1063	1273	1109	2520

C. MORTALITY

There were no test substance-related mortalities.

Four unscheduled deaths occurred during the study. In the F0 generation one male of the low dose group and one female of the mid dose group was killed for humane reasons on days 87 and 103, respectively. The male exhibited a mass of about 3 x 4 cm on the lower jaw. The female was in extremis following a suspected prolonged parturition. One high dose female was found dead on day 97 possibly due to complications during parturition.
In the F1 generation one control female was killed on day 99 following severe clinical signs (pallor of the extremities, lethargy, pilo-erection, hunched posture and staining around the ano-genital region); however the aetiology of the signs was not established.

D. CLINICAL OBSERVATIONS

No treatment-related clinical signs of toxicity were noted. Clinical signs observed in control and treated animals of the F0 and F1 generation are summarised in the tables below. These signs were considered unrelated to the test substance, since they were either commonly seen in laboratory rats, or caused by physical injury, or occurred in control and treated rats.

Table 5.6.1-4: Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat ([redacted], 2007): Observed clinical signs in F0 generation

Clinical sign	Number of rats affected in dose group*							
	Control (0 ppm)		Low (1500 ppm)		Mid (5000 ppm)		High (15000 ppm)	
	♂	♀	♂	♀	♂	♀	♂	♀
Abrasion to dorsal region	2/28	0/28	0/28	0/28	0/28	0/28	1/28	0/28
Generalised fur loss	5/28	5/28	3/28	5/28	2/28	6/28	2/28	3/28
Red/brown staining around snout	4/28	0/28	4/28	0/28	1/28	3/28	5/28	0/28
Red/brown staining of fur	1/28	0/28	1/28	0/28	2/28	2/28	1/28	0/28
Red/brown staining around eyes	1/28	0/28	1/28	1/28	0/28	0/28	3/28	0/28
Swollen face (due to overgrowth tooth)	1/28	0/28	0/28	0/28	0/28	0/28	0/28	0/28
Cranial abrasion	0/28	0/28	0/28	0/28	0/28	0/28	2/28	0/28
Red stained urine	0/28	0/28	0/28	0/28	0/28	0/28	1/28	0/28
Facial scab formation	1/28	0/28	0/28	0/28	1/28	0/28	0/28	0/28
Scab formation	1/28	0/28	1/28	0/28	1/28	0/28	0/28	0/28
Large mass under lower jar	0/28	0/28	1/28	0/28	0/28	0/28	0/28	0/28
Mass on dorsal region	0/28	0/28	0/28	0/28	1/28	0/28	0/28	0/28
Scab formation around right eye	0/28	0/28	0/28	0/28	1/28	0/28	0/28	0/28
Physical injury to tail apex	0/28	0/28	0/28	1/28	0/28	0/28	0/28	0/28
Stained fur on head	0/28	0/28	0/28	0/28	0/28	0/28	0/28	1/28
Red swollen ears	0/28	0/28	0/28	0/28	0/28	1/28	0/28	1/28
Blood seen without evidence of offspring born	0/28	1/28	0/28	0/28	028	0/28	0/28	0/28
Blood around vagina (suspected prolonged parturition, killed in extremis)	0/28	0/28	0/28	0/28	0/28	1/28	0/28	0/28
Piloerection	0/28	0/28	0/28	0/28	0/28	0/28	0/28	1/28
Exophthalmia	0/28	0/28	0/28	0/28	0/28	0/28	0/28	1/28

* x/y: number affected / total number of animals in group

Table 5.6.1-5: Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat ([redacted], 2007): Observed clinical signs in F1 generation

Clinical sign	Number of rats affected in dose group*							
	Control (0 ppm)		Low (1500 ppm)		Mid (5000 ppm)		High (15000 ppm)	
	♂	♀	♂	♀	♂	♀	♂	♀
Generalised fur loss	3/28	4/28	0/28	2/28	0/28	6/28	0/28	4/28
Red/brown staining around eyes	2/28	1/28	0/28	0/28	1/28	0/28	0/28	0/28
Red/brown staining of fur	0/28	1/28	2/28	0/28	0/28	2/28	1/28	1/28
Red/brown staining around snout	1/28	7/28	1/28	0/28	4/28	7/28	1/28	4/28
Scabbing and fur loss around eye	0/28	0/28	0/28	0/28	0/28	0/28	1/28	0/28
Protruding sternum	0/28	2/28	0/28	3/28	0/28	3/28	0/28	0/28
Lethargy	0/28	1/28	0/28	0/28	0/28	0/28	0/28	0/28
Hunched posture	0/28	1/28	0/28	0/28	0/28	0/28	0/28	0/28
Staining around ano-genital region	1/28	0/28	0/28	0/28	0/28	0/28	0/28	0/28
Pallor of extremities	1/28	0/28	0/28	0/28	0/28	0/28	0/28	0/28

* x/y: number affected / total number of animals in group

E. BODY WEIGHT

No adverse effect of body weight change was evident for treated animals in comparison to controls throughout the treatment period for both the F0 and F1 generations except for post-partum females treated

with 15000 ppm. During the final week of lactation, both the F0 and F1 generations showed statistically significant less body weight loss in comparison to controls (p < 0.001 and p < 0.01 respectively).

Table 5.6.1-6-: Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat ([redacted], 2007): Body weight changes during lactation (group mean values)

Dietary concentration (ppm)	No. of animals		Body weight Change (g) at day 4	7	14	21
			F0 Generation			
0 (Control)	26	mean SD	15 14	22 9	0 15	-23 10
1500	27	mean SD	16 9	16 9	3 13	-26 13
5000	26	mean SD	16 14	18 13	1 11	-23 11
15000	26	mean SD	18 11	18 12	1 14	-8*** 14
			F1 Generation			
0 (Control)	26	mean SD	14 11	9 13	9 14	-16 13
1500	27	mean SD	14 7	16 11	3 9	-21 17
5000	26	mean SD	17 12	10 10	5 13	-17 13
15000	26	mean SD	16 9	11 9	10 12	-4** 13

S standard deviation
** significantly different from control group p<0.01
*** significantly different from control group p<0.001

F. WATER CONSUMPTION

Daily visual inspection of water bottles showed no overt intergroup differences in water intake for treated males and females from the F0 or F1 generations, when compared to their concurrent controls.

G. REPRODUCTIVE PARAMETERS

Oestrus cycle

There were no toxicologically-significant effects on female oestrous cycles.

Mating Performance, Fertility and Gestation

There were no treatment-related effects on mating performance, fertility and gestation length for both F0 and F1 generation animals.

Table 5.6.1-7a: Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat ([redacted], 2007): Mating, fertility and gestation parameters (F0)

	Ppm	0		1500		5000		15000	
Oestrous cycle – prior to mating (F0)	Regular 4 or 5d cycles	24/28 (86%)		26/28 (93%)		20/28 (71%)		24/28 (86%)	
	Irregular cycle (a)	3/28 (11%)		1/28 (3.5%)		3/28 (11%)		2/28 (7%)	
	Acyclic (b)	1/28 (3.5%)		1/28 (3.5%)		3/28 (11%)		1/28 (3.5%)	
	Pseudo-pregnancy	0/28		0/28		2/28		1/28	
Pre-coital interval (F0)	1	9/28 (32%)		2/28 (7%)		8/28 (29%)		8/28 (29%)	
	2-4	16/28 (57%)		24/28 (86%)		17/28 (61%)		16/28 (57%)	
	>4	3/28		2/28+		3/28		4/28	
Mating		M	F	M	F	M	F	M	F

Table 5.6.1-7a: Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat ([redacted], 2007): Mating, fertility and gestation parameters (F0)

	Ppm		0		1500		5000		15000	
performance (F0)	# Paired		28	28	28	28	28	28	28	28
	# Successful mating		28/28 (100%)		28/28 (100%)		28/28 (100%)		28/28 (100%)	
	# Pregnant			27!		27		28		28
	# Females with live offspring			26		27		28		27++
Gestation length	Gestation length (days)	22		13 (50%)		11 (41%)		9 (32%)		8 (30%)
		22-23		12 (46%)		16 (59%)		19 (68%)		18 (67%)
		>23		1 (4%)		0 (0%)		0 (0%)		1 (3%)
Number of litters			261		27		278		261	
Pre-implantation loss (%) +/- SD			6.5 +/- 6.6		8.0 +/- 12.0		6.7 +/- 5.5		7.4 +/- 7.0	
Post-implantation loss (%) +/- SD			5.1 +/- 6.5		4.9 +/- 6.3		4.2 +/- 4.9		5.1 +/- 8.3	

(a) at least one cycle of two, three, or six to ten days; (b): extended oestrus or di-oestrus
! One female with implantation sites was not observed to give birth to a litter.
+ Male partner killed after first week of pairing and replaced.
++ Includes one female with total litter loss.
1 25 litters used in calculation of pre-implantation loss
$ 26 litters used to calculate pre and post implantation loss

Table 5.6.1-7b: Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat ([redacted], 2007): Mating, fertility and gestation parameters (F1)

	ppm		0		1500		5000		15000	
Oestrous cycle – prior to mating (F1)	Regular 4 or 5d cycles		21/24 (88%)		21/24 (88%)		17/24 (71%)		15/24 (62.5%)	
	Irregular cycle (a)		2/24 (8%)		2/24 (8%)		5/24 (21%)		6/24 (25%)	
	Acyclic (b)		1/24 (4%)		1/24 (4%)		2/24 (8%)		3/24 (12.5%)	
	Pseudo-pregnancy		0/24		0/24		0/24		1/24	
Pre-coital interval (F1)	1		5/24 (21%)		7/24 (29%)		2/24 (8%)		6/24 (25%)	
	2-4		19/24 (79%)		14/24 (58%)		17/24 (71%)		17/24 (71%)	
	>4		0/24 (0%)		3/24 (13%)		4/24 (17%)		1/24 (4%)	
Mating performance (F1)			M	F	M	F	M	F	M	F
	# Paired		24	24	24	24	24	24	24	24
	# Successful mating		24/24 (100%)		24/24 (100%)		24!/24 (100%)		24/24 (100%)	
	# pregnant			22		23		24!		23
Gestation length	Gestation length (days)	22		10 (45%)		11 (48%)		8 (33%)		14 (61%)
		>22		12 (55%)		12 (52%)		15 (62.5%)		9 (39%)
Number of litters (pre and post impl. calculated for)			22 (21)		23 (22)		24 (23)		23 (21)	
Pre-implantation loss (%) +/- SD			13.5 +/- 14.4		9.3 +/- 8.0		13.1 +/- 12.1		8.6 +/- 5.7	
Post-implantation loss (%) +/- SD			12.3 +/- 15.6		5.4 +/- 7.1		6.0 +/- 8.7		7.0 +/- 6.4	

! One female showed no signs of mating but went on to give birth to live offspring
(a) at least one cycle of two, three, or six to ten days; (b): extended oestrus or di-oestrus

H. LITTER DATA

Size and Viability
No overt differences in litter size and viability were detected. The mean numbers of corpora lutea and subsequent number of implantations did not indicate any adverse effect of dietary exposure and pre and post implantation loss for treated animals were essentially similar to controls. There were no toxicologically significant differences in sex ratio for both F0 - F1 and F1 - F2 litters.

Growth and Development
No adverse effects on mean offspring body weights, body weight change or development were detected for male and female offspring in comparison to their controls. Litter data (from F0-F1 and F1-F2): litter size, offspring survival indices, developmental maturation are tabulated in tables below.

Surface righting reflex was unaffected and all offspring displayed normal auditory and visual responses. The mid-air righting for F2 litters in the low-, mid- and high dose group were significantly reduced compared to the control group in a non-dose responsive manner, but was not observed in the F0-F1 offspring, this finding is considered to represent normal biological variation and was not considered to be test substance related. Physical maturation of the selected rats, as assessed by the age and body weight at completion of pinna unfolding, incisor eruption and eye opening was unaffected by the treatment. In F1 male offspring, sexual maturation (preputial separation) was delayed at 15000 ppm without any additional developmental retardation, hence a higher mean body weight was noted at attainment, see table below. No such effects were evident for females and there were no differences in mating performance, sperm changes and histopathological examinations did not reveal any changes in the testis or epididymis. In isolation, this finding was considered to be unrelated to treatment.

Table 5.6.1-8a: Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat ([redacted], 2007): Litter size – F0-F1

	Dose [ppm]	0	1500	5000	15000
# Litters (examined for a)		26 (25)	27	27	26 (25)
# Corpora lutea (a.)		17.4 ± 1.9	15.9 ± 2.3	17.6 ± 2.1	17.2 ± 2.1
# Implantation sites (b.)		16.3 ± 2.0	14.8 ± 3.1	16.3 ± 1.9	15.9 ± 2.2
# Offspring born		15.4 ± 1.7	14.1 ± 3.1	15.7 ± 2.2	15.1 ± 2.2
	Age (day)				
# Alive	1	15.2 ± 1.6	14.0 ± 3.1	15.4 ± 2.1	14.7 ± 2.2
	4	15.2 ± 1.6	13.8 ± 3.1	15.4 ± 2.1	14.5 ± 2.1
	7	15.1 ± 1.7	13.8 ± 3.1	15.2 ± 2.0	14.3 ± 2.0
	14	14.8 ± 1.7	13.5 ± 2.9	13.9 ± 2.4	13.8 ± 2.9
	21	14.8 ± 1.8	13.4 ± 2.9	13.8 ± 2.5	13.8 ± 2.9

Values expressed in group mean ± SD

Table 5.6.1-8b: Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat ([redacted], 2007): Survival indices – F0-F1

	Dose [ppm]	0	1500	5000	15000
# Litters examined		26	27	27	26
	Age (day)				
Live birth index	1	98.8 ± 2.9	99.2 ± 3.0	98.2 ± 3.3	97.2 ± 4.3
Viability index	1	99.5 ± 2.5	98.9 ± 2.9	100 ± 0.0	98.7 ± 2.7
	4	99.5 ± 1.9	99.8 ± 1.1	99.0 ± 3.3	99.1 ± 2.9
	7	98.5 ± 3.3	98.1 ± 4.2	92.0 ± 13.3	96.5 ± 15.4
	14	99.7 ± 1.4	99.8 ± 1.3	99.1 ± 2.7	100.0 ± 0.0
	21	97.2 ± 5.0	96.6 ± 5.4	90.5 ± 14.8	94.9 ± 16.1

Values expressed in group mean ± SD

Table 5.6.1-8c: Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat ([redacted], 2007): Litter size – F1-F2

	Dose [ppm]	0	1500	5000	15000
# Litters (examined for a.and b.)		22 (21)	23(22)	24(23)	23(21)
# Corpora lutea (a.)		18.0 ± 1.9	17.0 ± 1.7	18.2 ± 2.5	17.5 ± 1.7
# Implantation sites (b.)		15.5 ± 2.6	15.4 ± 2.1	15.7 ± 2.4	16.0 ± 1.7
# Offspring born		13.8 ± 3.5	14.7 ± 2.3	14.9 ± 2.8	15.1 ± 1.8
	Age (day)				
# Alive	1	13.7 ± 3.4	14.5 ± 2.3	14.5 ± 3.2	14.9 ± 1.8
	4	13.7 ± 3.4	14.3 ± 2.2	14.5 ± 3.1	14.7 ± 1.9
	7	13.5 ± 3.4	14.3 ± 2.2	14.2 ± 2.9	14.6 ± 2.0
	14	12.4 ± 3.8	13.9 ± 2.1	12.9 ± 3.8	13.6 ± 2.7
	21	12.2 ± 4.0	13.8 ± 2.0	12.8 ± 3.9	13.6 ± 2.7

Values expressed in group mean ± SD

Table 5.6.1-8d: Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat ([redacted], 2007): Survival indices – F1-F2

	Dose [ppm]	0	1500	5000	15000
# Litters examined		22	23	24	23
	Age (day)				
Live birth index	1	99.1 ± 2.3	98.3 ± 3.5	97.1 ± 8.7	98.6 ± 4.1
Viability index	1	100.0 ± 0.0	99.2 ± 2.1	99.6 ± 1.9	99.1 ± 2.5
	4	99.1 ± 2.4	99.3 ± 2.2	98.3 ± 3.9	99.1 ± 3.1
	7	92.0 ± 16.2	97.6 ± 6.4	91.6 ± 19.8	93.5 ± 16.4
	14	97.1 ± 7.7	99.7 ± 1.4	98.4 ± 5.4	100.0 ± 0.0
	21	89.5 ± 19.1	96.0 ± 8.2	89.0 ± 21.1	92.1 ± 17.0

Values expressed in group mean ± SD

Table CA 5.6.1-8-e: Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat ([redacted], 2007): Group mean (%) litter values with successful reflexological response– F0-F1 and F1-F2

Dose [ppm]	0	1500	5000	15000
F0-F1				
Number of litters	26	27	27	26
Surface righting	97.1	95.0	93.0	91.3
Mid-air righting	98.1	99.8	99.5	99.8
Pupil reflex	100.0	100.0	100.0	100.0
Startle reflex	100.0	100.0	100.0	100.0
F1-F2				
Number of litters	22	23	24	23
Surface righting	87.0	90.5	81.3	81.9
Mid-air righting	100.0	98.6*	94.2**	97.1*
Pupil reflex	100.0	100.0	100.0	100.0
Startle reflex	100.0	100.0	100.0	100.0

* significantly different from control group p<0.05
** significantly different from control group p<0.01

Table 5.6.1-9: Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat ([redacted], 2007): Balano-preputial separation of F1 males

Dietary concentration (ppm)	No. of animals		Age (days) at completion	Body weight (g) at attainment
0 (Control)	24	mean SD	43.0 2.3	210 23
1500	24	mean SD	43.3 1.6	216 22
5000	24	mean SD	43.5 2.3	219 22
15000	24	mean SD	45.9** 3.1	230** 28

SD standard deviation
** significantly different from control group p<0.01

Clinical signs
No clinical signs of toxicity were observed for offspring from treated animals.

I. PATHOLOGY
Necropsy
There were no toxicologically significant macroscopic abnormalities detected in the F0 and F1 animals, nor in the offspring.

Organ weights
F0 females treated with 15000 ppm displayed statistically significant increases in liver weights, both absolute and relative to terminal body weight ($p<0.001$). An increase in liver weights was also noted for F1 females treated with 15000 ppm (absolute: $p<0.05$, relative: $p<0.01$). In the absence of any histopathological changes in the liver, and as increased liver weights without histopathological changes were also noted in another repeated dose toxicity study this finding is considered as an adaptive response rather than an adverse effect. Furthermore, F0 females treated with 15000 ppm displayed an increase in kidney weights, both absolute ($p<0.001$) and relative to terminal body weight ($p<0.01$) (see Table 5.6.1-9). No such observations were detected for males treated with 15000 ppm from either generation.

Table 5.6.1-10: Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat ([redacted], 2007): Liver and kidney weights (relative and absolute) of females (group mean values)

Dietary concentration (ppm)	No. of animals		Organ weight (g)			
			Liver		Kidney	
			Absolute	Relative	Absolute	Relative
			F0 Generation			
0 (Control)	26	mean SD	15.0328 1.0493	4.3103 0.2864	2.4315 0.1706	0.6977 0.0548
1500	27	mean SD	15.1465 1.4948	4.3027 0.3435	2.5395 0.1602	0.7233 0.0560
5000	27	mean SD	15.8791 1.7649	4.3570 0.2810	2.5654* 0.2361	0.7062 0.0592
15000	26	mean SD	16.9704*** 1.7620	4.6806*** 0.2977	2.7096*** 0.2203	0.7490** 0.0521
			F1 Generation			
0 (Control)	22	mean SD	16.4887 2.0275	4.5970 0.4038	2.6792 0.4137	0.7483 0.1070
1500	23	mean	16.3848	4.6047	2.5777	0.7257

Dietary concentration (ppm)	No. of animals		Organ weight (g)			
			Liver		Kidney	
			Absolute	Relative	Absolute	Relative
		SD	1.7744	0.2858	0.2776	0.0647
5000	24	mean	17.2591	4.6543	2.8124	0.7585
		SD	2.0969	0.3628	0.5326	0.1229
15000	23	mean	18.0724*	4.9591**	2.7660	0.7578
		SD	1.2434	0.3130	0.2616	0.0517

SD standard deviation
* significantly different from control group p<0.05
** significantly different from control group p<0.01
*** significantly different from control group p<0.001

There were no toxicologically significant intergroup differences detected for the brain, spleen or thymus for offspring of either sex from either generation. Furthermore, there were no differences in uterus weights for treated females from either generation when compared to controls.

Sperm assessment

There were no toxicologically significant effects on the concentration, motility or morphology of samples of sperm from treated F0 and F1 generation males when compared to their controls. Furthermore, no abnormal sperm were detected in the control and treated males from either generation.

There were no toxicologically significant differences in homogenisation resistant spermatid counts for males treated with 15000 ppm from either the F0 or the F1 generation. The number of homogenisation resistant spermatid present in the cauda epididymis for F0 males treated at 15000 ppm were significantly lower than the controls (309 million/g vs. 400 million/g in the control). However, the statistical significance achieved was minimal (p<0.05). No difference in homogenisation resistant spermatid counts was in the testis or in F1 males (testis and cauda epididymides). In isolation, the reduction noted in F0 males was considered to have arisen incidentally.

Table 5.6.1-11a: Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat ([redacted], 2007): Sperm assessment and morphology – group mean values (F0)

	ppm	0		1500		5000		15000	
# Animals (No. of males used for sperm concentration only)		28 (24)		27 (23)		28 (27)		28 (22)	
Concentration (M/mL)		1145.8 ± 561.6		1288.5 ± 549.0		1124.5 ± 475.5		1291.4 ± 551.7	
Motile sperm (%)		77 ± 12		81 ± 11		77 ± 11		82 ± 13	
Progressively motile sperm (%)		3 ± 3		4 ± 4		3 ± 3		5 ± 5	
Cauda epididymis	Sperm count (106/g)	399.9 ± 151.2		-		-		309* ± 162.6	
Testis	Sperm count (106/g)	42.1 ± 12.9						41.3 ± 11.2	
Sperm morphology		#	%	#	%	#	%	#	%
	Normal	200	100%	200	100	200	100	200	100
	Decapitate	0	0	0	0	0	0	0	0
	Abnormal	0	0	0	0	0	0	0	0

* Significantly different from the control group p<0.05

Table 5.6.1-11b: Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat ([redacted], 2007): Sperm assessment and morphology – group mean values (F1)

	ppm	0	1500	5000	15000
# Animals (No. of males used for		24 (23)	24	24	24

sperm concentration only)									
Concentration (M/mL)		873.8 ± 469.6		1008.3 ± 472.7		1229.7 ± 573.2		872.3 ± 598.5	
Motile sperm (%)		59 ± 20		69 ± 14		70 ± 14		59 ± 23	
Progressively motile sperm (%)		2 ± 2		3 ± 2		3 ± 3		2 ± 2	
Cauda epididymis	Sperm count (106/g)	445.9 ± 213.1		-		-		417.0 ± 190.5	
Testis	Sperm count (106/g)	39 ± 13.6						40.2 ± 5.4	
Sperm morphology		#	%	#	%	#	%	#	%
	Normal	200	100	200	100	200	100	200	100
	Decapitate	0	0	0	0	0	0	0	0
	Abnormal	0	0	0	0	0	0	0	0

Oocyte assessment
There were no toxicologically significant differences in follicle numbers for F1 females treated with 15000 ppm when compared to controls.

Histopathology
No treatment-related changes were detected in the F0 generation animals.

In the F1 generation cortical vacuolation of the adrenal glands was observed with a lower incidence and with generally lower grades of severity among males treated with 15000 ppm ($p < 0.05$), 5000 ppm ($p < 0.05 - 0.01$), and 1500 ppm ($p < 0.1 - 0.05$) when compared to controls. The group distribution of incidence and of severity grades may also suggest a consequence of treatment. However, the absence of a dose-related response, may suggest that a higher than normal background incidence of the condition among control male rats may have contributed to the effect on this occasion (Table 5.6.1-11).

Table 5.6.1-12: Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat ([redacted], 2007): Incidence of adrenal cortical vacuolation in males at terminal kill

	Historical control data	Dietary concentration (ppm) 0		1500		5000		15000	
Generation	--	F0	F1	F0	F1	F0	F1	F0	F1
Animals examined	234	28	24	27	24	28	24	28	24
Adrenal cortical vacuolation									
Absent	153	20	7	--	14	--	16	16	14
Present	81	8	17	--	10*	--	8**	12	10***
Minimal	57	6	10	--	6	--	6	8	7
Slight	23	2	7	--	4	--	2	4	2
Moderate	1	0	0	--	0	--	0	0	1
% present	34.6	28.6	71	--	24	--	33	42.9	42

* significantly different from control group $p<0.1 – p<0.05$
** significantly different from control group $p<0.01 – p<0.05$
*** significantly different from control group $p<0.05$

All remaining morphological changes were those commonly observed in laboratory maintained rats of the age and strain employed and, since there were no differences in incidence or severity between control and treatment groups, all were considered to be without toxicological significance.

III. CONCLUSIONS

The oral administration of glyphosate technical to rats by dietary admixture at a maximum dose level of 15000 ppm for two successive generations resulted in possible treatment-related changes at 15000 ppm. The effects however were considered not to represent an adverse health effect, therefore the NOAEL was considered to be 15000 ppm (equivalent to 1063 and 1634 mg/kg bw/day, for males and females, respectively) for adult toxicity for both the F0 and F1 generations.
The NOAEL for reproductive and developmental toxicity, for both generations and offspring was considered to be 15000 ppm.

3. Assessment and conclusion

Assessment and conclusion by applicant:

In this study at the highest dose level of 15000 ppm increased organ weights in liver (F0 and F1 females) and kidneys (F0 females) were observed. The study authors stated that there is no toxicological concern regarding the significantly increased liver weights due to the absence of any histopathological changes in the liver. In fact, in the present study no evidence for histopathological examination of the liver was given. At this high dose level a significant decrease in homogenisation-resistant spermatids (HRS, cauda epididymis) was counted in F0 males (control: 400 million/gram; 15000 ppm: 309 million/gram**). Furthermore, in F1 male offspring sexual maturation (preputial separation) was delayed at 15000 ppm without any additional developmental retardation (e.g. body weight). The authors of the study considered this finding in F1 males (45.9 days versus control 43.0 day) to be unrelated to treatment, because no effects on sexual maturation were evident for females and there were no differences in mating performance. Sperm changes and histopathological examinations did not reveal any changes in the testis or epididymes. Although, the later onset of preputial separation in male offspring at 15000 ppm had obviously no impact on reproductive performance in week 29, a treatment-related effect on sexual maturation at a parental-toxic dose cannot be fully excluded. Therfore, the NOAEL for parental, reproductive and offspring toxicity is considered to be 5000 ppm (approx. equivalent to 351 mg/kg bw/day).

Assessment and conclusion by RMS:

1. Information on the study

Data point:	**CA 5.6.1/004**
Report author	[redacted]
Report year	2000
Report title	Glyphosate acid: Multigeneration reproduction toxicity study in rats
Report No	[redacted]/P/6332
Document No	Not reported
Guidelines followed in study	OECD 416 (2001), Annex V 67/548/EEC, 9.ATP 87/302/EEC OJEC, L133, 47-50 (1988), US-EPA OPPTS 870.3800 (1998)
Deviations from current test guideline (OECD 416, 2001)	Anogenital distance not examined as no treatment-related differences in sex ratio and sexual maturation were observed; thyroid weight not recorded; pre-implantation loss was not determined; pup development investigations restricted to body weight, vaginal opening and preputial separation.
Previous evaluation	Yes, accepted in RAR (2015)

GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Glyphosate acid was administered by dietary admixture to three groups of 26 male and female F0 generation Alpk:APfSD rats each, at dietary concentrations of 1000, 3000 and 10000 ppm. A further group of 26 male and 26 female F0 animals was exposed to basal laboratory diet to serve as a control.

After 10 weeks, the animals were mated and allowed to rear the F1A litters to weaning. The regime was repeated with the F1 parents (26 per sex and dose) selected from the F1A litters to produce the F2A litters after a 10 week pre-mating period. The remaining surviving F0 females and unselected offspring were terminated at Day 29 post partum, followed by the termination of all F0 male dose groups. At weaning of the F2 litters all surviving adults and their offspring were killed, followed by the termination of all F1 male dose groups. Diets containing glyphosate acid were fed continuously throughout the study. Clinical signs, body weight development, food and water consumption were monitored during the study.

Oestrous cycle assessment was performed daily for three weeks prior to mating for both the F0 and F1 generations. Observations for positive evidence of mating were recorded together with the start and completion of parturition. During the maturation phase of the F1 generation offspring, males and females were evaluated for sexual maturation. During the lactation phases daily clinical observations were performed on all surviving offspring, together with litter size. Litter weight, individual offspring weights and landmark developmental signs were also recorded on specific days post partum.

All animals at termination were subjected to a gross necropsy examination and histopathological evaluation of selective tissues was performed if necessary.

The body weights of the F1A pups in the 10000 ppm group were lower in comparison with the control group from Day 2 to Day 29 post partum although a similar effect was not observed for the F2A pups. In line with this, compound-related reductions in body weight and food consumption were evident only in F1 males given 10000 ppm. No further treatment related effects were observed.

I. MATERIALS AND METHODS

A. Materials

Test Material:

Identification:	Glyphosate acid, technical
Description:	White solid
Lot/Batch #:	Y04707/082
Purity:	97.6 % (w/w)
Stability of test compound:	At least 10 years at ambient temperature
Vehicle:	Plain diet
Test Animals:	
Species:	Rat
Strain:	Alpk:APfSD (Wistar-derived)

Source:	[redacted]
Age:	At least 5 weeks old
Sex:	Males and females
Weight at dosing:	Males: approx. 160 g; females: approx. 140 g
Acclimation period:	At least 14 days
Diet/Food:	CT1 diet (Special Diet Services Ltd., Witham, Essex, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Rats were house in pairs (same sex) in multiple rat racks (with rats of the same group in adjacent cages). During mating animals were house one male : one female. Mated females were housed individually during gestation and lactation and provided with bedding material. After day 29 females separated from their litter were house in pairs until termination. Males were housed up to four per cage after being used for mating.
Environmental conditions:	Temperature: 22 ± 3° C Humidity: 50 ± 20 % Air changes: at least 15/hour 12 hours light/dark cycle

B. STUDY DESIGN

In life dates: Not reported

Animal assignment and treatment:
In a two-generation reproduction study groups of 26 Alpk:APfSD rats per sex of the F0 generation received daily dietary doses of 0, 1000, 3000 and 10000 ppm glyphosate acid in diet. The dose levels were chosen based on results of a previously conducted 2-year dietary toxicity study.
After 10 weeks of treatment pairing of animals within each dose group was undertaken on a one male: one female basis, to produce the F1 litters. On day 29 post partum, groups of twenty-six male and twenty-six female offspring from each dose group of the F0 generation were selected to form the F1 generation. F0 males were terminated after the completion of littering and females were terminated on or soon after day 29 of lactation. Unselected offspring were terminated at day 29 post partum. The offspring selected for the F1 generation were dosed for at least 10 weeks and then paired within each dose group to produce the F2 litters. F2 litters were weaned off on day 29 post partum and terminated thereafter.

Diet preparation and analyses
For preparation of diet mixtures (60 kg) a known amount of the test substance was mixed with a small amount of basal diet in a mortar using a pestle. Further milled diet was added to give a pre-mix of 1000 g. Each pre-mix was grounded at a constant speed for 15 min with an automatic pestle and mortar. This pre-mix was then added to a larger amount of basal diet and blended for further 6 minutes in a Pharma Matrix Blender Model PMA 150S (TK Fielder). Control diet was treated in the same way but without addition of the test substance. The stability and homogeneity of the test material in diet were determined in the lowest and the highest dose. Dietary admixtures were analysed for achieved concentration at a 2 month interval.

Clinical observations
A check for clinical signs of toxicity, ill-health or behavioural changes was made once daily.

Body weight
Individual body weights were recorded for F0 adults immediately prior to treatment and weekly thereafter

throughout the pre-mating period. F0 males were weighed weekly thereafter until termination. Successfully mated F0 females were weighed on day 1, 5, 8, 15 and 22 of gestation and on day 1, 5, 8, 15, 22 and 29 post partum. Initial body weights for the F1 adults were recorded at selection on day 29 post partum and weekly thereafter throughout the pre-mating period. F1 males were weighed weekly thereafter until termination. Successfully mated F1 females were weighed on day 1, 5, 8, 15 and 22 of gestation and on day 1, 5, 8, 15, 22 and 29 post partum. All rats were weighed at termination.

Food consumption and compound intake
Food consumption for each cage was recorded throughout the pre-mating period and calculated on a weekly basis. Food utilisation was calculated as the body weight gained by the rats in the cage per 100 g of food eaten. Food consumption was also recorded for females during gestation and lactation and calculated on a weekly basis.

Reproduction parameters
Oestrus cycle
Prior to pairing of females for the F0 and F1 mating phases, a vaginal smear was taken daily for twenty-one days and examined microscopically to determine the stage of oestrous. A vaginal smear was also taken and examined from all F0 and F1 females at termination.

Reproductive performance
The success of mating (production of viable litter) was established. Length of gestation was measured in days from the date of the positive smear to the date of birth. Pre-coital interval was measured as the number of days from the date of pairing to the date of the positive smear.

Litter data
The following litter data were recorded:
The number of offspring born and the number of offspring alive were counted within 24 h after parturition and thereafter on day 5, 8, 15, 22 and 29 post partum. The sex and the litter weight was also recorded at these times. Any clinical findings were recorded. Litters were examined for dead or moribund pups at least once daily.

Physical and sexual development
All selected F1 offspring were observed for sexual development and the body weight for each individual animal at the time of sexual maturation was recorded.

Sacrifice and pathology
All surviving adult females and surviving offspring, except offspring selected to form the F1 generation, were sacrificed on day 29 post partum. Males were sacrificed at completion of the littering. All adult animals and offspring, including those dying during the study, were subjected to a full external and internal examination, and any macroscopic abnormalities were recorded. For F0 and F1 females the uterine implantation sites were counted.

The following organs of F0 males and females from each dose group that were sacrificed at the end of the study were sampled, weighed and preserved:
adrenal gland, brain, left and right epididymides and caudae, kidneys, liver, ovaries, prostrate, pituitary, seminal vesicles (with coagulating gland and fluids), spleen, testes, uterus (with cervix and oviducts).
The following organs from one male and one female offspring from the F1 pairings were weighed: brain, spleen and thymus.

The following tissues were preserved from all F0 males and females from each dose group in 10 % buffered formalin, except for the left epididymis, left testis, which were fixed in Bouins fixative: adrenals, brain, coagulating gland, left epididymis, ovary, left testis, pituitary, prostrate, seminal vesicle, uterus (with oviducts) and cervix, vagina and all gross lesions.

Beside all pups killed in extremis (age 18-29 days) 3 male and 3 female per F2-litter were given a macroscopic examination at termination on day 29 post partum. One of the 3 pups/sex/litter was used for organ weight determination as described above. Following tissues were stored from these pups: brain, spleen, thymus, salivary gland. Abnormal tissue from all these pups were taken and fixed as described earlier.

The reproductive organs from animals suspected of reduced fertility were processed for histopathological examination.

Semen assessment
At necropsy of adult F0 and F1 males sperm were taken from the right distal cauda epididymis. At least 200 individual sperms were evaluated for motility, motility characteristics, and morphology. In addition, samples of the right testis of the control and high dose animals were homogenised and examined for homogenisation resistant spermatids.

Evaluation of the oocyte number
Primordial and small growing follicles were quantified in the left ovary of all F1 females from the control and high dose groups. Quantification was done using five 5 µm thick sections cut from the central third of each ovary and taken at least 100 µm apart and as evenly spaced as possible.

Statistics
One or a combination of the following statistical methods were applied for the evaluation of the measured parameters: analyses of variance (ANOVA), analyses of covariance, ANOVA followed by analyses of covariance, as well as ANOVA following the double arcsine transformation of Freeman and Tukey (1950), or ANOVA following a square root formation, or Fisher's Exact Test.

All analyses were carried out in SAS (1996). For Fisher's Exact Tests the proportion in each treated group was compared to the control group proportion. Analyses of variance and covariance, with the exception of pup organ weights, allowed for the replicate structure of the study design.

Least-squares means for each group were calculated using the LSMEAN Option in SAS PROC MIXED. Unbiased estimates of differences from control were provided by the difference between each treatment group least-squares mean and the control group least-squares mean. Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean using a Student's t-test, based on the error mean square in the analysis.
All statistical tests were two sided.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS
The chemical stability of glyphosate acid in the diet at nominal concentrations of 1,000 and 10,000 ppm was consistent for at least 6 weeks (at room temperature). Homogeneity of the test substance in the dietary mixture was satisfactory, percentage deviations from the overall mean were within 4%. The mean achieved concentrations of glyphosate acid in the preparations were within 9% of the nominal concentrations and the overall mean concentrations were within 3% of the nominal concentrations.

B. TEST COMPOUND INTAKE
The group mean achieved dosages are summarised in Table 5.6.1-13 below.

Table 5.6.1-13: Glyphosate acid: Multigeneration reproduction toxicity study in rats ([redacted], 2000): Group mean achieved dose levels F0 and F1 generation

Group	Dietary concentration (ppm)	Mean achieved dose level (mg/kg bw/day)				
		Males		Females		
		F0	F1	Maturation	Gestation	Lactation
Control	0	0	0	0	0	0
Low	1000	99.4	116.5	113.9	90.4	227.9
Intermediate	3000	292.6	351.8	346.8	277.9	752.4
High	10000	984.7	1161.0	1136.2	910.9	2424.8

C. MORTALITY

There were no test substance related mortalities.

Seven unscheduled deaths occurred during the study. In the F0 generation one control male was killed for humane reasons during week 9 because it was found to have a ruptured eyeball. In the low level dose group one female was killed for humane reasons during week 14 having failed to litter on time, dead foetuses were present in the uterus. In the intermediate level dose group one female was killed in week 14 on gestation day 23 due to difficulties with parturition. In the high-level dose group one female with an imperforate vagina and one male having a subcutaneous mass were killed in week 15 and 18, respectively. In the F1 generation two control animals were killed in extremis. One male due to an accidental injury in week 2 and one female in week 15 due to difficulties with parturition (one dead foetus present in uterus).

D. CLINICAL OBSERVATIONS

No treatment-related clinical signs of toxicity were noted.

During the pre-mating period, annular constrictions were visible on the tails of the F0 and F1 male and female rats. Almost all males and approximately half of the females, in all groups, were affected. Scaly tail was also observed in some of the animals. These findings were considered incidental to the administration of glyphosate acid in the diet. Other recorded changes in clinical condition were either isolated occurrences or of an incidence comparable with that of the control group.

These signs were considered unrelated to the test substance, since they were either commonly seen in laboratory rats, or caused by physical injury, or occurred in control and treated rats.

E. BODY WEIGHT

There was no effect of glyphosate acid on body weight adjusted for initial weight for the F0 rats, males and females, during the pre-mating period. For the F1 males given 10000 ppm, body weight was slightly lower at week 1, in comparison with the control group. Thereafter, body weights adjusted for initial weight remained lower than the controls for the duration of the pre-mating period and were statistically significant different from week 2 through to week 8 (see Table 5.6.1-14-). There was no effect of 10000 ppm on the body weight of the F1 females and no effect of 3000 or 1000 ppm on the body weight of the F1 males or the F1 females (see **Error! Reference source not found.**). There was no effect of glyphosate acid on body weight adjusted for initial weight for either the F0 or F1 rats during gestation or lactation.

Table 5.6.1-14-: Glyphosate acid: Multigeneration reproduction toxicity study in rats ([redacted], 2000): Body weight during the pre-mating period - F1 generation (group mean values, adjusted for initial weight)

	Body weight (g)			
F1 generation	Control (0 ppm)	Low (1000 ppm)	Mid (3000 ppm)	High (10000 ppm)

Week	♂ (n=25)	♀ (n=26)	♂ (n=26)	♀ (n=26)	♂ (n=26)	♀ (n=26)	♂ (n=26)	♀ (n=26)
1	80.2	74.8	81.1	75.2	78.1	74.2	75.3	73.4
2	130.1	115.4	132.3	115.7	128.6	114.7	127.6*	115.2
3	188.5	152.6	190.7	154.7	186.5	151.2	183.3*	152.3
4	246.2	178.3	247.6	180.2	242.8	176.5	237.3**	179.4
5	300.3	201	304.1	202.7	296.5	199.7	289.5**	202.1
6	345	219.8	347.5	224.1	334.5	217.2	328.7**	218.4
7	377.2	231.7	382.4	237.1	369	228.3	360.5**	234.4
8	403.6	241.9	410.1	245.1	395.3	237.2	387.0*	245.6
9	425	250.3	433.3	253.6	416.3	245.1	411.8	252.5
10	443.4	259.7	453.1	263.8	435.1	251.7	431.6	258.1
11	461.7	265.7	471.3	271.2	455.5	258.8	449.7	266.9

* significantly different from control group p<0.05
** significantly different from control group p<0.01

F. FOOD CONSUMPTION, FOOD UTILISATION

There was no effect of glyphosate acid on food consumption for the F0 generation as well as all F1 females and F1 males of the low and intermediate level dose group during the pre-mating period. Only F1 males of the high-level dose group showed significantly lower food consumption throughout the pre-mating period. There was no effect of glyphosate acid on food utilisation for the F0 generation, all F1 females and F1 males of the low and intermediate dose group during the pre-mating period. Food utilisation was slightly higher for F1 males given 10000 ppm glyphosate acid, the difference from control being statistically significant for weeks 5-8 only. There was no effect of glyphosate acid on food consumption for either the F0 or F1 rats during gestation or lactation.

Table 5.6.1-15: Glyphosate acid: Multigeneration reproduction toxicity study in rats ([redacted], 2000): Food consumption and food utilisation during the pre-mating period - F1 generation males (group mean values)

Parameter	Dietary dose level			
	Control (0 ppm)	Low (1000 ppm)	Mid (3000 ppm)	High (10000 ppm)
N	13	13	13	13
Food consumption (g/animal/day)				
Week				
1	19.3	19.7	19.0	18.1*
2	27.2	27.8	27.0	25.9*
3	31.1	31.9	30.9	29.3**
4	34.6	35.5	33.9	32.6**
5	35.9	36.4	35.8	33.9*
6	36.5	36.6	34.7	33.2**
7	36.1	36.6	34.6	33.2**
8	35.5	36.1	34.1	33.0**
9	34.8	35.5	33.8	32.3**
10	35.5	35.7	34.1	33.0**
Food utilisation (g growth/100 g food)				
Weeks				
1 – 4	28.44	28.22	27.99	28.11
5 – 8	12.35	12.57	12.31	13.25*

Table 5.6.1-15: Glyphosate acid: Multigeneration reproduction toxicity study in rats ([redacted], 2000): Food consumption and food utilisation during the pre-mating period - F1 generation males (group mean values)

Parameter	Dietary dose level			
	Control (0 ppm)	Low (1000 ppm)	Mid (3000 ppm)	High (10000 ppm)
N	13	13	13	13
Food consumption (g/animal/day)				
Week				
9 -10	7.22	7.61	8.24	8.37
Overall (weeks 1 – 10)	16.78	16.93	16.91	17.38

* significantly different from control group p<0.05
** significantly different from control group p<0.01

G. REPRODUCTIVE PARAMETERS

Oestrus cycle

There were no consistent toxicologically-significant effects on female oestrous cycles.

Mating Performance, Fertility and Gestation

There were no treatment-related effects on pre-coital interval, mating performance, and gestation length for both F0 and F1 generation animals.

Table 5.6.1-16a: Glyphosate acid: Multigeneration reproduction toxicity study in rats ([redacted], 2000): Mating, fertility and gestation parameters (F0)

	ppm		0		1000		3000		10000	
Oestrous cycle – prior to mating (F0)	Regular 4 or 5d cycles		15/26 (58%)		14/26 (54%)		19/26 (73%)		13/26 (50%)	
	Irregular cycle (a)		11/26 (42%)		12/26 (46%)		7/26 (27%)		13/26 (50%)	
	Acyclic (b)		0/26		0/26		0/26		0/26	
	Mean number of cycles		4.73		4.46		4.88		5.38*	
Pre-coital interval (F0)	1		7/25 (28%)		7/26 (27%)		7/26 (27%)		11/26 (42%)	
	2-4		17/25 (68%)		19/26 (73%)		18/26 (69%)		14/26 (54%)	
	>4		1/25 (4.0%)		0/26 (0.0%)		1/26 (3.8%)		1/26 (3.8%)	
Mating performance (F0)			M	F	M	F	M	F	M	F
	# Paired		26	26	26	26	26	26	26	26
	# Successful mating		23/26 (88.5%)		22/26 (84.6%)		22/26 (84.6%)		24/26 (92.3%)	
Gestation length and # of pups (F0)	# pregnant			23		22		22		24
	Gestation length (days)	<22		1 (4.3%)		0 (0%)		0 (0%)		0 (0%)
		22		14 (61%)		16 (73%)		18 (82%)		20 (83%)
		>22		8 (35%)		6 (27%)		4 (18%)		4 (17%)
	Pups born live			277/295 (94.9%)		253/261 (96.5%)		261/264** (99.1%)		291/301 (97.2%)
	Mean # of pups born			12.8		11.9		12		12.5
Post-implantation loss				12/307		18/279		24/288*		10/311
Prop. of litters affected				9/23		12/22		15/22		6/24

(a) at least one cycle of two, three, or six to ten days; (b): at least ten days without oestrus

Table 5.6.1-16-b: Glyphosate acid: Multigeneration reproduction toxicity study in rats ([redacted], 2000): Mating, fertility and gestation parameters (F1)

	ppm		0		1000		3000		10000	
	mg/kg bw/day		0							
Oestrous cycle – prior to mating (F1)	Regular 4 or 5d cycles		23/26 (88.5%)		25/26 (96%)		23/26 (88.5%)		23/26 (88.5%)	
	Irregular cycle (a)		3/26 (11.5%)		1/26 (4%)		3/26 (11.5%)		3/26 (11.5%)	
	Acyclic (b)		0/26		0/26		0/26		0/26	
	MEAN		4.35		4.46		4.62		4.50	
Pre-coital interval (F1)	1		5/26 (19%)		6/25 (24%)		2/24 (8%)		3/26 (11.5%)	
	2-4		18/26 (69%)		19/25 (76%)		21/24 (88%)		19/26 (73%)	
	>4		3/26 (12%)		0/25 (0.0%)		1/24 (4%)		4/26 (15.4%)	
Mating performance (F1)			M	F	M	F	M	F	M	F
	# paired		26	26	26	26	26	26	26	26
	# successful mating		22/26 (84.6%)		23/26 (88.5%)		21/26 (80.8%)		25/26 (96.2%)	
Gestation length and # of pups (F1)	# pregnant			22		23		22		25
	Gestation length (days)	22		15 (68%)		19 (83%)		18 (86%)		25** (100%)
		>22		7 (32%)		4 (17%)		3 (14%)		0** (0%)
	Pups born live			226/239 95%		254/263 96.3%		251/254** 98.7%		296/304 96.9%
	Mean # of pups born			10.9		11.4		12.4		12.6
Post-implantation loss				6/245		11/274		6/260		11/315
Prop. of litters affected				5/22		7/23		4/21		6/25

(a) at least one cycle of two, three, or six to ten days; (b): at least ten days without oestrus

H. LITTER DATA

Size and Viability

No overt effects of glyphosate acid on pup survival or on litter size during lactation were detected.

In both generations the incidence of whole litter losses was low and similar across all groups. Glyphosate acid treatment did not affect the percentage of post-implantation loss. The proportion of F1A and F2A pups born live was slightly higher in the glyphosate acid groups than in the control group. There was no effect of glyphosate acid on litter size at birth or during the time of lactation for either the F1A or F2A pups. The proportion of litters with all pups surviving and the proportion of pups surviving during lactation were also unaffected by the treatment. An increased proportion of litters with all pups surviving noted for the F1A litters in the 10000 ppm group in comparison with the control group were not present for the F2A litters since the F2A controls showed an improvement over the F1A controls. Sex distribution within the litters was not altered by the administration of glyphosate acid. Individual pup body weights were recorded within 24 hours of births and on days 5, 8, 15, 22 and 29 post partum. As pups were not individually identified, the data were recorded by sex and litter.

Growth and Development

There was no effect of glyphosate acid on pup weight at birth for the F1A or F2A pups. Thereafter, the adjusted mean body weights of the F1A pups in the 10000 ppm group were lower in comparison with the control group. The differences from control were statistically significant for males from day 8 through to day 29, and for females from day 5 through to day 29 (see table below). A similar effect was neither observed for the F2A pups in the 10000 ppm group or for the F1A pups of the low and intermediate dose level groups. There was no effect of glyphosate acid on total litter weight of either generation. Also the day

of age when preputial separation or vaginal opening occurred in the F1 parents was unaffected by treatment.

Table 5.6.1-17a: Glyphosate acid: Multigeneration reproduction toxicity study in rats ([redacted], 2000): mean pup body weights – F1A

ppm	0	1000	3000	10000	0	1000	3000	10000
Sex	Males				Females			
Age (d)								
1	5.8 ± 0.6	6.1 ± 0.6	6.0 ± 0.8	6.1 ± 0.6	5.4 ± 0.6	5.8 ± 0.6	5.6 ± 0.6	5.7 ± 0.6
N	23	22	22	24	23	22	22	24
5	8.8 ± 1.6	9.2 ± 1.7	9.0 ± 1.4	8.6 ± 1.2	8.7 ± 1.1	8.9 ± 1.8	8.3 ± 1.4	8.1 ± 1.4
Adj. mean	9.2	9.1	8.9	8.5	9.0	8.5	8.4	8.1**
N	22	22	21	23	21	21	21	24
8	13.3 ± 1.6	13.5 ± 2.7	13.3 ± 1.9	12.8 ± 1.6	13.0 ± 1.6	13.2 ± 2.4	12.4 ± 2.1	12.2 ± 1.5
Adj. mean	13.8	13.4	13.2	12.6*	13.3	12.8	12.4	12.1**
N	21	22	21	23	21	20	21	23
15	26.3 ± 2.6	26.2 ± 5.1	25.9 ± 3.6	24.9 ± 2.8	25.7 ± 2.6	25.8 ± 4.4	24.3 ± 3.6	24.0 ± 2.5
Adj. mean	26.8	26.1	25.8	24.6*	26.1	25.2	24.5	23.8*
N	21	22	21	23	21	20	21	23
22	42.5 ± 4.8	42.7 ± 8.1	41.7 ± 6.2	39.7 ± 5.6	41.3 ± 4.8	41.4 ± 7.2	39.1 ± 5.9	38.3 ± 5.3
Adj. mean	43.4	42.4	41.4	39.2*	41.9	40.3	39.4	37.7*
N	21	22	21	23	21	20	21	23
29	80.3 ± 8.3	79.9 ± 12.1	79.8 ± 10.5	75.4 ± 8.4	76.1 ± 8.0	75.6 ± 9.9	73.5 ± 8.8	70.8 ± 7.4
Adj. mean	81.7	79.5	79.6	74.6*	77.1	74.0	74.1	69.9**
N	21	22	21	23	21	20	21	23

Values expressed as group mean ± SD
* statistically significant difference from control group p<0.05
** statistically significant difference from control group p<0.01

Table 5.6.1-17b: Glyphosate acid: Multigeneration reproduction toxicity study in rats ([redacted], 2000): Mean pup body weights – F2A

ppm	0	1000	3000	10000	0	1000	3000	10000
Sex	Males				Females			
Age (d)								
1	6.3 ± 0.5	6.3 ± 0.5	6.3 ± 0.6	6.2 ± 0.4	6.1 ± 0.5	5.9 ± 0.6	5.9 ± 0.6	5.8 ± 0.4
N	22	23	21	25	22	23	19	25
5	9.8 ± 1.1	10.0 ± 1.1	9.4 ± 1.0	9.3 ± 0.9	9.6 ± 1.4	9.6 ± 1.3	9.1 ± 1.1	8.9 ± 0.9
Adj. mean	9.7	9.9	9.3	9.5	9.3	9.6	9.1	9.1
N	21	22	19	25	20	21	19	24
8	14.5 ± 1.9	14.8 ± 1.5	13.9 ± 1.6	14.0 ± 1.5	14.3 ± 2.4	14.2 ± 1.9	13.4 ± 1.9	13.4 ± 1.7
Adj. mean	14.3	14.7	13.8	14.2	13.8	14.2	13.4	13.7
N	21	22	19	25	20	21	19	24
15	27.8 ± 4.0	28.5 ± 3.2	26.5 ± 3.8	27.1 ± 3.3	27.6 ± 4.9	27.5 ± 3.8	25.8 ± 4.4	25.9 ± 3.5
Adj. mean	27.4	29.3	26.4	27.5	26.7	27.5	25.8	26.5
N	21	22	19	25	20	21	19	24
22	45.3 ± 7.2	46.7 ± 5.8	43.1 ± 6.6	44.1 ± 5.8	44.4 ± 7.9	44.7 ± 6.3	41.7 ± 7.2	41.9 ± 6.0

Table 5.6.1-17b: Glyphosate acid: Multigeneration reproduction toxicity study in rats ([redacted], 2000): Mean pup body weights – F2A

ppm	0	1000	3000	10000	0	1000	3000	10000
Sex	Males				Females			
Adj. mean	44.5	46.2	43.1	44.9	42.7	44.8	41.8	42.9
N	21	22	19	25	20	21	19	24
29	84.2 ±11.2	86.8 ± 9.8	80.6 ± 10.1	81.5 ± 8.9	80.1 ± 11.3	80.7 ± 10.1	75.5 ±10.1	75.9 ± 8.8
Adj. mean	83.0	86.0	80.6	82.8	77.7	80.6	75.6	77.4
N	21	22	19	25	20	21	19	24

Values expressed in group mean ± SD

Table 5.6.1-18: Glyphosate acid: Multigeneration reproduction toxicity study in rats ([redacted], 2000): Sexual maturation – age group mean as developmental landmark (F1A)

Dose	ppm	0	1000	3000	10000
Vaginal opening	Day of age	35.3 ± 1.3	35.3 ± 1.5	35.2 ± 1.6	35.9 ± 1.6
Balano-preputial separation	Day of age	47.1 ± 2.3	46.6 ± 1.7	47.2 ± 1.6	48.0 ± 2.1

Clinical signs
No clinically observable signs of toxicity were noted for offspring from treated animals.

I. PATHOLOGY
Necropsy
No macroscopic findings that could be attributed to the treatment with glyphosate acid were observed in any animal of the F0 and F1 generation.

The incidence of unilateral pelvic dilatation was slightly higher (9/69) in F2A females in the 10,000 ppm group compared with the other groups. Unilateral pelvic dilatation is a very common spontaneous change in the Alpk:APfSD strain of rat. There was no increase in incidence in the F0 or F1 adults or in the F1A pups and, as an isolated observation, it is considered incidental to treatment with glyphosate acid.

Organ weights
The treatment of rats with glyphosate acid did not affect the weight of the adrenal glands, brain, right cauda epididymis, epididymides, kidney, liver, ovary, pituitary gland, prostate gland, spleen, seminal vesicles, testes or uterus. For the F0 males given 10000 ppm glyphosate acid, liver and kidney weights adjusted for body weight were statistically significantly greater than in the control group. Similar changes were not observed in the F1 males given 10000 ppm glyphosate acid. Absolute and relative values were comparable with the control group (see table below). The weight changes seen in the liver and kidney of the F0 males were therefore considered not to be treatment related. For the F0 males given 3000 or 10000 ppm glyphosate acid, brain weight adjusted for body weight was statistically significantly greater than in the control group. Absolute values were comparable with the control group. Similar changes were not observed in the F1 animals. The weight changes seen in the brain of the F0 males were therefore considered to be incidental to treatment.

Table 5.6.1-19a: Glyphosate acid: Multigeneration reproduction toxicity study in rats ([redacted], 2000): Liver, kidney and brain weights (relative and absolute) of males (group mean values)

Dietary concentration (ppm)	No. of animals		Organ weight (g)					
			Liver		Kidney		Brain	
			Absolute	Relative	Absolute	Relative	Absolute	Relative
			F0 Generation					
0 (Control)	25	mean	19.3	3.4	3.20	0.57	2.14	0.38
		SD	2.6	0.2	0.38	0.04	0.09	0.03
1000	26	mean	19.1	3.5	3.17	0.58	2.12	0.39
		SD	2.3	0.2	0.36	0.04	0.08	0.03
3000	26	mean	18.7	3.5	3.11	0.58	2.12	0.40
		SD	1.9	0.2	0.27	0.03	0.07	0.03
10000	25	mean	19.7	3.6	3.23	0.59	2.13	0.40
		SD	2.7	0.2	0.38	0.04	0.07	0.05
			F1 Generation					
0 (Control)	25	mean	21.4	3.7	3.42	0.6	2.11	0.37
		SD	2	0.3	0.31	0.05	0.07	0.02
1000	26	mean	21.4	3.7	3.45	0.59	2.12	0.37
		SD	3.3	0.4	0.37	0.04	0.07	0.02
3000	26	mean	20.1	3.6	3.32	0.6	2.1	0.38
		SD	2.6	0.3	0.31	0.04	0.07	0.03
10000	26	mean	19.7*	3.6	3.36	0.62	2.1	0.39
		SD	2.3	0.3	0.28	0.04	0.07	0.03

SD standard deviation
* significantly different from control group p<0.05

Table 5.6.1-19b: Glyphosate acid: Multigeneration reproduction toxicity study in rats ([redacted], 2000): Liver, kidney and brain weights (organ weight adjusted for body weight) of males (group mean values)

Dietary concentration (ppm)	No. of animals		Organ weight adjusted for body weight		
			Liver	Kidney	Brain
			F0 Generation		
0 (Control)	25	mean	18.8	3.13	2.09
1000	26	mean	19.1	3.18	2.12
3000	26	mean	19.0	3.16	2.13*
10000	25	mean	19.8**	3.25*	2.13*
			F1 Generation		
0 (Control)	25	mean	20.9	3.37	2.11
1000	26	mean	20.5	3.35	2.11
3000	26	mean	20.6	3.38	2.10
10000	26	mean	20.5	3.45	2.11

SD standard deviation
* significantly different from control group p<0.05
** significantly different from control group p<0.01

There was no effect of glyphosate acid on brain, spleen or thymus weight.

For the F1A female pups in the 10000 ppm group absolute thymus weight was statistically significantly lower than in the control group. There was no effect of glyphosate acid on the thymus weight of the F2A pups. The observation in the F1A females is therefore considered incidental to treatment with glyphosate acid.

Sperm assessment
In F0 and F1 males no effect of glyphosate acid on the number of sperm, sperm motility parameters or sperm morphology was observed.

Table 5.6.1-20a: Glyphosate acid: Multigeneration reproduction toxicity study in rats ([redacted], 2000): Sperm assessment and morphology – group mean values (F0)

	ppm	0	1000	3000	10000
# Animals		25	25	26	25
Average Path Velocity (µm/sec)		98.9 ± 8	97.5 ± 10.2	96.8 ± 10.6	99.5 ± 11.6
Straightness (%)		55.7 ± 5.3	56 ± 7.9	57.5 ± 6.6	55.9 ± 6.5
Motile sperm (%)		85.9 ± 8.8	81.5 ± 11.1	83.5 ± 16.1	85 ± 8.5
Cauda epididymis (Right)	Weight (g)	0.258 ± 0.031	0.238** 0.025	0.247 ± 0.033	0.256 ± 0.024
	Total (106)	131 ± 40	112 ± 51	117 ± 44	138 ± 70
	Sperm count (106/g)	513 ± 160	469 ± 213	477 ± 182	550 ± 310
Testis	Total (million) sperm	91 ± 12			90 ±15
	Sperm count (106/g)	55 ± 7			53 ± 10
Sperm morphology Head (%)	Detached head	1.72 ± 1.47	1.50 ± 1.74	3.67 ± 7.61	1.58 ± 1.59
	Abnormal shaped head	0.11 ± 0.2	0.09 ± 0.22	0.05 ± 0.15	0.10 ± 0.19
Tail (%)	Coiled/Kinked	0.09 ± 0.18	0.07 ± 0.17	0.23 ± 0.36	0.10 ± 0.25
	Abnormal sized tail	0.22 ± 0.27	0.27 ± 0.34	0.14 ± 0.26	0.37 ± 0.55
Sperm (%)	Normal	97.9 ± 1.7	98.1 ± 1.9	95.9 ± 7.8	97.9 ± 2.1
	Abnormal	2.1 ± 1.7	1.9 ± 1.9	4.1 ± 7.8	2.1 ± 2.1

Table 5.6.1-20b: Glyphosate acid: Multigeneration reproduction toxicity study in rats ([redacted], 2000): Sperm assessment and morphology – group mean values (F1)

	ppm	0	1000	3000	10000
# Animals		25	26	26	26
Average Path Velocity (µm/sec)		92.1 ± 15.5	91.1 ± 22.0	88.1 ± 16.1	88.1 ± 13.1
Straightness (%)		52.1 ± 8.3	49.6 ± 13.0	51.6 ± 10.0	51.1 ± 8.7
Motile sperm (%)		78.1 ± 15.9	82.7 ± 18.8	79.7 ± 14.2	78.8 ± 11.9
Cauda epididymis (Right)	Weight (g)	0.255 ± 0.034	0.263 ± 0.037	0.259 ± 0.030	0.255 ± 0.031
	Total (106)	112 ± 44	129 ± 72	116 ± 50	103 ± 56
	Sperm count (106/g)	444 ± 183	503 ± 308	447 ± 177	419 ± 259
Testis	Total (million) sperm	96 ± 14			92 ±20
	Sperm count	56 ± 7			55 ± 12

Table 5.6.1-20b: Glyphosate acid: Multigeneration reproduction toxicity study in rats ([redacted], 2000): Sperm assessment and morphology – group mean values (F1)

	ppm	0	1000	3000	10000
	(106/g)				
Sperm morphology Head (%)	Detached head	0.65 ± 0.48	0.75 ± 0.93	0.80 ± 0.68	0.59 ± 0.51
	Abnormal shaped head	0.17 ± 0.23	0.13 ± 0.25	0.13 ± 0.22	0.02** ± 0.10
Tail (%)	Coiled/Kinked	0.11 ± 0.20	0.04 ± 0.13	0.10 ± 0.24	0.07 ± 0.22
	Abnormal sized tail	0.11 ± 0.24	0.09 ± 0.23	0.07 ± 0.17	0.09 ± 0.24
Sperm (%)	Normal	99.0 ± 0.8	99.0 ± 1.0	98.9 ± 0.8	99.2 ± 0.6
	Abnormal	1.0 ± 0.8	1.0 ± 1.0	1.1 ± 0.8	0.8 ± 0.6

** significantly different from control group p<0.01

Oocyte assessment
There was no effect of 10000 ppm glyphosate acid on the number of primordial and small growing follicles in the left ovary of the F1 parent animals.

Histopathology
No treatment-related changes were detected in the F0 and F1 generations.

III. CONCLUSIONS

The oral administration of glyphosate acid to rats by dietary admixture at a maximum dose level of 10000 ppm for two successive generations of the Alpk:APfSD rat resulted in possible treatment-related changes at 10000 ppm, where a reduction in the body weight of the F1A pups in the 10000 ppm group with a subsequent reduction in body weight of the selected F1 parent males for the duration of the pre-mating period was observed. Therefore the 'No Observed Adverse Effect Level' (NOAEL) was considered to be 3000 ppm (equivalent to 322 and 459 mg/kg bw/day for males and females, respectively) for maternal and offspring for both the F0 and F1 generations.

3. Assessment and conclusion

Assessment and conclusion by applicant:
This study is considered valid in spite of some deviations from the most recent OECD 416 (2001). The oral administration of glyphosate acid to rats by dietary admixture at a maximum dose level of 10000 ppm for two successive generations of the Alpk:APfSD rat resulted in treatment-related changes at 10000 ppm, where a reduction in the body weight of the F1A pups in the 10000 ppm group with a subsequent reduction in body weight of the selected F1 parent males for the duration of the pre-mating period was observed. Therefore, the NOAEL was considered to be 3000 ppm (equivalent to 322 and 459 mg/kg bw/day for males and females, respectively) for maternal and offspring for both the F0 and F1 generations. The NOAEL for reproduction is considered to be 10000 ppm (equivalent to 1072 / 911 – 2425 mg/kg bw/day).

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.6.1/005
Report author	[redacted]

Report year	1997
Report title	HR-001: A two-generation reproduction study in rats
Report No	[redacted] 96-0031
Document No	Not reported
Guidelines followed in study	OECD 416 (1981), US-EPA FIFREA Guidelines Subdivision F (1984), Japan MAFF Guideline 59 NohSan No. 4200 (1985)
Deviations from current test guideline (OECD 416, 2001)	Number of animals used are not in line with the recommendation by the guideline; pup development investigations restricted to body weight; developmental landmarks (vaginal opening and preputial separation) not examined; anogenital distance was not determined; thyroid weight and histopathology were not determined; pre- and post-implantation loss were not reported; number of corpora lutea was not given; time to mating was not reported.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

To evaluate the potential effects of glyphosate on reproduction groups of 24 Sprague-Dawley rats per sex were fed diets containing test substance concentrations of 0, 1200, 6000 and 30000 ppm for two consecutive generations. Clinical signs, body weight development, food consumption were monitored during the study. Reproductive parameters (oestrus cycle, mating, fertility and gestation indices, sperm assessment) were also evaluated. Gross pathological examinations were performed on all animals. Organ weight determinations and histopathological examinations were also performed on designated animals. Litter data determined covered the total number of live and dead pups, the number of males and females, viability indices, body weights and clinical signs.

There were no treatment-related signs of toxicity noted in parental animals of the low- and mid-dose groups. At 30000 ppm treatment-related adverse effects consisted of defecation of loose stool in F0 and F1 males and females, and lower body weights in F0 and F1 males than in the respective control group males. Also in the high-dose group distension of the caecum and increased liver and kidney weights in F0 and F1 males and a decreased prostrate weight in F1 males were observed at necropsy.

Reproductive performance was not affected by the treatment in any dose group. The slightly lower gestation indices observed in F1 females of the mid- and high-dose group were considered unrelated to treatment, as shown by the results of the reciprocal crosses of F1 animals with untreated rats.

No treatment-related alterations were observed in offspring of the low- and mid-dose groups. In the high-dose group pups of both sexes of the F1 and F2 generation showed significant decreased body weight gains and a significant increase in the incidence of distension of the caecum.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material:

Identification:	Glyphosate technical, Code: HR-001
Description:	White crystal
Lot/Batch #:	T-950308

Purity:	94.61 % (w/w)
Stability of test compound:	Not reported
Vehicle:	Plain diet
Test Animals:	
Species:	Rat
Strain:	Sprague-Dawley; Crj:CD (SD)
Source:	[redacted]
Age:	5 weeks
Sex:	Males and females
Weight at dosing:	Males: 132 - 148 g; females: 112 - 126 g
Acclimation period:	7 days
Diet/Food:	Certified pulverised feed (MF Mash, Oriental Yeast Co., Ltd), *ad libitum*
Water:	Filtered, sterilised well water, *ad libitum*
Housing:	During acclimatisation in groups of five per sex in suspended wire-mesh stainless steel cages). During pre-mating, and mating periods animals were housed in groups o 3/sex/cage. During mating one male and one female were housed in aluminium cages with wire-mesh floors and fronts. Mated females were housed individually during gestation and lactation and provided with bedding material. After day 29 females separated from their litter were house in pairs until termination. Males were housed up to four per cage after being used for mating.
Environmental conditions:	Temperature: 22 ± 2 °C Humidity: 55 ± 10 % Air changes: 15/hour 12 hours light/dark cycle

B. STUDY DESIGN

In life dates: 1996-04-16 - 1997-03-31

Animal assignment and treatment:
In a two-generation reproduction study groups of 24 Sprague-Dawley rats per sex of the F0 generation received daily dietary doses of 0, 1200, 6000 and 30000 ppm HR-001 in diet. The dose levels were chosen based on results of a preliminary reproductive study in Crj:CD (SD) rats. In the preliminary study, Crj : CD (SD) rats, 8/sex/group, were given diets containing glyphosate at dose levels of 0, 1000, 3000, 10000, or 30000 pprn during the 3–week-rearing and subsequent breeding periods. In the 30000 ppm group, treatrnent related adverse -effects on parental animals were evident, and the incidence of loose stool was significantly higher than that in the control grqup. Moreover, decreases in the body weights and food consumption were observed. The adverse effects on the body weights and food consumption were more prominent in males than in females, and statistically significant differences from controls were detected in the mean body weights of males on weeks 5, 6, and 8 of treatrnent. Necropsy of parental animals at termination of test substance treatment revealed distention of the cecum in males with a statistically significant increase in the incidence. In the 1000, 3000, and 10000 ppm groups, no treatment-related adverse effects on parental animals were detected. As for the reproductive performance of parental animals, no treatrnent-related adverse effects were detected in any treated groups. In offspring in the 30000 ppm group, treatment-related adverse effects were evident. The rnean body weights of both sexes on days 14 and 21 of lactation in this dose group were lower than those in the control group, and the differences on day 21 of lactation were

statistically significant. Necropsy of pups after weaning revealed distention of the cecum with a significantly increased incidence. In offspring in the 10000 ppm group, the following treatment-related adverse effects were also observed: a decrease in the body weights on day 21 of lactation and a low incidence of distention of the caecurn. In the 1000 and 3000 ppm group, no treatment-related abnormalities were detected in any pups. Based on these results, the dose level of 30000 ppm was selected for the high-dose level in the present study and 1200 and 6000 pprn for the low- and middle-dose levels, respectively.

After 10 weeks of treatment pairing of animals within each dose group was undertaken on a one male: one female basis, to produce the F1 litters. The day of proved copulation was designated day 0 of gestation. Copulated females were placed individually into breeding boxes with nestle material. The day of completed parturition was designated day 0 of lactation. On day 4 post partum, litter sizes were reduced to a maximum of 8 pups, preferable to 4 males and 4 females, and the remaining pups were culled. Weaning was done on day 21 of lactation and all F0 parental animals were sacrificed. Groups of 24 male and 24 female offspring from each dose group of the F0 generation were selected to form the F1 parents. Unselected offspring were sacrificed and subjected to a gross necropsy.

The offspring selected for the F1 generation were dosed for 10 weeks and then paired within each dose group to produce the F2 litters. F2 litters were weaned on day 21 of lactation and terminated together with F1 parental animals. F1 parental rats which failed to produce F2 offspring (10 males and 10 females with normal external genitalia and oestrus cycle) were mated with untreated rats of the same strain and sacrificed thereafter for fertility assessment (reproductive performance).

Diet preparation and analyses
Diets were prepared monthly during the pre-mating period, and biweekly during the breeding period. For each dose level a specified amount of the test substance was mixed with a small amount of basal diet in a mortar. This pre-mix was stirred into the remaining part of the diet. The diets were stored at about 4 °C in the dark. Analyses for homogeneity were done for each dose level of the first diet preparation. Analyses for achieved concentration were done for all prepared diets.

Clinical observations
A check for clinical signs of toxicity and mortality was made once daily on all F0 and F1 parental animals. A detailed physical examination was performed on males prior to treatment, and weekly during pre-mating and breeding periods and at necropsy. Females were examined prior to treatment, weekly during pre-mating periods and on gestation days 0, 7, 14 and 20, and on days 0, 7, 14 and 21 of lactation, and at necropsy.

Body weight
Individual body weights F0 and F1 males adults were determined prior to treatment, and weekly during pre-mating and breeding periods and at necropsy. F0 and F1 females were weighed prior to treatment, weekly during pre-mating periods and on gestation days 0, 7, 14 and 20, and on days 0, 7, 14 and 21 of lactation, and at necropsy.

Food consumption and compound intake
Food consumption for each cage was recorded and daily food consumption was calculated. Determination of food consumption was made on a weekly basis during the pre-mating period for males and females and during the breeding period for males. In addition, for females total food consumption was determined at the following intervals: day 0-7, 7-14, 14-20 of gestation and of days 0-7, 7-14 and 14-21 of lactation.
Compound intakes in parental animals were calculated during the pre-mating periods for each sex on a weekly basis.

Reproduction parameters
Oestrus cycle
The oestrus cycle was checked daily by microscopically examination of vaginal smears. Examinations were done for each female for one week prior to mating until copulation was confirmed.

Reproductive performance
Mating indices for males and females were calculated separately after copulation was confirmed. In addition, fertility and gestation indices, the length of gestation, as well as the number of implantation sites were determined.

Sperm assessment
An assessment of motility and morphology of epididymal sperm was done at necropsy for 10 males per group, which were selected for the organ weight measurement, as well as for males that failed to impregnate females.

Litter data
Total number of live and dead pups, and the number of males and females per litter were determined on day 0 of lactation. The sex ratio was calculated for each group. Viability indices were determined for each litter on lactation days 0, 4 and 21. Body weights were determined on lactation days 0, 4, 7, 14 and 21.
A check for clinical signs of toxicity and mortality was made once daily during the lactation period on all F1 and F2 pups. A detailed physical examination was done on lactation days 0, 4, 7, 14 and 21.

Sacrifice and pathology
All surviving parental F0 and F1 males and females were sacrificed on day 21 post partum and subjected to a gross pathological examination. Animals of all generations that died, were found dead or were killed moribund during the study period were necropsied as soon as possible. The following organs and tissues were preserved: adrenals, aorta, brain, caecum, colon, duodenum, epididymis, eyes, gross lesions, head (incl. nasal cavity, paranasal sinuses, buccal mucosa and ears), heart, ileum, jejunum, kidneys, larynx, liver, lung, mammary gland, oesophagus, ovaries, pancreas, pharynx, pituitary, prostrate, rectum, seminal vesicles, spleen, stomach, testes, thymus, thyroid/parathyroid, tongue, trachea, urinary bladder, uterus (cornua and cervix) and vagina.

F1 and F2 pups that were not selected on day 4 of lactation were also killed and necropsied on that day. In addition, F1 weanlings that were not selected for parental animals of the F1 generation and all F2 weanlings were necropsied at 22-26 and 21-26 days of their age, respectively. The same organs, as described above, were preserved from one animal per sex per litter of the F1 and F2 weanlings necropsied.

The following organs weights of 10 F0 and F1 males and females from each dose group that were sacrificed at the end of the study, as well as from pairs of parental animals that failed to mate: adrenal gland, brain, epididymides, kidneys, liver, ovaries, prostrate, pituitary, seminal vesicles (with coagulating gland and fluids), testes, uterus.
A histopathological examination was performed on the reproductive organs and pituitary of the control and high dose F0 and F1 parental animals that survived until scheduled termination. A histopathological examination of the reproductive organs and pituitary in the low and mid-dose group was only performed on pairs of animals that had failed to produce offspring.
In addition, a histopathological examination was performed on organs with significant weight change, and on all organs with gross pathological changes.

Statistics
One or a combination of the following statistical methods were applied for the evaluation of the measured parameters: Bartlett´s test for equality of variance (p=0.05) followed by parametric analyses of variance in one-way classification (p=0.05) or Dunnett´s t-test or Scheffé ´s multiple comparison test (p=0.05, 0.01 or 0.001); or Bartlett´s test followed by Kruskal-Wallis test (P=0.05) and Dunnett-type mean rank test or Scheffe-type mean rank test ((p=0.05, 0.01 or 0.001). Fisher´s exact probability test ((p=0.05, 0.01 or 0.001) and Mann-Whitney´s U-test (p=0.05 or 0.01) were also used.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS

Based on the results of the dose-finding study the chemical stability of the test substance in the was given for 5 weeks (at room temperature) in sealed plastic bags in the dark, and for at least 2 weeks after being released from the plastic bags.
Homogeneity of the test substance in the dietary mixtures was satisfactory, percentage deviations from the overall mean were within 4%. The mean achieved concentrations of HR-001 in the diet preparations were in the range of 90 – 105% of the nominal and therefore acceptable.

B. FOOD CONSUMPTION AND TEST COMPOUND INTAKE

F0 and F1 males

In F0 males, mean food consumption at treatment week 13 in the 1200 ppm group was significantly higher than that in the control group. Since there was no such increase observed in the mid- and high-dose groups throughout the study, this change was not thought to be treatment-related.
In F1 males in the 30000 ppm group, mean food consumption at treatment week 4 was significantly lower than that in the control group, but the values on the other treatment weeks in this dose group were comparable to the controls. In the 1200 and 6000 ppm groups, mean food consumption of F1 males was comparable to the controls throughout the study.

F0 and F1 females

In F0 females, the values on treatment weeks 2-4 in the 30000 ppm group were significantly higher than the controls. Inversely, the value on lactation days 7-14 in this dose group was significantly lower than those in the control group. So it was unclear these changes were treatment-related or not. In the 1200 and 6000 ppm groups, mean food consumption of F0 females was comparable to the controls throughout the study.
In F1 females in the 1200 and 6000 groups, mean food consumption on lactation days 14-21 were significantly higher than those in the control group. However, these changes were thought to be incidental because no such increase was observed in the highest dose group. In the 30000 ppm group, mean food consumption of F1 females was comparable to the controls throughout the study.

The group mean achieved dosages are summarised in Table 5.6.1-21 below.

Table 5.6.1-21: HR-001: A two-generation reproduction study in rats ([redacted], 1997): group mean achieved dose levels F0 and F1-generation

Group	Dietary concentration (ppm)	Mean achieved dose level (mg/kg bw/day)			
		Males		Females	
		F0	F1	F0	F1
Control	0	0	0	0	0
Low	1200	83.6	91.7	96.9	104.8
Intermediate	6000	417	458	485	530
High	30000	2150	2411	2532	2760

C. MORTALITY

F0 and F1 males

There were no treatment related mortalities in F0 or F1 males. During the study period, one F0 male and one Fl male in the control groups and one Fl male in the 6000 ppm group showed malocclusion of the incisors, respiratory wheezing, and red sebum. One of these animals (in the 6000 ppm group) also showed distention of the abdomen. These animals were euthanatised within several days after discovery due to unfavorable prognosis. Necropsy revealed a fracture of the facial bones in all cases, suggesting that the

alterations were caused by an accident in a cage.

F0 and F1 females
During the study period, no deaths occurred in any females of the F0 and Fl generations.

D. CLINICAL OBSERVATIONS
F0 and F1 males
There were no treatment-related clinical signs observed in the 1200 and 6000 ppm groups.
At 30000 ppm F0 and F1 parental males exhibited loose stool with incidences during the pre-mating growth and breeding periods of 3/24 and 2/24 for the F0 generation, and of 13/24 and 0/24 for the F1 generation, respectively, with a significant difference in the value for the pre-mating growth period of the F1 generation. Since this finding was not observed in other groups including control, defecation of loose stool was considered to be treatment-related.
Statistically significant differences were also observed in the incidence of hair loss during the breeding period for F0 males in all test substance groups. However, the occurrence of this change in the treated groups was rather lower than controls, and was considered to be incidental.
During the study period, one F0 male and one F1 male in the control group and one F1 male in the 6000 ppm group showed malocclusion of the incisors, respiratory wheezing, and red sebum. The aforementioned one F1 male of the 6000 ppm group also showed distension of the abdomen. These animals were euthanatised within several days after discovery due to unfavourable prognosis. Necropsy noted a fracture of the facial bones in all cases, suggesting that the alterations were caused by an accident in the cage. Accident malocclusion of incisors was also observed in one F1 male in the 1200 ppm group. However, test substance treatment of this animal was continued until termination of the study because its condition was improved.

F0 and F1 females
There were no treatment-related clinical signs observed in the 1200 and 6000 ppm groups. In F0 and F1 parental females, loose stool was also observed at 30000 ppm. The incidences during the pre-mating growth period and the lactation and post-weaning period were 1/24 and 6/24 for the F0 generation, and 4/24 and 2/24 for the F1 generation, respectively, with a significant difference in the value for the lactation and post-weaning period of the F0 generation.

Table 5.6.1-22a HR-001: A two-generation reproduction study in rats ([redacted], 1997): Observed clinical signs in male rats (F0 and F1 generation)

Clinical sign	Number of male rats affected in dose group (ppm)							
	Pre-mating growth period				Breeding period			
	0	1200	6000	30000	0	1200	6000	30000
F0								
No. of animals examined	24	24	24	24	23	24	24	24
Swelling of the right auricle	0	0	0	0	0	1	0	1
Red sebum	1	0	0	0	0	0	0	0
Lacrima	0	0	0	1	0	0	0	1
Malocclusion	1	0	0	0	0	0	0	0
Wound: head, neck or back	2	0	0	0	2	0	0	0
Hair loss: head, neck, back, etc.	2	1	1	0	6	1	0**	0**
Soiled fur perianal region	0	0	0	2	0	0	0	1
Loose stool	0	0	0	3	0	0	0	2
Killed in extremis	1	0	0	0	0	0	0	0
F1								
No. of animals examined	24	24	24	24	23	24	23	24
Soiled fur perinasal region	1	1	1	0	0	1	1	0
Red sebum	1	1	2	0	0	2	1	0
Malocclusion	1	1	1	0	0	1	0	0

Distention of the abdomen	0	0	1	0	0	0	0	0
Hair loss: head, neck, back, etc.	1	1	1	0	1	0	2	0
Soiled fur perianal region	0	0	0	4	0	0	0	1
Erosion in the perianal region	0	0	0	2	0	0	0	0
Loose stool	0	0	0	13**	0	0	0	0
Killed in extremis	1	0	1	0	0	0	0	0

* Significantly different from control at p<0.05
** Significantly different from control at p<0.01

Table 5.6.1-22b: HR-001: A two-generation reproduction study in rats ([redacted], 1997): Observed clinical signs in female rats (F0 and F1 generation)

Clinical sign	Number of female rats affected in dose group (ppm)							
	Pre-mating growth period				Mating/gestation and Lactation/post-weaning period			
	0	1200	6000	30000	0	1200	6000	30000
F0								
No. of animals examined	24	24	24	24	24	24	24	24
Red sebum	0	0	0	0	0	0	1	0
Hair loss: head, neck, back, etc.	2	0	0	1	2/2	0	0	3/3
Mass on the chest	0	0	0	0	0	0	0	1
Scab on the chest	0	0	0	0	0	0	0	1
Loose stool	0	0	0	1	0	0	0	6**
F1								
No. of animals examined	24	24	24	24	23	23	21	19
Red sebum	0	0	0	1	0	1	0	0
Hair loss: head, neck, back, etc.	1	1	1	0	1/2	1/1	1	0
Mass on the chest	0	0	0	0	0	1/1	0	0
Soiled fur perianal region	0	0	0	1	0	0	0	0
Erosion in the perianal region	0	0	0	0	0	0	0	1
Loose stool	0	0	0	4	0	0	0	2

* Significantly different from control at p<0.05
** Significantly different from control at p<0.01

E. BODY WEIGHT

F0 and F1 males

Mean body weights of F0 and F1 males in the 30000 ppm group were consistently lower than those in the control group from treatment week 1 to the day of necropsy, and the differences from controls at treatment weeks 1-12 and 14 for the F0 generation, and treatment weeks 1-6 for the F1 generation were statistically significant. In the 1200 and 6000 ppm groups, mean body weights of F0 and F1 parental males were comparable to the controls throughout the study.

Table 5.6.1-23: HR-001: Dietary Two Generation Reproduction Study in the Rat ([redacted], 1997): Selected body weights throughout treatment period – F0 and F1 males (group mean values)

Dietary concentration (ppm)	No. of animals		Body weight (g) at Week				
			0	5	10	14	18
			F0 Generation				
0 (Control)	241	mean	140	366	454	498	531
		SD	4	34	52	60	64
1200	24	mean	140	373	463	516	549
		SD	4	31	44	53	58
6000	24	mean	140	363	456	501	532

		SD	4	24	37	40	44
30000	24	mean SD	140 4	341* 25	417* 35	457* 39	486 44
			F1 Generation				
0 (Control)	242	mean SD	71 6	351 32	484 44	528 46	558 51
1200	24	mean SD	73 7	355 32	485 54	632 70	567 72
6000	24	mean SD	71 7	349 23	487 43	540 52	578 55
30000	24	mean SD	67** 6	326** 24	464 39	511 45	554 46

1 initial group size, reduced to 23 from week 4 onwards
2 initial groups size, reduced to 23 from week 8 onwards
SD standard deviation
* significantly different from control at p≤0.05
** significantly different from control at p≤0.01

F0 and F1 females

There were no significant differences in mean body weights of F0 females in any treatment group when compared to control. In F1 females in the 30000 ppm group, mean body weight on lactation day 0 was significantly higher than that in the control group. In the 1200 and 6000 ppm groups, mean body weights of F1 parental females were comparable to the controls throughout the study.

Table 5.6.1-24: HR-001: Dietary Two Generation Reproduction Study in the Rat ([redacted], 1997): Selected body weights of F0 and F1 females during pre-pairing (group mean values)

Dietary concentration (ppm)	No. of animals		Body weight (g) at Week 0	5	10
			F0 Generation		
0 (Control)	24	mean SD	119 4	225 12	264 16
1200	24	mean SD	119 4	228 15	268 19
6000	24	mean SD	119 4	224 13	268 17
30000	24	mean SD	119 4	225 14	263 18
			F1 Generation		
0 (Control)	271	mean SD	66 5	218 13	276 20
1200	24	mean SD	67 5	226 16	283 22
6000	24	mean SD	66 5	218 19	278 25
30000	24	mean SD	63 6	213 16	270 22

SD standard deviation

Table 5.6.1-25: HR-001: Dietary Two Generation Reproduction Study in the Rat ([redacted], 1997): Body weights of F0 and F1 females during gestation (group mean values)

Dietary concentration (ppm)	No. of		Body weight (g) at Gestation Day

	animals		0	7	14	20
			F0 Generation			
0 (Control)	24	mean SD	271 16	298 15	331 19	404 24
1200	24	mean SD	273 19	300 20	330 21	401 25
6000	24	mean SD	273 20	301 18	332 19	407 22
30000	24	mean SD	271 18	297 19	325 20	397 28
			F1 Generation			
0 (Control)	23	mean SD	280 23	308 22	339 22	411 27
1200	23	mean SD	286 21	318 21	349 22	425 27
6000	21	mean SD	282 29	314 25	345 25	416 31
30000	19	mean SD	274 18	310 19	343 19	416 28

SD standard deviation

Table 5.6.1-26: HR-001: Dietary Two Generation Reproduction Study in the Rat ([redacted] 1993): Body weights of F0 and F1 females during lactation (group mean values)

Dietary concentration (ppm)	No. of animals		Body weight (g) at Day 0	7	14	21
			F0 Generation			
0 (Control)	23	mean SD	288 21	333 21	355 23	327 18
1200	24	mean SD	300 25	334 23	351 17	330 19
6000	24	mean SD	299 22	335 17	353 17	328 20
30000	24	mean SD	301 22	328 17	342 19	339 20
			F1 Generation			
0 (Control)	23	mean SD	310 26	344 24	360 22	326 24
1200	23	mean SD	321 26	350 23	361 22	337 20
6000	21	mean SD	324 25	345 23	360 25	342 21
30000	19	mean SD	325 22	348 18	362 20	349** 17

SD standard deviation
** significantly different from control at $p \leq 0.01$

F. REPRODUCTIVE PARAMETERS

F0 males and females

Reproductive performance of F0 parental animals was not adversely affected by test substance treatment, and no significant differences were observed in such parameters as percentage of females having normal oestrous cycle, mating index, fertility index, gestation index, duration of gestation, number of implantation sites, and number, motility and morphology of epididymal sperm between the control group and the treated groups (Table 5.6.1-27a).

F1 males and females
In F1 parental animals, reproductive parameters in the treated groups were also comparable to the controls with the exception of gestation index and number of implantation sites, on which some biases were occasionally observed.
The significant higher number of implantation sites at 1200 ppm when compared to control was considered to be unrelated to treatment, since there was no increase noted at 6000 and 30000 ppm.
A similar bias was also found in the fertility index. The fertility indices in the control, 1200, 6000 and 30000 ppm groups were 95.8 (23/24), 95.8 (23/24), 87.5 (21/24) and 79.2% (23/24), respectively, with somewhat low values in the 2 higher dose groups. However, these decreases were considered to be incidental because the differences between the control and treated groups were not statistically significant, and because, as described below, normal reproduction results were obtained in the F1 parental animals, which had failed to produce offspring in this study, after remating with untreated animals.

Among the total of ten F1 females mated with untreated males, only one female in the 30000 ppm group did not undergo pregnancy. Histopathological of this female showed no abnormalities in the reproductive organs and pituitary. So the cause of infertility of this female was not known. The other nine F1 females were proved to have normal reproductive performance. One F1 male in each of the 1200, 6000 and 30000 ppm groups could not successfully impregnate untreated females mated. These 3 males had histopathological abnormalities in the testes and epididymides, and abnormalities in the sperm parameters, as a cause of infertility. However, the other 7 males were proved to have normal reproductive performance. Thus, the majority of F1 males and females which had failed to produce offspring were proved to have normal reproductive performance (see tables below).

Table 5.6.1-27a: HR-001: A two-generation reproduction study in rats ([redacted], 1997): Reproductive parameters and litter data (F0)

	ppm	0	1200	6000	30000
Oestrous cycle – prior to mating (F0)	Regular 4 or 5d cycles	24/24 (100 %)	23/24 (95.8 %)	24/24 (100 %)	24/24 (100 %)
Mating Index	Males	23/23 (100 %)	24/24 (100 %)	24/24 (100 %)	24/24 (100 %)
	Females	24/24 (100 %)	24/24 (100 %)	24/24 (100 %)	24/24 (100 %)
Fertility Index		24/24 (100 %)	24/24 (100 %)	24/24 (100 %)	24/24 (100 %)
Duration of gestation (days)		22.4	22.3	22.1	22.3
Number of implantation sites		14.9 ± 1.6	14.9 ± 1.6	15.1 ± 1.1	14.8 ± 2.6
# of pups delivered (mean ± SD)		13.8 ± 1.6	13.2 ± 1.8	14.1 ± 1.4	13.6 ± 2.6
Sex ratio		0.459	0.491	0.499	0.446
Viability Index on lactation day	0	97.5	97.9	95.9	97.4
	4	95.5	99.7	97.8	98.9
	21	100.0	98.4	99.5	99.5

Table 5.6.1-27b: HR-001: A two-generation reproduction study in rats ([redacted], 1997): Reproductive parameters and litter data (F1)

	ppm	0	1200	6000	30000
Oestrous cycle – prior to mating (F1)	Regular 4 or 5d cycles	24/24 (100 %)	24/24 (100 %)	24/24 (100 %)	24/24 (100 %)
Mating Index	Males	23/23 (100 %)	24/24 (100 %)	23/23 (100 %)	24/24 (100 %)
	Females	24/24 (100 %)	24/24 (100 %)	24/24 (100 %)	24/24 (100 %)

	ppm	0	1200	6000	30000
Fertility Index		23/24 (95.8 %)	23/24 (95.8 %)	21/24 (87.5 %)	19/24 (79.2 %)
Duration of gestation (days)		22.2	22.4	22.2	22.2
Number of implantation sites		13.9 ± 2.1	15.7 ±1.4*	13.6 ±2.5	14.5 ± 2.1
# of pups delivered (mean ± SD)		12.8 ± 2.1	13.7 ± 2.3	13.0 ± 2.7	13.1 ± 2.7
Sex ratio		0.481	0.514	0.551	0.500
Viability Index on lactation day	0	98.8	98.9	98.9	98.1
	4	99.3	99.7	98.7	99.7
	21	100.0	100.0	100.0	99.3

* Significantly different from control at p<0.05

Table 5.6.1-28a: HR-001: A two-generation reproduction study in rats ([redacted], 1997): Sperm assessment and morphology – group mean values (F0)

F0	ppm	0	1200	6000	30000
# animals		10	10	10	10
Sperm motility (%)		77.9 ± 6.8	77.8 ± 9.5	75.7 ± 5.4	81.9 ± 5.9
Sperm count(106)	per cauda epididymis	198 ± 22	193 ± 44	206 ± 41	198 ± 15
	per gram cauda epididym.	660 ± 117	631 ± 122	640 ± 128	665 ± 60
Sperm morphology	Normal	97.7 ± 1.3	98.1 ± 1.6	98.0 ± 1.3	98.2 ± 1.2
	Decapitate	n.r.	n.r.	n.r.	n.r.
	Abnormal	n.r.	n.r.	n.r.	0

n.r. Not reported.

Table 5.6.1-28b HR-001: A two-generation reproduction study in rats ([redacted], 1997): Sperm assessment and morphology – group mean values (F1)

F1	ppm	0	1200	6000	30000
# animals		10	10	10	10
Sperm motility (%)		74.5 ± 5.3	73.9 ± 6.4	76.0 ± 4.4	74.8 ± 8.8
Sperm count(106)	per cauda epididymis	185 ± 37	199 ± 21	215 ± 45	177 ± 28
	per gram cauda epididym.	618 ± 124	673 ± 92	666 ± 117	547 ± 73
Sperm morphology	Normal	96.1 ± 3.7	95.0 ± 3.9	94.0 ± 3.5	95.1 ± 2.9
	Decapitate	0	0	0	0
	Abnormal	0	0	0	0

n.r. Not reported
Values represent mean ± S.D.

G. LITTER DATA

Number of pups delivered
Mean number of F1 and F2 pups delivered in the 1200, 6000 and 30000 ppm groups were comparable to the controls.

Sex ratio
Sex ratios of F1 and F2 pups in the 1200, 6000 and 30000 ppm groups were comparable to the controls.

Viability index
The viability indices of F1 and F2 pups in the 1200, 6000 and 30000 ppm groups were comparable to the controls.

Body weights

F1 pups

There were no effects on mean body weight noted in the low- and mid-dose group when compared to controls. F1 pups of both sexes in the 30000 ppm group, showed significantly higher mean body weights on lactation day 0 than the controls. However, mean body weights on days 14 and 21 were significantly decreased when compared controls.

F2 pups

There were no effects on mean body weight noted in the low- and mid-dose group when compared to controls during the lactation period. In F2 pups in the 30000 ppm group, mean body weights of both sexes on day 21 of lactation were significantly lower than those in the control group.

Table 5.6.1-29a: HR-001: A two-generation reproduction study in rats ([redacted] 1997): Mean pup body weights (F1)

ppm	0	1200	6000	30000	0	1200	6000	30000
Sex	Males				Females			
Age (d)								
0	6.7 ± 0.6	6.8 ± 0.5	6.7 ± 0.4	7.2* ± 0.7	6.3 ± 0.6	6.4 ± 0.5	6.4 ± 0.5	6.8* ± 0.6
N	24	24	23	24	24	24	23	24
4	11.6 ± 1.2	11.6 ± 1.2	11.7 ± 1.0	11.6 ± 1.2	11.1 ± 1.2	11.2 ± 1.1	11.3 ± 0.9	11.3 ± 1.2
N	23	24	24	24	23	24	24	24
7	19.5 ± 1.7	19.1 ± 2.0	19.5 ± 1.6	19.3 ± 1.2	18.6 ± 1.8	18.4 ± 1.9	18.8 ± 1.5	18.3 ± 1.6
N	23	24	24	24	23	24	24	24
14	39.5 ± 3.2	39.4 ± 2.6	39.3 ± 2.6	36.6** ± 2.6	38.4 ± 3.6	37.9 ± 2.6	38.2 ± 2.2	35.4** ± 2.6
N	23	24	24	24	23	24	24	24
21	63.9 ± 4.4	63.8 ± 4.1	62.4 ± 3.7	55.1 ± 3.5***	61.0 ± 4.8	60.6 ± 3.9	59.8 ± 3.1	53.2*** ± 4.0
N	23	24	24	24	23	24	24	24

Values expressed in group mean ± SD

* statistically significant difference from control group p <0.05

** statistically significant difference from control group p <0.01

*** statistically significant difference from control group p <0.001

Table 5.6.1-29b: HR-001: A two-generation reproduction study in rats ([redacted], 1997): Mean pup body weights (F2)

ppm	0	1200	6000	30000	0	1200	6000	30000
Sex	Males				Females			
Age (d)								
0	7.0 ± 0.5	6.9 ± 0.6	7.3 ± 0.7	7.1 ± 0.5	6.6 ± 0.5	6.6 ± 0.7	6.8 ± 0.6	6.8 ± 0.6
N	23	23	21	19	23	23	21	19
4	12.0 ± 1.2	12.1 ± 1.5	12.5 ± 1.5	12.5 ± 1.3	11.6 ± 1.2	11.5 ± 1.6	12.0 ± 1.5	12.1 ± 1.1
N	23	23	21	19	23	23	21	19
7	19.8 ± 1.5	20.0 ± 1.9	20.4 ± 2.2	20.6 ± 1.7	18.9 ± 2.0	19.1 ± 2.1	19.6 ± 2.2	19.9 ± 1.4
N	23	23	21	19	23	23	21	19
14	40.1 ± 3.0	39.0 ± 2.8	38.7 ± 2.9	39.1 ± 2.8	38.7 ± 3.5	38.0 ± 2.2	37.5 ± 2.9	38.1 ± 2.9

ppm	0	1200	6000	30000	0	1200	6000	30000
Sex	Males				Females			
N	23	23	21	19	23	23	21	19
21	58.6 ± 5.1	59.4 ± 4.4	58.3 ± 4.3	53.1** ± 4.4	56.4 ± 5.5	57.1 ± 4.4	56.2 ± 4.5	51.8* ± 4.2
N	23	23	21	19	23	23	21	19

Values expressed in group mean ± SD
* statistically significant difference from control group p<0.05
** statistically significant difference from control group p<0.01

Clinical signs
There were no treatment-related abnormalities noted in F1 and F2 pups of any dose group.

During the lactation period, deaths and loss due to maternal cannibalism occurred in several pups in all groups including the control. However, the incidences in the treated groups were comparable to the control.

I. PATHOLOGY
Necropsy
F0 and F1 generation
Necropsy of parental animals of both sexes noted several findings in all groups including the control group. Among these alterations, the incidences of distension of the caecum in F0 and F1 males and females of the 30000 ppm group were significantly higher than those of the controls, and were considered treatment-related. Statistically significant differences from controls were also found in the incidences of hair loss in F0 males of the 1200, 6000 and 30000 ppm groups. However, the values were rather lower than controls and were considered to be incidental. Other findings were low in their incidences and considered not treatment-related.

F1 and F2 pups
Necropsy of stillbirths found on lactation days 0, pups found dead during lactation days 1-4, and pups killed to reduce the litter size on lactation day 4 demonstrated no treatment-related abnormalities in any of the F1 and F2 pups.
During days 5-21 of lactation, only 2 F1 pups in the 1200 ppm group were found dead. Necropsy of these dead pups were not performed due to advanced autolysis.
Necropsy of F1 and F2 weanlings in the 30000 ppm group noted distension of the caecum, suggesting a treatment-related occurrence. In the 1200 and 6000 ppm groups, no treatment-related abnormalities were observed in any of the F1 and F2 weanlings.

Organ weights
F0 and F1 males:
There were no effects in the absolute and relative organ weights in F0 and F1 males of the low- and mid-dose groups. At 30000 ppm relative weights of the liver and kidneys of F0 and F1 males were significantly higher than the control values. These increases were considered treatment-related. In F1 males in the high-dose group, there was also a significant decrease noted in the absolute and relative weights of the prostate. Besides these changes, the relative brain weight of F0 males in the 30000 ppm group was significantly higher than the control value. However, this finding was considered to be the change associated with the low body weights in this group (Table 5.6.1-29a).

F0 and F1 females
In F0 females, the absolute and relative weights of all organs were comparable between the control and treated groups. In F1 females in the 30000 ppm group, the absolute and relative weights of the liver and kidneys were significantly higher than the controls, and these increases were considered treatment-related. Significantly higher-than-control value was also observed in the absolute kidney weight in the 6000 ppm group. However, this increase was not considered treatment-related because statistical significance in the difference between the control and 6000 ppm groups disappeared when all F1 females were subjected to

the weighing of the kidneys fixed in 10% neutral buffered formalin. The significant lower relative ovarian weight observed in F1 females in the 1200 ppm group was considered to be an incidental finding because no such decrease was observed in the mid- and high-dose groups (Tables 5.6.1-30a and -30b).

Table 5.6.1-30a: HR-001: A two-generation reproduction study in rats ([redacted], 1997): Selected organ weights of males

Dietary concentration (ppm)		bw (g)	Organ weight (absolute weights in mg$) Brain absolute	Brain relative	Kidney absolute	Kidney relative	Liver absolute	Liver relative	Prostate absolute	Prostate relative
			F0 Generation							
0	mean	538	2197	0.411	1656	0.308	16900	3.13	642	0.1204
	SD	54	111	0.030	184	0.021	2382	0.20	208	0.0389
1200	mean	533	2154	0.406	1631	0.307	16351	3.08	606	0.1141
	SD	43	68	0.025	115	0.028	1094	0.24	159	0.0287
6000	mean	538	2179	0.409	1690	0.316	16568	3.07	707	0.1332
	SD	55	59	0.040	120	0.033	2335	0.19	126	0.0305
30000	mean	473*	2123	0.451*	1617	0.342	15617	3.29	569	0.1204
	SD	45	142	0.035	178	0.021	2410	0.26	94	0.0164
			F1 Generation							
0	mean	569	2219	0.393	1717	0.303	18163	3.19	662	0.1178
	SD	50	46	0.035	150	0.023	2136	0.18	185	0.0355
1200	mean	532	2170	0.413	1694	0.318	17638	3.28	493	0.0931
	SD	66	81	0.049	288	0.035	3655	0.33	85	0.0158
6000	mean	559	2261	0.406	1796	0.322	17509	3.12	582	0.1039
	SD	47	114	0.031	180	0.019	2380	0.22	196	0.0319
30000	mean	567	2217	0.393	1942	0.344	20523	3.62**	450*	0.0797*
	SD	50	131	0.029	175	0.038	2252	0.23	153	0.0296

$ Relative organ weights to body weights are shown as percent of body weights (considering 10 rats in every group)
SD standard deviation
* significantly different from control group $p<0.05$
** significantly different from control group $p<0.01$

Table 5.6.1-30b: HR-001: A two-generation reproduction study in rats ([redacted], 1997): Selected organ weights of females

Dietary concentration (ppm)		bw (g)	Organ weight (absolute weights in mg$) Brain absolute	Brain relative	Kidney absolute	Kidney relative	Liver absolute	Liver relative	Ovaries absolute	Ovaries relative
			F0 Generation							
0	mean	306	2014	0.658	1147	0.374	13491	4.39	61.1	0.0199
	SD	18	110	0.031	120	0.030	1909	0.41	7.3	0.0021
1200	mean	304	1979	0.652	1143	0.376	13219	4.36	66.6	0.0219
	SD	10	66	0.028	74	0.025	1053	0.36	10.3	0.0030
6000	mean	301	1967	0.655	1110	0.369	13090	4.36	60.0	0.0200
	SD	12	82	0.033	84	0.026	1005	0.29	5.9	0.0022
30000	mean	313	2013	0.646	1202	0.385	14009	4.47	64.6	0.0207
	SD	21	73	0.041	125	0.031	1761	0.38	6.3	0.0014
			F1 Generation							
0	mean	314	2035	0.650	1114	0.355	13436	4.28	68.4	0.0218
	SD	19	112	0.050	125	0.028	1598	0.41	7.0	0.0025
1200	mean	326	2026	0.624	1211	0.372	14611	4.49	61.3	0.0189*
	SD	19	64	0.034	99	0.020	1240	0.32	7.6	0.0027
6000	mean	327	2038	0.628	1242*	0.381	15528	4.73	68.0	0.0209

Dietary concentration (ppm)		bw (g)	Organ weight (absolute weights in mg$)							
			Brain		Kidney		Liver		Ovaries	
			absolute	relative	absolute	relative	absolute	relative	absolute	relative
	SD	28	71	0.056	102	0.025	2830	0.53	7.6	0.0023
30000	mean	319	2073	0.652	1304	0.409***	16394**	5.13***	69.5	0.0218
	SD	16	94	0.051	108**	0.030	1835	0.44	7.6	0.0027

$ Relative organ weights to body weights are shown as percent of body weights (considering 10 rats in every group)
SD standard deviation
* significantly different from control group p<0.05
** significantly different from control group p<0.01
*** significantly different from control group p<0.001

Histopathology
F0 and F1 generations
In all F0 and F1 males and females in the 30000 ppm group, histopathological examinations of the reproductive organs and pituitaries did not indicate any treatment-related alterations.

No treatment-related histopathological alterations were also evident in the following organs in which significant weight changes were detected: kidneys of F1 females in the 6000 ppm group; kidneys of F0 males and F1 males and females in the 30000 ppm group; and liver of F1 males and females in the 30000 ppm group.

III. CONCLUSIONS

Oral dietary administration of 0, 1200, 6000 and 30000 ppm glyphosate to Sprague-Dawley rats for two successive generations resulted in treatment-related signs of toxicity in parental rats at 30000 ppm. Therefore, the NOAEL for maternal toxicity is considered to be 6000 ppm, equivalent to 417-458 mg/kg bw/day and 485-530 mg/kg bw/day for males and females, respectively.
The NOAEL for reproduction is 30000 ppm, since the reproductive performance was not affected in any dose group. Based on the body weight effects and increased incidences of caecum distension the NOAEL for offspring is considered to be 6000 ppm.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Parental toxicity was observed at highest dose of 30000 ppm (> 2000 mg/kg bw/day) only and consisted of loose stool (F0/F1, m/f), reduced body weight (F0/F1, m) caecum distension (F0/F1, m/f), increased liver and kidney weights (F0/F1, m/f), decreased prostate weight (F1). Histopathological alterations were not detected.

Offspring toxicity was observed at highest dose level only and confined to reduced body weight and caecum distension in both sexes. Based on the results the NOAEL for parental and offspring toxicity was considered to be 6000 ppm (417-458 / 485-530 mg/kg bw/day) and 30000 ppm (2150-2411 / 2532-2760) for reproductive toxicity.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	**CA 5.6.1/006**
Report author	[redacted]
Report year	1993
Report title	Two Generation Reproduction Study in Wistar Rats
Report No	TOXI 885-RP-G2
Document No	Not reported
Guidelines followed in study	OECD 416 (1983)
Deviations from current test guideline (OECD 416, 2001)	Missing endpoints: estrous cycle monitoring, pre-coital interval, sperm analysis, organ weights, monitoring of physical and sexual offspring development. Deviations from the current version of OECD 416 (2001) are basically due to the fact that the study was aligned to an older version of the OECD test guideline 416.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities1,2	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

To evaluate the potential effects of glyphosate technical on reproduction groups of 30 Wistar rats per sex were fed diets containing test substance concentrations of 0, 100, 1000, and 10000 ppm (equivalent to 0, 7.7, 77 and 770 mg/kg bw/day) for two consecutive generations up to weaning of third (F2) generation.

First parental (F0) animals were treated for at least 8 weeks before mating. The first pairing produced the F1 litter from which the second parental generation was selected. All F2 litters were sacrificed at weaning. All groups were observed for clinical signs, body weight development, food consumption, mating behaviour, vaginal smear and pups observation (number, sex, survival and body weight). During gestation and lactation periods the dams were observed for body weight and food consumption. The data were statistically analysed. From all groups of parental (F0 and F1) rats, major visceral organs and reproductive organs were subjected to detailed gross necropsy. Lesions if any found in reproductive organs/tissues in all groups were subjected to histopathological examination. All pups were subjected to detailed gross pathology.

Dietary administration of glyphosate at up to 10000 ppm for two successive generations in Wistar rats showed no treatment and dose related significant and consistent changes in the incidence of clinical signs, mortality rate, body weight change and food consumption during treatment, gestation and lactation periods. No treatment and dose related consistent effects on number of pups born (combined and individual sex) and their growth were observed; however the total litter weight and female pup weight tended to be higher in treatment groups occasionally.

The reproductive performance parameters like (a) number of dams littered (b) number of dead pups at first observation (c) mean litter size; pup survival parameters like live birth index and survival index for 24 hours, day 4, 7, 14 and 21 and fertility index parameters for sires and dams have though shown some incidental significant changes compared to the control group; however, these changes were not consistent in both the generations and there was no relationship with treatment or the dose of the test compound in the diet.

In the two generation reproduction study conducted on glyphosate at dietary dose levels of 0, 100, 1000 and 10000 ppm no major effects on general health, growth of parents, gestation/lactation period, body weight and food consumption, gross necropsy findings of pups and parents were observed. The test compound did not cause any treatment or dose related consistent changes in parental mortality, parturition performance, mean litter size, pup weight and male and female fertility index.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material:

Identification:	Glyphosate technical
Chemical name	N-(Phosphonomethyl) glycine
Description:	Odourless, white crystal
Batch #:	60
Purity:	96.8 %
Date of receipt	11/9/1990
Expiry date:	July 1992
Stability of test compound:	More than two years at ambient temperature
Vehicle:	Plain diet
Test Animals:	
Species:	Rat
Strain:	Wistar rats (Random bred)
Source:	[redacted]
Age at start of treatment (F0):	8 weeks
Sex:	Males and females
Mean body weight at initiation of dosing:	Males: 160 - 190 g; females: 141 - 160 g
Acclimation period:	7 days
Diet/Food:	Standard "Gold Mohur" brand powdered rat feed manufactured by M/s Lipton India Limited, Bangalore, India
Water:	Deep bore well water passed through activated charcoal filter and exposed to UV rays (Aquaguard on-line water filter cum-purifier manufactured by M/s Eureka Forbes Limited, Bombay, India) was provided in glass bottles *ad libitum*
Housing:	Groups of five/three rats of same sex per cage depending on the size of the animals were accommodated in standard polypropylene rat cages (size: L 430 x W 270x H 150 mm) with stainless steel top grill; bedding material (paddy husk) was changed three times per week.
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 40-70 % Air changes: 10-15/hour 12 hours light/dark cycle

B. STUDY DESIGN

In life dates: May 1991 to April 1992 (not further specified)

Animal assignment and treatment:
In a two-generation reproduction study groups of 30 Wistar rats per sex of the F0 generation received daily dietary doses of 0, 100, 1000 and 10000 ppm glyphosate technical in diet.
After at least 8 weeks of treatment pairing of animals within each dose group was undertaken on a one male:one female basis, to produce the F1 litters. The day of proved copulation (vaginal smear) was designated day 0 of gestation. On day 4 post partum, litter sizes were reduced to a maximum of 8 pups, preferable to 4 males and 4 females, and the remaining pups were culled. Weaning was done on day 21 of lactation and all F0 parental animals were sacrificed. Groups of 30 male and 30 female offspring from each dose group of the F0 generation were selected to form the F1 parents.
The offspring selected for the F1 generation were paired within each dose group to produce the F2 litters. F2 litters were weaned on day 21 of lactation and terminated together with F1 parental animals.

Diet preparation and analyses
The required quantities of test compound were weighed and mixed manually with 1.0 kg of powdered rat feed to prepare the premix. The premixes were added to the bulk of remaining quantities of feed and mixed in ribbon mixer. Prepared feed bulks were sampled at different intervals for assaying test compound concentration in experimental diet. Frequency of feed mixing was once a fortnight.

Clinical observations
All animals were observed daily throughout the study and any visible clinical signs were recorded with details on type, severity, time of onset and duration. Any animal found dead or sacrificed in extremis was necropsied and macroscopically abnormal tissues were retained.

Body weight
Males were weighed weekly until termination. Females were weighed weekly during pre-mating, on gestational days 0, 6, 13, and 20 and on days 1, 4, 7, 14 and 21 of lactation.
Offspring were weighed sex-wise as litters on days 1, 4, 7, 14 and 21 post partum.

Food consumption and compound intake
Food consumption for each cage of males was recorded weekly until termination. Food consumption of females was recorded weekly during pre-mating and at the following intervals: days 0-6, 6-13, 13-20 of gestation and days 1-4, 4-7, 7-14 and 14-21 of lactation.

Mating procedure and vaginal smears
After the scheduled period of treatment (minimum of 8 weeks), females were paired on a one-to-one basis with males from the same treatrnent group. Each morning following pairing, a vaginal srnear was prepared from each fernale and examined for the presence of spermatozoa indicating mating. The day on which evidence of mating was found was designated as day 0 of gestation. Once mating had occurred, rnales were separated from females and vaginal smearing was discontinued.
Females not mated within seven days of pairing were removed and placed with another male of the same treatment group. This was repeated once again, if needed, thus allowing each female a maximum of 21 days to get rnated . Matings of siblings were avoided by pairing males and females of different litters in each generation . Same mating procedure was adopted in Fl animals after 13 weeks of dietary regime.

Pre-coital interval
The time elapsed between initial pairing and detection of mating was noted.

Duration of gestation
The time elapsed between detection of mating and parturition was recorded.

Reproduction parameters

Reproductive performance
The following reproductive indices were recorded: Male and female fertility index, fecundity index, mean number of implantations, parturition percentage, percentage mortality of pregnant dams, percentage of live pups born, in females the pre-coital interval (time elapsed between initial pairing and detection of mating) and duration of gestation.

Litter data
Total number of live and dead pups, viability indices (mean viable litter size on day 0, live birth index), litter weight, individual sex and observations on individual pups (if any) were determined within 24 hours after birth. Survival indices were determined on days 2, 4, 7, 14 and 21 of lactation. Body weights were determined on Lactation days 0, 4, 7, 14 and 21. A check for clinical signs of toxicity and mortality was made once daily during the lactation period on all F1 and F2 pups. On day 4 post partum, offspring were culled to reduce litter size to eight.

Assessment of development and reproductive performance of progeny
Following weaning, 30 male and 30 female offspring from the Fl litters were selected to form the second generation (P1) parents. When possible, one male and one female were selected from each litter using random numbers within litters after grossly atypical animals were excluded. When litters were insufficient to provide 30 males and 30 females in each group, additional offspring were randomly selected as above from litters already used within each group.The experimental conditions and serial observations made were same as described for the first generation (P0).

Sacrifice and pathology
All surviving parental F0 and F1 males and females and the non-selected weanlings from F1 and all F2 weanlings were sacrificed and subjected to a gross pathological examination. Tissue collection was done for animals of the parent generations only. Animals of all generations that died, were found dead or were killed moribund during the study period were necropsied as soon as possible.
The following organs and tissues were preserved from all F0 and F1 parents of all groups: Ovaries, uterus, vagina, testes, epididymides, seminal vesicles, prostate, coagulation glands, pituitary, adrenals, liver and kidneys. The organs were examined for gross pathological changes and those found abnormal were examined histopathologically.
Females failing to get mated within 21 days and females failing to produce a viable litter by day 25 post coitum were necropsied and any macroscopically abnormal tissue was retained for histopathological examination. The presence of corpora lutea, implantations and resorptions was examined in females which had failed to produce a viable litter.
On day 4 post partum, offspring were culled to reduce litter size to eight, where possible; culled offspring or found dead were necropsied. All F2 pups were sacrificed at weaning.

Statistics
One or a combination of the following statistical methods were applied for the evaluation of the measured parameters: Dunnett's t-test (for body weight, food consumption, litter number, litter weight, gestation and lactation period), Z Test (for mating performance, fertility index, gestation index, live birth index, viability index, lactation index, pups survival data, number of dead pups at birth, survival indices, number littered) and t/r test (for dose-response relationship).

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS
In-house stability study for glyphosate technical was carried out at 0, 2000 and 20000 ppm. Chemical stability was given for 30 days at room temperature with a loss of less than 7% at 0, 2000 and 20000 ppm levels in experimental diet when stored in polyethylene lined stainless steel drums.
The mean achieved concentrations of glyphosate in the diet preparations were analysed; the achieved concentrations were in the range of 96-100 % of the nominal and therefore acceptable.

B. TEST COMPOUND INTAKE

Test compound intake at 100, 1000 and 10000 ppm was estimated to be 7.7, 77 and 770 mg/kg bw/day, respectively.

C. MORTALITY

F0 and F1 males

There were no mortalities in male animals.

F0 females

In the females, there were three mortalities, two in the low dose group, (one dystokia and one suppurative pneumonia) and one in the high dose group (cause of death not ascertained).

F1 females

One dam in low dose group died of dystokia.

D. CLINICAL OBSERVATIONS

F0 generation

Nasal discharge and snuffling and cannibalism were seen in all groups. No other treatment related changes in clinical signs were observed.

F1 generation

The incidence of clinical signs was low and not treatment or dose related. There was no incidence of total cannibalism and the incidence of partial cannibalism was similar in all study groups.

Table 5.6.1-31: Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat ([redacted] 1993): Observed clinical signs in F0 generation

Clinical sign	Number of rats affected in dose group*							
	Control (0 ppm)		Low (100 ppm)		Mid (1000ppm)		High (10000 ppm)	
	♂	♀	♂	♀	♂	♀	♂	♀
General affections								
Weakness/loss of body weight	1/30	0/30	3/30	4/30	0/30	0/30	0/30	0/30
Respiratory affections								
Nasal discharge	12/30	9/30	22/30	14/30	10/30	12/30	20/30	8/30
Snuffing	7/30	5/30	6/30	5/30	5/30	8/30	8/30	6/30
GIT affection								
Soft stool	0/30	0/30	1/30	0/30	0/30	0/30	0/30	0/30
Skin affections								
Biting injury	0/30	0/30	0/30	0/30	1/30	0/30	0/30	0/30
Urogenital affections								
Urine incontinency	0/30	0/30	1/30	1/30	0/30	0/30	0/30	0/30
Parturition performance								
Prolonged gestation	NA	0/30	NA	0/30	NA	0/30	NA	0/30
Prolonged parturition	NA	0/30	NA	0/30	NA	0/30	NA	0/30
Mating not confirmed	NA	0/30	NA	0/30	NA	0/30	NA	0/30
Not littered	NA	0/30	NA	4/30	NA	2/30	NA	0/30
Total cannibalism of pups	NA	1/30	NA	0/30	NA	0/30	NA	0/30
Partial cannibalism of pups	NA	3/30	NA	4/30	NA	5/30	NA	4/30
Dystokia death	NA	0/30	NA	1/30	NA	0/30	NA	0/30
Subsequent total cannibalism	NA	1/30	NA	4/30	NA	1/30	NA	1/30
Death during treatment	NA	0/30	NA	0/30	NA	0/30	NA	0/30

Table 5.6.1-31: Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat ([redacted] 1993): Observed clinical signs in F0 generation

Clinical sign	Number of rats affected in dose group*							
	Control (0 ppm)		Low (100 ppm)		Mid (1000ppm)		High (10000 ppm)	
	♂	♀	♂	♀	♂	♀	♂	♀
Death during gestation	NA	0/30	NA	0/30	NA	0/30	NA	0/30
Death during lactation	NA	0/30	NA	1/30	NA	0/30	NA	1/30
Mortality	0/30	0/30	0/30	2/30	0/30	0/30	0/30	1/30

* x/y number affected / total number of animals in group
NA not applicable for male animals

Table 5.6.1-32 Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat ([redacted] 1993): Observed clinical signs in F1 generation

Clinical sign	Number of rats affected in dose group*							
	Control (0 ppm)		Low (100 ppm)		Mid (1000ppm)		High (10000 ppm)	
	♂	♀	♂	♀	♂	♀	♂	♀
General affections								
Weakness/loss of body weight	3/30	1/30	0/30	1/30	0/30	5/30	1/30	3/30
Neurological affections								
Circling disease	0/3	0/3	0/3	0/3	0/3	0/3	0/3	2/3
Respiratory affections								
Nasal discharge	0/30	2/30	3/30	4/30	0/30	2/30	2/30	0/30
Snuffing	3/30	4/30	6/30	3/30	2/30	2/30	5/30	4/30
GIT affection								
Soft stool	0/30	0/30	9/30	0/30	10/30	0/30	6/30	0/30
Skin affections								
Alopecia	0/30	0/30	0/30	0/30	0/30	2/30	0/30	1/30
Urogenital affections								
Urine incontinency	0/30	0/30	1/30	0/30	1/30	0/30	5/30	1/30
Parturition performance								
Prolonged gestation	NA	0/30	NA	0/30	NA	0/30	NA	0/30
Prolonged parturition	NA	0/30	NA	0/30	NA	0/30	NA	0/30
Mating not confirmed	NA	0/30	NA	0/30	NA	0/30	NA	0/30
Not littered	NA	4/30	NA	2/30	NA	8/30	NA	2/30
Partial cannibalsm of pups	NA	12/30	NA	7/30	NA	3/30	NA	12/30
Total cannibalism of pups	NA	0/30	NA	0/30	NA	0/30	NA	0/30
Dystokia death	NA	0/30	NA	1/30	NA	0/30	NA	0/30
Subsequent total cannibalism	NA	0/30	NA	0/30	NA	0/30	NA	0/30
Mortality	0/30	0/30	0/30	1/30	0/30	0/30	0/30	0/30

*x/y number affected / total number of animals in group
NA not applicable for male animals

E. BODY WEIGHT

F0 males

Initial body weight of treatment groups was higher compared to the control group and this trend continued during the entire treatment period. The absolute weight gain (difference between initial and terminal) during entire treatment period was similar to control group in low and high dose while in mid dose it was slightly higher.

F0 females

No significant treatment related differences were noted between treated and control groups.

F1 males
Mid dose group body weight (both initial and in subsequent weeks) was more than control. In high dose group initial body weight (Week 0) was higher than control but at Week 2 and 3 it was less. However in this group the body weight tended to be higher (not significant) during last seven weeks.

F1 females
The body weight of all treatment groups at selection (Week 0) was higher than in the control group and continued to be significantly higher than in the control group for up to Week 10 in mid and high dose groups. Body weights of the high dose group dams on Days 0, 6 and 13 of gestation period were significantly higher compared to controls but the body weight gain was statistically not significantly different. Another incidental significant finding was higher body weight (Gestational Day 0-20) of mid dose group dams compared to controls. Absolute body weight of mid dose group on Lactation Days 1 and 4 and that of high dose group during all periods of lactation was significantly higher than in control group. The mid dose group had lost body weight during Days 7-14, 14-21 and 1-21 of lactation period as compared to control.

Table 5.6.1-33 Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat ([redacted] 1993): Selected body weights throughout treatment period – F0 and F1 males (group mean values)

Dietary concentration (ppm)	No. of animals		Body weight (g) at Week			
			0	5	10	16/18*
			F0 Generation			
0 (Control)	30	mean	160	244	285	290
		SD	9.7	18.1	19.0	14.3
100	30	mean	182+	271+	311+	317+
		SD	19.6	19.3	23.8	32.2
1000	30	mean	175+	262+	307+	328+
		SD	19.8	20.3	20.9	22.9
10000	30	mean	190+	282+	312+	321+
		SD	24.3	32.4	36.7	35.6
			F1 Generation			
0 (Control)	30	mean	70	183	279	338
		SD	15.9	25.0	24.2	31.5
100	30	mean	69	206+	293	339
		SD	13.6	22.4	29.9	37.4
1000	30	mean	84+	213+	305+	364+
		SD	13.8	24.5	29.8	29.6
10000	30	mean	72	186	282	353
		SD	10.3	21.9	28.2	32.6

SD - standard deviation
\+ - significantly higher than control
* - values for F0 generation are from week 16, values for F1 generation are from week 18

Table 5.6.1-34 Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat ([redacted] 1993): Selected body weights of F0 and F1 females during pre-pairing (group mean values)

Dietary concentration (ppm)	No. of animals		Body weight (g) at Week			
			0	5	9	12
			F0 Generation			
0 (Control)	30	mean	142	175	199	Not reported

		SD	10.2	9.6	11.9	
100	30	mean	160+	182+	199	Not reported
		SD	12.7	14.5	12.8	
1000	30	mean	142	178	193	Not reported
		SD	11.9	14.8	19.6	
10000	30	mean	141	186+	200	Not reported
		SD	11.0	17.2	15.9	
			F1 Generation			
0 (Control)	30	mean	66	143	182	197
		SD	15.9	13.4	13.0	15.2
100	30	mean	67	153+	186	195
		SD	13.3	16.2	14.9	15.7
1000	30	mean	69	159+	197+	202
		SD	12.8	12.8	15.4	17.3
10000	30	mean	78+	158+	194+	207
		SD	8.8	15.1	16.6	19.4

SD - standard deviation
\+ - significantly higher than control

Table 5.6.1-35 Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat ([redacted] 1993): Body weights of F0 and F1 females during gestation (group mean values)

Dietary concentration (ppm)	No. of animals		Body weight (g) at Gestation Day			
			0	6	13	20
			F0 Generation			
0 (Control)	30	mean	205	224	244	289
		SD	16.9	15.4	14.2	22.7
100	27	mean	203	218	237	278
		SD	14.0	15.1	17.0	22.1
1000	28	mean	199	216	237	284
		SD	16.6	18.8	23.5	28.9
10000	30	mean	200	218	239	288
		SD	13.3	15.5	19.6	26.5
			F1 Generation			
0 (Control)	26	mean	205	220	242	299
		SD	16.6	17.4	22.0	25.8
100	28	mean	203	219	242	295
		SD	17.8	18.1	20.0	26.7
1000	22	mean	208	226	250	316
		SD	19.0	22.3	20.9	26.9
10000	28	mean	219+	236+	258+	316
		SD	19.5	19.9	21.2	29.3

SD standard deviation
Not reported not applicable
\+ significantly higher than control

Table 5.6.1-36 Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat ([redacted] 1993): Body weights of F0 and F1 females during lactation (group mean values)

Dietary concentration (ppm)	No. of animals		Body weight Change (g) at Day			
			1	4	14	21
			F0 Generation			

0 (Control)	30	mean SD	214 18.0	229 13.9	243 19.6	233 18.5
100	30	mean SD	214 17.2	223 15.2	226- 25.4	226 19.9
1000	30	mean SD	213 20.4	227 24.2	238 25.3	233 19.0
10000	30	mean SD	223 21.2	230 22.6	248 19.7	238 22.9
			F1 Generation			
0 (Control)	26	mean SD	221 25.1	230 20.7	249 17.2	248 16.8
100	27	mean SD	222 25.9	233 22.7	248 19.1	239 16.2
1000	22	mean SD	244+ 21.3	249+ 22.0	259 23.0	244 27.9
10000	28	mean SD	244+ 21.4	251+ 20.3	268+ 24.0	269+ 22.0

SD - standard deviation
+/- - significantly higher/ lower than control

F. FOOD CONSUMPTION

F0 parents

Mean food consumption of males was similar to the controls throughout the study. High dose female animals tended to consume significantly more food than controls during gestation. During lactation low and mid-dose females consumed significantly less than controls, especially for the Periods 7-14 and 14-21. High dose females consumed significantly more food for Lactation Days 4-7 as compared to controls.

F1 males

Treatment groups did not show consistent and dose related changes as compared to control group. However initially (Weeks 0-2) mid and high dose groups consumed significantly less feed and later on a few occasions mid dose group showed increased consumption.

F1 females

Treatment group dams did not show treatment and dose related consistent difference from control group; on a few occasions the treatment groups showed both increased/decreased food consumption over control. During gestation there was no statistically significant inter group difference in feed consumption between control and treatments during gestation period. Low dose dams consumed significantly less food than controls during different lactation periods (except for Day 7 and Period 7-14). Mid and high dose group dams did not show any treatment and dose related changes over control except for an incidental finding of increased and decreased feed consumption on Day 7, 14 and Period 7-14 and 14-21 respectively in mid dose group.

Table 5.6.1-37 Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat ([redacted] 1993): Food consumption throughout treatment period – F0 and F1 males (selected group mean values)

Dietary concentration (ppm)	No. of animals (No. of cages)		Food consumption (g) in Week 0	5	9	16/18*
			F0 Generation			
0 (Control)	30 (6)	mean SD	21.2 1.47	18.3 0.82	19.0 1.41	20.7 4.27
100	30 (6)	mean SD	19.2 2.14	18.8 0.75	18.8 2.71	19.7 2.23

1000	30 (6)	mean SD	17.2 0.41	19.7 0.82	21.3 1.21	18.2 1.17
10000	30 (6)	mean SD	18.8 0.75	19.2 1.33	19.2 2.56	19.0 1.79
			F1 Generation			
0 (Control)	30 (6)	mean SD	15.7 0.88	17.5 0.77	19.0 0.89	24.3 2.01
100	30 (6)	mean SD	15.9 0.80	17.5 0.63	22.1 5.17	22.8 1.00
1000	30 (6)	mean SD	14.3- 0.52	18.9 1.46	22.5 2.43	22.9 1.14
10000	30 (6)	mean SD	13.2- 0.93	17.8 1.13	19.3 0.88	23.3 0.86

SD - standard deviation
\- - significantly lower than control
* - values for F0 generation are from week 16, values for F1 generation are from week 18

Table 5.6.1-38 Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat ([redacted] 1993): Food consumption of F0 and F1 females during pre-pairing (selected group mean values)

Dietary concentration (ppm)	No. of animals (No. of cages)		Food consumption (g) in Week 0	5	9	12
			F0 Generation			
0 (Control)	30 (6)	mean SD	21.3 1.51	18.3 0.82	18.5 2.07	Not reported
100	30 (6)	mean SD	19.0 5.22	18.0 1.67	18.0 2.37	Not reported
1000	30 (6)	mean SD	14.0 1.41	17.0 0.89	17.7 2.73	Not reported
10000	30 (6)	mean SD	17.8 0.75	18.8 1.94	18.8 3.25	Not reported
			F1 Generation			
0 (Control)	30	mean SD	15.8 0.94	16.9 1.50	16.0 2.10	22.5 6.42
100	30	mean SD	14.0 1.30	17.4 0.58	26.2+ 8.65	23.1 5.01
1000	30	mean SD	13.8 1.03	18.3 1.54	22.4+ 5.29	21.0 1.35
10000	30	mean SD	13.5 1.10	18.2 0.68	19.5 0.71	20.7 0.59

SD standard deviation
\+ significantly higher than control

Table 5.6.1-39 Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat ([redacted] 1993): Food consumption of F0 and F1 females during gestation (group mean values)

Dietary concentration (ppm)	No. of animals		Food Consumption per Day (g) during Period			
			0-6	6-13	13-20	0-20
			F0 Generation			
0 (Control)	30	mean	17	18	21	19
		SD	4.0	4.0	4.7	3.5
100	27	mean	14-	17	19	17
		SD	3.3	3.2	4.7	2.9
1000	28	mean	18	19	23	20
		SD	4.7	5.1	6.1	4.0
10000	30	mean	22+	23+	22	23+
		SD	9.1	6.1	6.9	5.5
			F1 Generation			
0 (Control)	26	mean	21	20	24	22
		SD	3.5	2.7	3.1	2.6
100	28	mean	21	21	23	22
		SD	4.0	3.5	4.3	3.1
1000	22	mean	22	22	23	25
		SD	4.6	3.6	2.4	2.8
10000	28	mean	21	22	25	23
		SD	5.3	3.8	4.3	3.5

SD standard deviation
+/- significantly higher/ lower than control

Table 5.6.1-40 Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat ([redacted] 1993): Food consumption of F0 and F1 females during lactation (group mean values)

Dietary concentration (ppm)	No. of animals		Food Consumption per Day (g) during Period			
			1-4	4-7	14-21	1-21
			F0 Generation			
0 (Control)	28	mean	35	36	55	42
		SD	11.7	9.5	12.3	5.4
100	22	mean	33	37	44-	36-
		SD	13.4	17.5	13.7	7.5
1000	27	mean	30	41	48	37-
		SD	13.8	16.2	9.6	7.2
10000	28	mean	36	49+	59	45
		SD	14.9	16.4	13.6	9.8
			F1 Generation			
0 (Control)	30	mean	38	46	60	42
		SD	15.5	15.2	7.3	6.7
100	30	mean	28-	36-	53-	37-
		SD	13.7	8.1	9.4	6.7
1000	30	mean	34	40	50-	39
		SD	11.7	10.7	7.4	4.8
10000	30	mean	30	40	59	41
		SD	6.7	11.2	6.5	3.8

SD standard deviation
+/- significantly higher/ lower than control

G. REPRODUCTIVE PARAMETERS

Reproductive performance parameters of F0 parental animals such as female fertility index, number of implantations, gestation index, duration of gestation and live birth index were not significantly different between treated and control groups. Male fertility index was significantly higher in low and high dose groups over control.

F0 generation
On Day 1 of lactation, mean litter size was significantly less than control in low and mid dose groups and the mean viable litter size at birth was significantly less in low dose group; the number of live pups on Day 1 was significantly lower in the mid-dose group.

F1 generation
Reproductive performance parameters of F1 parental animals such as male and female fertility index, fecundity index, parturition percentage and mortality of pregnant dams was not different between treatment and control groups. The incidence of dams not littered tended to be higher in the mid-dose group compared to controls. A significantly decreased number of implantations was observed in low and mid dose groups; the percentage of live pups born was significantly reduced in the in mid dose group and significantly increased in the high dose group.

Table 5.6.1-41 Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat ([redacted] 1993): Reproduction data F0 and F1 animals

	Group 1 - control 0 ppm		Group 2 100 ppm		Group 3 1000 ppm		Group 4 10000 ppm	
	F0	F1	F0	F1	F0	F1	F0	F1
Number of dams in group	30	30	30	30	30	30	30	30
Number of dams mated	30	30	30	30	30	30	30	30
Number of dams pregnant	30	26	27	27+1*	29	25	30	28
Number of dams littered	29	26	26	27+1*	28	22	30	28
Female fertility index (%)	100.0	86.7	90.0	93.3	96.7	83.3	100.0	93.3
Male fertility index (%)	86.7	86.7	100.0+	86.7	96.7	96.7	100.0+	83.3
Mean number of implantations	12.1	13.4	11.2	11.6-	11.0	12.0-	12.3	12.9
Mean litter size	11.3	11.7	9.8-	10.4	9.9-	10.9	10.4	11.9
Mean viable litter size at birth	11.0	11.7	9.7-	10.4	9.9	10.9	9.9	11.9
Number of pups alive on day 1	320	305	253	281	276-	239	296	334
Number of dead pups on day 1	9	0	2	0	0	0	15	0
Percentage of live pups born [%]	87.9	87.6	83.5	86.5	86.5	79.7-	80.0-	92.8+

- significantly decreased; + significantly increased
* one dam died due to dystocia

H. LITTER DATA

Number of pups delivered

Mean number of F1 and F2 pups delivered and mean litter sizes in the 100, 1000 and 10000 ppm groups were comparable to the controls.

Sex ratio
Sex ratios of F1 and F2 pups in the 100, 1000 and 10000 ppm groups were comparable to the controls.

Viability index
F1 pups
In the low dose group the pup survival index for Days 4, 14 and 21 was significantly lower than in controls. In the mid dose group the live birth index and Day 14 survival index were higher and Day 4 survival index was lower compared to controls. In the high dose group on Day 14 and 21 survival index was higher than in controls. Dose response relationship was not seen in these parameters.

F2 pups
There were no statistically significant inter group differences between control and treatment groups in parameters of F2 litters at first observation including incidence of external abnormalities in pups. The mean number of pups (combined and individual sex) during different periods of lactation did not show statistically significant differences compared to control group.
The group mean values of pup survival data parameters like: live birth index, 24 hours survival index and survival index for Days 4, 7, 14 and 21 did not show any significant inter group difference between control and treatment groups.

Body weights
F1 pups
Mean litter weight of combined sex and female pups in treatment groups were significantly more than control group on Day 1 and 4, respectively. On Day 7 combined sex litter weight and male pup weight was significantly less than control in low dose group while in high dose group it was more than control group. On Day 21 the mean body weight of complete litter and individual sex pups of mid dose group were more than control group. None of these showed any apparent dose response relationship.

F2 pups
Combined sex litter weight on day one and that of female pups of all treatment groups was higher than in controls; in addition combined sex litter weight in low and mid dose groups and that of male and female in mid dose group was higher than control on Day 4. In high dose group the male pup body weight on Day 14 and 21 was lower than control. None of these parameters showed any dose response relations.

Clinical signs
There were no treatment-related abnormalities noted in F1 and F2 pups of any dose group.
During the lactation period, deaths and loss due to maternal cannibalism occurred in several pups in all groups including the control. However, the incidences in the treated groups were comparable to the control.

Table 5.6.1-42 Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat ([redacted] 1993):
Litter data F1 and F2 litters

	Group 1 - control 0 ppm		Group 2 100 ppm		Group 3 1000 ppm		Group 4 10000 ppm	
	F1	F2	F1	F2	F1	F2	F1	F2
Number of litters examined	29	26	26	27	28	22	30	28
Mean litter size at birth	11.3	11.7	9.8-	10.4	9.9-	10.9	10.4	11.9
Mean viable liter size at birth	11.0	11.7	9.7-	10.4	9.9	10.9	9.9	11.9
Pups with external	1	0	5	0	0	0	0	0

abnormalities								
Live birth index (%)	97.3	100	99.2	100	100.0+	100	95.2	100
Day 4 survival index	98.8	95.4	94.5-	98.2	95.3-	97.5	98.3	97.3
Day 7 survival index	99.1	100	100.0	100	99.0	98.8	99.5	98.6
Day 14 survival index	95.2	97.0	89.9-	99.0	99.0+	98.8	99.1+	95.5
Day 21 survival index	94.8	96.0	87.8-	99.0	97.5	98.2	99.1+	95.0

- significantly decreased; + significantly increased

Table 5.6.1-43 Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat ([redacted] 1993): pup body weights of F1 and F2 pups during lactation (group mean values)

Dietary concentration (ppm)	No. of litters		Body weight (g) at Day 1	4	14	21
			F1 Generation			
0 (Control)	29	mean	5.5	7.8	21.8	31.2
		SD	0.6	0.9	3.2	5.7
100	26	mean	5.8+	8.5	20.6	30.0
		SD	0.7	1.3	3.5	6.0
1000	28	mean	6.1+	8.6+	23.3	36.3+
		SD	1.1	1.4	3.0	6.0
10000	30	mean	6.0+	8.6	22.6	32.7
		SD	0.7	1.1	2.4	4.4
			F2 Generation			
0 (Control)	26	mean	5.4	7.4	22.9	35.0
		SD	0.6	1.2	2.2	3.8
100	27	mean	5.7+	8.1+	23.1	33.8
		SD	0.7	1.2	2.6	4.4
1000	22	mean	5.9+	8.4+	22.7	33.3
		SD	0.6	1.2	3.0	6.1
10000	28	mean	5.6+	7.6	21.6	32.0
		SD	0.4	0.7	3.0	4.2

SD standard deviation
+ significantly higher than control

I. PATHOLOGY

Necropsy

F0 generation

The gross pathological lesions seen were consolidated lungs with ecchymoses, chronic liver changes, kidneys with cysts and dilated pelvis, and hypoplastic testes (1 in the control group, 2 in the mid-dose and 1 in the high-dose group). The incidence was low and did not appear to be compound or dose related.

F1 generation

The gross pathological lesions seen were consolidated and collapsed lungs with emphysema, hydronephrotic kidneys, and unilateral hypoplastic testes. The lesions observed were few and appeared to be incidentally. A single incidence of unilateral testicular hypoplasia was observed in each of the three treatment groups, hydronephrosis was seen in two animals in the high dose group.

F1 pups

A higher incidence of emaciated pups was recorded for the mid and high dose groups compared to controls. A low incidence of minor developmental abnormalities like kinked tail, rudimentary tail, kidney hydronephrosis and dilated pelvis occurred without dose-response relation.

Table 5.6.1-44 Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat ([redacted] 1993): Summary of necropsy findings F1 pups

	Dietary concentration (ppm)							
	0		100		1000		10000	
Sex	M	F	M	F	M	F	M	F
Animals examined	125	135	102	84	94	115	123	119
Cannibalism/Missing	5	7	15	9	7	10	2	5
General Observations								
Emaciation	0	2	0	0	3	2	2	4
Autolysis	5	7	8	8	1	0	8	7
Developmental malformations								
External malformations								
Kinked tail	0	0	2	3	0	0	0	0
Rudimentary tail	0	1	0	0	0	0	0	0
Visceral organ pathology								
Kidney-hydronephrosis	0	0	0	0	2	0	1	1
Kidney – pelvis dilated	1	1	0	0	0	0	0	0

F2 pups
A higher incidence of emaciation has been observed in pups of high dose group. Occasional not treatment and dose related incidence of hydronephrosis and dilated pelvis in kidney have been recorded.

Table 5.6.1-45 Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat ([redacted] 1993): Summary of necropsy findings F2 pups

	Dietary concentration (ppm)							
	0		100		1000		10000	
Sex	M	F	M	F	M	F	M	F
Animals examined	167	138	133	148	134	105	161	173
Cannibalism/Missing	10	11	5	3	5	2	6	12
General Observations								
Emaciation	0	0	0	0	1	0	5	3
Autolysis	0	1	0	0	0	1	2	0
Developmental malformations								
External malformations	0	0	0	0	0	0	0	0
Visceral organ pathology								
Kidney-hydronephrosis	0	2	0	0	0	0	0	0
Kidney – pelvis dilated	0	0	0	0	0	0	1	0

Histopathology
F0 generation
Reproductive organs showing gross pathological changes were recorded as outlined in the following: testes from one control animal, two mid dose and one high dose animal. The control and high dose animals showed degenerative changes in the seminiferous tubules while the mid dose group did not show this finding. These changes appeared to be incidental and not compound related.

Table 5.6.1-46 Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat ([redacted] 1993): Summary of histpopathological fings – F0 males

Observations	Dietary concentration (ppm)			
	0	100	1000	10000
No. of tissues evaluated	1	0	2	1
Testes				
Degenerative changes – seminiferous tubules	1	0	0	1

F1 generation
Reproductive organs showing gross pathological changes were recorded as outlined in the following: testes from one animal in each of the three treatment groups; the testes in the low and mid dose groups showed unilateral degenerative changes and giant cell formation in the seminiferous tubules and focal chronic inflammation. The testes in the high dose were normal though unequal in size. The changes appeared to be incidental and not compound related.

Table 5.6.1-47 Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat ([redacted] 1993): Summary of histpopathological fings – F1 males

Observations	Dietary concentration (ppm)			
	0	100	1000	10000
No. of tissues evaluated	0	1	1	1
Testes				
Degenerative changes - seminiferous tubules	0	1	1	0
Giant cell formation - seminiferous tubules	0	1	1	0
Infammation – chroni focal	0	1	1	0

III. CONCLUSIONS

Study conclusion:
The oral administration of glyphosate to rats by dietary admixture at a maximum dose level of 10000 ppm for two successive generations of Wistar rats resulted in no parental toxicity. The NOAEL for reproduction is considered to be 10000 ppm, since the reproductive performance was not affected in a dose-related manner. The NOAEL for offspring is 10000 ppm, since no treatment-related effects on offspring were observed.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The highest dose of 10000 ppm is considered to be the NOAEL for parental, reproductive and offspring toxicity. This dietary level would correspond to a mean daily compound intake of 700-800 mg/kg bw/day.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	**CA 5.6.1/007**
Report author	[redacted]
Report year	1992
Report title	The Effect of Dietary Administration of Glyphosate on Reproductive Function of Two Generations in the Rat (Vol. 1)
Report No	CHV 47/911129
Document No	Not reported
Guidelines followed in study	OECD 416 (1983), US-EPA FIFRA 83-4 (1982)
Deviations from current test guideline (OECD 416, 2001)	No determination of pre-mating oestrous cycles, no sperm analysis, some organ weights of parental animals missing, organ weights of offspring missing
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

1. Information on the study

Data point:	**CA 5.6.1/008**
Report author	[redacted]
Report year	1992
Report title	The Effect of Dietary Administration of Glyphosate on Reproductive Function of Two Generations in the Rat (Vol. 2)
Report No	CHV 47/911129
Document No	Not reported
Guidelines followed in study	OECD 416 (1983), US-EPA FIFRA 83-4 (1982)
Deviations from current test guideline (OECD 416, 2001)	No determination of pre-mating oestrous cycles, no sperm analysis, some organ weights of parental animals missing, organ weights of offspring missing
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Glyphosate technical was administered by dietary admixture to three groups of 28 male and female F0 generation Sprague-Dawley rats each at dietary concentrations of 1000, 3000 and 10000 ppm (equivalent to a mean achieved dosages of 66.4 – 76.1, 196.8 – 230.2 and 668.1 – 771.3 mg/kg bw/day for males and 75.3 – 82.1, 226 – 244.9 and 752.3 – 841.1 mg/kg bw/day for females, respectively). An additional group of 28 male and 28 female F0 animals was exposed to basal laboratory diet to serve as a control.

Each parent generation was mated to produce two litters. Offspring from the first litters of the F0 parents (F1A litters) were selected to be parents for the F2 generation (24/sex/group). Offspring not included in the selection procedure and offspring from the second litter of each generation (F1B and F2B) were sacrificed at Day 21 post partum and given a gross post mortem examination. Parent animals were sacrificed shortly after termination of the second litter.

Clinical signs, body weight development, food and water consumption, mating performance, pregnancy rate, and length of gestation of adults were monitored during the study. Litter weight, individual offspring weights and landmark developmental signs were also recorded on specific days post partum. All animals at termination were subjected to a gross necropsy examination and weighing of selected organs and histopathological evaluation of selected tissues for the F0 and F1 adults was performed.

Treatment at 10000 ppm produced marginal signs of toxicity in parent animals and minimal histological changes in the target organ (salivary gland). Histological changes in the salivary gland were also noted at 3000 ppm. This finding is considered to be an adaptive response due to oral irritation from the ingestion of glyphosate, an organic acid, in the diet and is not considered to be adverse. In the offspring no treatment-related effects were apparent at dietary administration up to 10000 ppm.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material:

Identification:	Glyphosate technical
Description:	White solid
Lot/Batch #:	206-Jak-119-1
Purity:	99.2%
Stability of test compound:	Stable during the treatment period.
Vehicle:	Plain diet
Test Animals:	
Species:	Rat
Strain:	Sprague-Dawley Crl:CD (SD) BR VAF/Plus
Source:	[redacted]
Age:	Approximately 6 weeks
Sex:	Males and females
Weight at dosing:	Males: 143 – 201 g; females: 106 – 175 g
Acclimation period:	At least 15 days
Diet/Food:	Biosure Laboratory Animal Diet No.2, ad libitum
Water:	Tap water, ad libitum

Housing:	During pre-mating periods, animals were housed in groups of four in metal cages with wire mesh front, floor and top. During the first week of F1A and contingency animals of F2B animals were housed in plastic cages. During mating animals were housed on an 1:1 basis in plastic cages where females stayed after mating for breeding. Males were re-housed in former metal cages.
Environmental conditions:	Temperature: 23 ± 4°C Humidity: 45 ± 24% Air changes: not reported 12 hours light/dark cycle

B. STUDY DESIGN
In life dates: 1990-03-14 to 1991-03-22

Animal assignment and treatment:
In a two-generation reproduction study groups of 28 Sprague-Dawley rats per sex of the F0 generation received daily dietary doses of 0, 1000, 3000 and 10000 ppm glyphosate technical. The dose levels were chosen based on results of a previously conducted study (CA 5.6.1/009). In this study, treatment at 3000, 10000 and 30000 ppm was associated with signs of maternal toxicity, whilst effects on development of the offspring to 6 weeks of age was not affected up to 10000 ppm. After at least 70 days of treatment pairing of animals within each dose group was undertaken on a 1:1 basis to produce the F1 litters. At Day 21 post partum of offspring from the F0 mating phase, groups of 24 male and 24 female offspring from each dose group were selected to form the F1A generation. The remaining pups were sacrificed. Approximately 10 days following the weaning of all F1A pups, F0 males and females were re-mated. At Day 21 post partum all F1B pups were sacrificed. F0 males and females were terminated shortly after weaning of F1B pups.
The selected F1A animals were dosed from approximately Week 4 of age for at least 84 days and then mated on a 1:1 basis (sibling pairings were avoided). On Day 4 post partum F2A litters were standardised to 8 pups per litter. The remaining pups were sacrificed. On or shortly after Day 21 post partum all F2A pups were sacrificed. Approximately 10 days following the weaning of all F2A pups, F1 males and females were re-mated. On Day 4 post partum F2B litters were standardised to 8 pups per litter. The remaining pups were sacrificed. On or shortly after Day 21 post partum all F2B pups were sacrificed. F1 males and females were terminated shortly after weaning of F2B pups.

Diet preparation and analyses
For the weekly preparation of diet mixtures a known amount of the test substance was mixed with a small amount of basal diet. This pre-mix was then added to larger amount of basal diet and blended for further 7 minutes in a rotary double-cone-blender. Fresh diet was prepared weekly and fed for no more than 15 days from the date of preparation.
The stability and homogeneity of the test material in diet were determined. Dietary admixtures were analysed for achieved concentration throughout the study.

Clinical observations
A check for clinical signs or ill health was made once daily and recorded daily for the first week of treatment and on a weekly basis thereafter. Rats showing marked signs of ill health or reaction to treatment were killed and subjected to necropsy.

Body weight
Individual body weights were recorded at the start of each generation (F0: Week 6 of age; F1A: Week 4 of age) and subsequent at weekly intervals. Females were weighed daily during mating and continued until parturition. Weights were reported for Days 0, 7, 14, 17 and 20 of pregnancy. Females with live litters were weighed on Days 0, 7, 14 and 21 post partum.

Food consumption and compound intake
Food consumption was recorded on a weekly basis from allocation throughout the first pre-mating phase of each generation. During this period food conversion ratios and achieved intake (mg/kg bw/day) were calculated.

Water consumption
Water intake was observed daily during the initial and final two weeks of the first pre-mating period for each generation and from allocation for the F0 generation.

Reproduction parameters
Vaginal smears were taken daily during the 20–day mating period to examine the oestrus cycle and median pre-coital time. Additionally, date of mating and duration of gestation was recorded.

Litter data
The number of offspring born and the number of offspring alive were recorded daily. Pups were weighed on Days 0 and 4 and all litters containing more than eight pups were culled to eight on Day 4 post partum, retaining, where possible, ideally 4 pups per sex. The remaining pups were also weighed on Days 8, 12, 16 and 21. Dead and culled young were subjected to necropsy.

Sacrifice and pathology
All adult animals were subjected to a full external and internal examination, and any macroscopic abnormalities were recorded.

The following organs were weighed of adults: adrenals, brain, heart, kidneys, liver, lungs, ovaries, prostrate (with seminal vesicles and coagulating gland), testes (with epididymides), thymus.

The following tissues were preserved from all adults: adrenals, aorta, bone (femur and joint), bone marrow (sternum), brain, cranial vault (for lachrymal glands, teeth, nasal turbinates, inner ear), caecum, colon, duodenum, eyes, heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes (cervical/mesenteric), mammary gland, macroscopically abnormal tissues, oesophagus, ovaries*, pancreas, pituitary*, prostate with seminal vesicles (with coagulating gland)*, rectum, salivary gland, sciatic nerve, skeletal muscle, skin, spinal column (vertebral column), spleen, stomach, testes (with epididymides)*, thymus, thyroids (with parathyroids), tongue, trachea (with larynx and pharynx), urinary bladder, uterus (with cervix)* and vagina*.

Histology of the reproductive tract was restricted to adults of the control and high-dose group and any apparently infertile animals at the lower dietary concentrations and confined to tissues marked with an asterisk (*).

Statistics
Two tailed significance tests were performed on adult parameters (water consumption, food consumption, body weight, organ weights) and litter data. Evaluation of other parameters were found not to be useful. Significances at 1% and 5% were reported.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS
Stability analyses indicated that the dose preparations at nominal concentrations of 500 and 30000 ppm were stable for up to 18 days during storage under animal room conditions.
Analyses for homogeneity at nominal concentrations of 500 and 30000 ppm indicated that the dose preparations were homogeneous.
Analyses for achieved concentration performed at 4-5 weekly intervals demonstrated that the prepared

dietary admixture concentrations given to the animals were within ± 15% of the nominal concentration in all groups.

B. TEST COMPOUND INTAKE

The group mean intakes of glyphosate are summarised in the tables below.

Table 5.6.1-48 Glyphosate technical: The Effect of Dietary Administration of Glyphosate on Reproductive Function of Two Generations in the Rat ([redacted], 1992): Group mean achieved intakes of glyphosate - F0 generation

Group	Dietary concentration (ppm)	Mean intakes (Week 1 - 10) (mg/kg bw/day)	
		Males	Females
Control	0	0	0
Low	1000	66.4	75.3
Intermediate	3000	196.8	226.0
High	10000	668.1	752.3

Table 5.6.1-49Glyphosate technical: The Effect of Dietary Administration of Glyphosate on Reproductive Function of Two Generations in the Rat ([redacted], 1992): Group mean achieved intakes of glyphosate - F1 generation

Group	Dietary concentration (ppm)	Mean intakes (Week 5 - 16) (mg/kg bw/day)	
		Males	Females
Control	0	0	0
Low	1000	76.1	82.1
Intermediate	3000	230.2	244.9
High	10000	771.3	841.1

C. MORTALITY

There were no test substance related mortalities.

Four unscheduled deaths occurred during each generation.

In the F0 generation one female of the low dose group and one male of the high dose group were killed for humane reasons during Week 15 and 23, respectively. The female exhibited pilo-erection and thin appearance and the necropsy noted thickened forestomach, invaginated stomach and abnormal contents in the gastro-intestinal tract. The male was unable to use hind limbs, exhibiting aberrations of brain and spinal cord at necropsy. Another male of the high dose group died during Week 3, with effects on pancreas and liver noted at necropsy. One control male was sacrificed during Week 16 following poor condition, however, the actiology of the signs was not established.

In the F1 generation one female of the low dose group was killed following a procedural error. In the mid dose group one male died during Week 34 but autolytic changes precluded a valid necropsy. Moreover, one male and one female died and were sacrificed, respectively, during Week 23. Necropsies failed to identify a specific cause of death.

D. CLINICAL OBSERVATIONS

No treatment-related clinical signs of toxicity were noted. General signs were observed in occasional animals from both generations and were not related to treatment.

E. BODY WEIGHT

No adverse effect of body weight change was evident for treated animals in comparison to controls for both generations.

However, absolute mean body weights in high dose F1 males were slightly lower as compared to control. In addition it was noted that during the first mate of each generation, body weight gains during the initial stages of pregnancy tended to be slightly lower than controls at all dietary levels. Since no consistent dose-response was apparent these effects cannot conclusively be attributed to treatment.

Table 5.6.1-50 Glyphosate technical: The Effect of Dietary Administration of Glyphosate on Reproductive Function of Two Generations in the Rat ([redacted], 1992): Group mean body weights (g) - F0 generation

Week	Males Dietary concentration (ppm)				Females Dietary concentration (ppm)			
	0	1000	3000	10000	0	1000	3000	10000
0	233	233	236	235	173	171	171	170
5	452	454	459	449	272	275	270	270
9	535	539	546	530	304	308	304	304
10[1]	550	556	565	546	311	314	312	312
15	595	603	615	591	352	347	360	351
19	641	650	664	638	349	355	349	347
20[2]	650	661	674	649	353	360	353	351
25	664	672	692	664	410	400	408	403
28	704	710	732	702	381	381	377	370

1 First mating commenced
2 Second mating commenced

Table 5.6.1-51 Glyphosate technical: The Effect of Dietary Administration of Glyphosate on Reproductive Function of Two Generations in the Rat ([redacted], 1992): Group mean body weights (g) - F1 generation

Week	Males Dietary concentration (ppm)				Females Dietary concentration (ppm)			
	0	1000	3000	10000	0	1000	3000	10000
4	108	104	106	100	95	93	98	93
10	425	429	423	412	266	265	270	267
15	526	542	521	512	316	313	325	319
16[1]	540	555	538	528	324	321	335	326
21	605	619	600	591	373	370	373	381
26	659	677	659	649	367	368	380	370
27[2]	673	692	671	663	374	372	390	376
32	710	727	709	699	413	424	436	424
37	762	781	746	742	415	410	424	417

1 First mating commenced
2 Second mating commenced

F. FOOD AND WATER CONSUMPTION

Apart from a slightly higher but not statistically significant food consumption of high dose F1 females during the second half of the pre-mating period, there were no marked intergroup differences in food consumption of males or females. Across both generations, there were no clear or consistent adverse effects of treatment on the food conversion ratio, indicating that treatment with glyphosate does not overtly affect the efficiency of food utilisation into body weight of either sex at dietary concentrations of up to 10000

ppm.

Apart from a slight increase among high dose F1 females (attaining statistical significance in Week 16), no overt intergroup differences in water intake for treated males and females from the F0 or F1 generations when compared to their concurrent controls.

Table 5.6.1-52Glyphosate technical: The Effect of Dietary Administration of Glyphosate on Reproductive Function of Two Generations in the Rat ([redacted], 1992): Group mean weekly food consumption (g/rat/week) - F0 generation

Week	Males Dietary concentration (ppm)				Females Dietary concentration (ppm)			
	0	1000	3000	10000	0	1000	3000	10000
1	188	191	190	185	136	134	134	129
5	188	191	191	193	136	136	135	135
10	184	185	186	184	125	128	129	128

Table 5.6.1-53 Glyphosate technical: The Effect of Dietary Administration of Glyphosate on Reproductive Function of Two Generations in the Rat ([redacted], 1992): Group mean weekly food consumption (g/rat/week) - F1 generation

Week	Males Dietary concentration (ppm)				Females Dietary concentration (ppm)			
	0	1000	3000	10000	0	1000	3000	10000
5	133	125	134	124	108	106	111	108
10	190	195	189	187	140	139	144	147
13	190	193	191	191	135	137	140	143
16	181	184	184	180	128	128	131	132

G. REPRODUCTIVE PARAMETERS

There were no treatment-related effects on mating performance, fertility and gestation length for both F0 and F1 generation animals.

Table 5.6.1-54 Glyphosate technical: The Effect of Dietary Administration of Glyphosate on Reproductive Function of Two Generations in the Rat ([redacted], 1992): Summary of adult performance - F0 generation

Parameter	Dietary concentration (ppm)			
	0	1000	3000	10000
Males				
Initial group size	28	28	28	28
Died (pre-mating 1)	-	-	-	1
Sacrificed (pre-mating 2)	1	-	-	-
Sacrificed (post-mating 2)	-	-	-	1
Failed to induce pregnancy in either female partner	-	-	-	1
Females				

Table 5.6.1-54 Glyphosate technical: The Effect of Dietary Administration of Glyphosate on Reproductive Function of Two Generations in the Rat ([redacted], 1992): Summary of adult performance - F0 generation

Parameter	Dietary concentration (ppm)			
	0	1000	3000	10000
First mating				
Initial group size/mated	28	27	28	28
Median pre-coital time (days)	2.0	3.0	2.5	3.0
Mean duration of pregnancy (days)	22.0	22.2	22.0	22.1
No young born	-	-	1	2
Non-pregnant[1]	1	2	-	1
Pregnancy rate (%)	96.4	92.9	96.4	89.3
Sacrified post partum	-	1	-	-
Rearing young to weaning	27	25	27	25
Second mating				
Initial group size/mated	28	27	28	28
Median pre-coital time (days)	2.5	3.0	3.0	3.0
Mean duration of pregnancy (days)	22.1	21.9	21.9	22.0
No young born	2	2	-	1
Non-pregnant1	1	2	-	1
Pregnancy rate (%)	89.3	85.2	100.0	92.9
Total litter loss post partum	1	1	-	1
Rearing young to weaning	24	22	28	25
Failed to mate with both male partners	1	2	-	1

1 No implantation sites detected by Salewski technique at termination following second mating

Table 5.6.1-55 Glyphosate technical: The Effect of Dietary Administration of Glyphosate on Reproductive Function of Two Generations in the Rat ([redacted], 1992): Summary of adult performance – F1 generation

Parameter	Dietary concentration (ppm)			
	0	1000	3000	10000
Males				
Initial group size	24	24	24	24
Died (pre-mating 2)	-	-	-	11
Died (post-mating 2)	-	-	1	-
Failed to induce pregnancy in either female partner	3	1	3	31
Females				
First mating				
Initial group size/mated	24	24	24	24
Median pre-coital time (days)	3.0	3.0	6.0	3.0
Mean duration of pregnancy (days)	22.2	22.0	22.1	22.1
No young born	1	3	3	2
Non-pregnant2	3	3	7	3
Pregnancy rate (%)	83.3	75.0	58.3	79.2
Total litter loss post partum	1	1	-	-
Rearing young to weaning	19	17	14	19

Table 5.6.1-55 Glyphosate technical: The Effect of Dietary Administration of Glyphosate on Reproductive Function of Two Generations in the Rat ([redacted], 1992): Summary of adult performance – F1 generation

Parameter	Dietary concentration (ppm)			
	0	1000	3000	10000
Second mating				
Sacrificed prior to mating	-	13	-	1
Group size of females for mating	24	23	24	23
Median pre-coital time (days)	3.5	2.5	4.5	4.0
Mean duration of pregnancy (days)	22.3	22.1	22.2	22.3
No young born	5	2	1	3
Non-pregnant2	3	3	7	3
Pregnancy rate (%)	66.7	78.3	66.7	73.9
Total litter loss post partum	-	1	1	-
Rearing young to weaning	16	17	15	17
Failed to mate with both male partners	3	3	7	3

1 Failed to mate with partner at first mating, included in failed to mate
2 No implantation sites detected by Salewski technique at termination following second mating
3 Female mistakenly placed in male cage after first mating and became pregnant

H. LITTER DATA

Size and Viability

No overt differences in litter viability were detected.

In the high-dose group total litter size at birth was consistently, but not significantly, lower than controls across all four matings and remained lower than controls at Day 4 in three of the four matings. Since the mean litter size at birth within each mating, was not always the lowest litter size recorded, this finding could not be clearly attributed to treatment.

Growth and Development

No adverse effects on mean offspring body weights, body weight change or development were detected for male and female offspring in comparison to their controls.

Table 5.6.1-56 Glyphosate technical: The Effect of Dietary Administration of Glyphosate on Reproductive Function of Two Generations in the Rat ([redacted], 1992): Litter data F0 generation

Parameter	Dietary concentration (ppm)			
	0	1000	3000	10000
First Mating				
Mean litter size				
at birth (live pups)	15.0	14.0	14.3	14.0
at day 4 post partum (pre-cull)	14.5	13.3	14.0	13.7
at day 8 post partum	7.9	7.6	7.9	7.8
at day 21 post partum	7.9	7.6	7.9	7.8
Mean pup weight (g)				
at birth	6.2	6.2	6.2	6.4
at day 4 post partum (pre-cull)	10.0	9.8	10.1	10.3
at day 8 post partum	19.6	18.5	19.3	19.3
at day 21 post partum	60.7	56.6*	57.6*	56.0**
Percentage male pups(%)				
at birth	49.9	50.8	57.7	54.4

Table 5.6.1-56 Glyphosate technical: The Effect of Dietary Administration of Glyphosate on Reproductive Function of Two Generations in the Rat ([redacted], 1992): Litter data F0 generation

Parameter	Dietary concentration (ppm)			
	0	1000	3000	10000
At day 21 post partum	50.9	49.4	52.2	51.8
Second Mating				
Mean litter size				
at birth (live pups)	15.0	15.7	14.6	15.1
at day 4 post partum (pre-cull)	14.7	15.2	14.4	14.9
at day 8 post partum	7.9	7.8	7.6	7.8
at day 21 post partum	7.9	7.7	7.5	7.8
Mean pup weight (g)				
at birth	6.2	6.3	6.6	6.4
at day 4 post partum (pre-cull)	10.0	9.8	10.4	10.4
at day 8 post partum	19.6	19.4	20.1	20.5
at day 21 post partum	60.9	59.2	60.4	59.0
Percentage male pups(%)				
at birth	52.3	54.5	53.7	47.8
At day 21 post partum	49.6	52.0	50.0	49.0

*/** significant at P<0.05/0.01; Kruskal-Wallis 'H' statistic followed by intergroup comparison with the controls (Shirley's test)

Table 5.6.1-57Glyphosate technical: The Effect of Dietary Administration of Glyphosate on Reproductive Function of Two Generations in the Rat ([redacted], 1992): Litter data F1 generation

Parameter	Dietary concentration (ppm)			
	0	1000	3000	10000
First Mating				
Mean litter size				
at birth (live pups)	13.8	12.4	14.1	13.2
at day 4 post partum (pre-cull)	13.6	12.2	13.9	12.9
at day 8 post partum	7.8	7.6	8.0	7.6
at day 21 post partum	7.8	7.6	8.0	7.6
Mean pup weight (g)				
at birth	6.4	6.5	6.4	6.7
at day 4 post partum (pre-cull)	10.1	10.7	10.2	11.1
at day 8 post partum	19.2	20.0	19.0	20.8
at day 21 post partum	57.2	56.6	56.4	58.7
Percentage male pups(%)				
at birth	53.0	53.0	44.9	51.3
At day 21 post partum	47.8	51.2	48.2	51.8
Second Mating				
Mean litter size				
at birth (live pups)	14.7	12.8	15.0	13.1
at day 4 post partum (pre-cull)	13.9	12.6	14.7	13.0
at day 8 post partum	7.9	7.5	7.8	7.5
at day 21 post partum	7.9	7.4	7.4	7.4
Mean pup weight (g)				
at birth	6.4	6.7	6.4	6.7
at day 4 post partum (pre-cull)	10.1	11.0	9.9	11.3
at day 8 post partum	19.1	20.6	18.3	21.2
at day 21 post partum	60.4	62.4	59.5	60.4
Percentage male pups(%)				

Table 5.6.1-57Glyphosate technical: The Effect of Dietary Administration of Glyphosate on Reproductive Function of Two Generations in the Rat ([redacted], 1992): Litter data F1 generation

Parameter	Dietary concentration (ppm)			
	0	1000	3000	10000
at birth	57.9	51.6	51.6	56.1
At day 21 post partum	50.4	51.0	54.2	55.2

Table 5.6.1-58 Glyphosate technical: The Effect of Dietary Administration of Glyphosate on Reproductive Function of Two Generations in the Rat ([redacted], 1992): Mean sexual maturation landmarks F1 generation

Parameter	Dietary concentration (ppm)			
	0	1000	3000	10000
Balanopreputial separation (day of age)	41.81	42.5	42.2	42.6
Vaginal opening (day of age)	33.7	33.5	33.2	33.9

1 In the report, this value was given incorrectly as 39.9. This was already noted by the RMS in RAR 2015. Recalculation by the RMS resulted in the given value (double-checked for accuracy).

Clinical signs
No clinically observable signs of toxicity were observed for offspring from treated animals.

I. PATHOLOGY

Necropsy
There were no toxicologically significant macroscopic abnormalities detected in the F0 and F1 animals, or offspring.

Organ weights
There were no overt or statistically significant treatment-related changes in any organ weights analysed in either generation.

Histopathology
No treatment-related changes in tissues associated with the reproductive tract were detected in the F0 or F1 generation animals.

Examination of two previously identified target organs, the parotid and submaxillary salivary glands, was initially performed only in the control and high-dose groups. Due to effects seen in the parotid gland, examination was extended to the remaining treatment groups. For the submaxillary gland, examination was extended to only the F0 and F1 females in the low- and mid dose group. The findings are summarised in the table below.

Table 5.6.1-59 Glyphosate technical: The Effect of Dietary Administration of Glyphosate on Reproductive Function of Two Generations in the Rat ([redacted], 1992): Incidence of salivary gland findings

Observation	Dietary concentration (ppm)							
	Males				Females			
	0	1000	3000	10000	0	1000	3000	10000
F0 Generation								
Animals examined	27	28	28	26	28	27	28	28
Hypertrophy of acinar cells with prominent granular cytoplasm (minimal)								
parotid	2	2	3	12	0	2	5	17
submaxillary	0	-	-	0	0	1	4	14
F1 Generation								
Animals examined	24	24	23	23	24	23	24	23
Hypertrophy of acinar cells with prominent granular cytoplasm (minimal)								
parotid	1	0	4	11	0	0	4	9
submaxillary	0	-	-	0	0	0	0	3

- = not examined

Treatment-related minimal changes were apparent in the parotid salivary gland of both F0 and F1 males and females in the mid- and high-dose groups and the submaxillary salivary gland of the F0 females in the mid- and high-dose groups and F1 females in the high-dose group. This finding is similar to those seen occasionally in other subchronic and long-term dietary studies and is considered to be an adaptive response due to oral irritation from the ingestion of glyphosate, an organic acid, in the diet and is not considered to be adverse (see CA 5.8.2/002). There were no effects on the salivary glands noted in the low-dose group.

III. CONCLUSIONS

Study conclusion:

The oral administration of glyphosate technical to rats by dietary admixture at a maximum dose level of 10000 ppm for two successive generations resulted in minimal effects consisting of increased food and water consumption of F1 females, possibly reduced body weights of F1 males and minimal histological changes in the target organ (salivary glands) in F0 and F1 adults at 10000 ppm. The only findings associated with treatment at 3000 ppm were minimal histopathological changes of the salivary glands in F0 and F1 adults. No effects were apparent at 1000 ppm. Thus, the parental reproductive and offspring NOAELs are considered to be 10000 ppm, corresponding to 668 and 752 mg/kg bw/day in males and females, respectively.

3. Assessment and conclusion

Assessment and conclusion by applicant:

No evidence of reproductive effects was observed. The only findings associated with treatment at 3000 ppm were minimal histopathological changes of the salivary glands in F0 and F1 adults considered to be an adaptive response due to oral irritation from the ingestion of glyphosate, an organic acid, in the diet and is not considered to be adverse (for mechanistical studies on salivary gland effects see also section 5.8.2). No effects were apparent at 1000 ppm. Thus, the parental reproductive and offspring NOAELs are considered to be 10000 ppm, corresponding to 668 and 752 mg/kg bw/day in males and

females, respectively.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	**CA 5.6.1/009**
Report authors	[redacted], A.J. *et al.*
Report year	1991
Report title	Dietary range finding study of glyphosate in pregnant rats and their juvenile offspring
Report No	CHV 42/90619
Document No	Not reported
Guidelines followed in study	Not applicable for this dose-range finding study
Deviations from current test guideline	Not applicable
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No (dose-range finding study)
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

In this preliminary assessment of the effect of glyphosate on pregnancy of the rat and postnatal development of the offspring, glyphosate was present in the diet of 10 time-mated females per group at 0 (Control), 3000, 10000 and 30000 ppm from Day 3 of pregnancy through to termination of the study. All females were allowed to litter and rear their young to weaning, when 10 male and 10 female offspring per group were selected and reared on their respective diets to six weeks of age. The duration of the study was 10 weeks. Diets were not analysed for homogeneity, stability or achieved concentration of the test compound as satisfactory data concerning homogeneity and stability in rodent diet were already available to the Sponsor.

Treatment at 30000 ppm was associated with the following maternal findings: one mortality, signs of soft faeces and yellow stained sawdust in all animals, slightly increased water consumption towards the end of pregnancy retarded body weight gain during the first two weeks of pregnancy and the second half of lactation, macroscopic salivary gland changes in 8/9 animals, macroscopic gastro-intestinal changes in 8/9 animals marked granular basophilic cytoplasm of acinar cells and moderate hypertrophy of acinar cells in all animals and a prominent mitoses in acinar cells of 2/9 animals. Offspring findings included a reduction in litter and mean pup weight, most evident from Day 8 post partum through to Day 21 post partum. Among offspring retained to six weeks of age, loose faeces, reduced food intake and food conversion ratio and, in males only, a slight continuation of the reduced weight gain evident from birth to weaning. Findings at post mortem examination included macroscopic changes to the salivary gland in 5/10 males and 2/10 females and gastro-intestinal disturbances in 7/10 males and 9/10 females.

Treatment at 10000 ppm was associated with the following maternal findings: signs of soft faeces and yellow stained sawdust in all animals, slightly lower body weight gain by Day 14 of pregnancy and during the second half of lactation, macroscopic salivary gland changes in 6/10 animals, macroscopic gastro-intestinal changes in 8/10 animals, moderate/marked granular basophilic cytoplasm of acinar cells and

minimal/moderate hypertrophy of acinar cells in all animals. No adverse effects on development of the offspring from birth to 6 weeks of age were noted, although 4 pups sacrificed at weaning had congested salivary glands.

Treatment at 3000 ppm was associated with the following maternal findings: slightly lower body weight gain by Day 14 of pregnancy, macroscopic salivary gland changes in 2/9 animals, macroscopic gastro-intestinal changes in 5/9 animals, minimal granular basophilic cytoplasm of acinar cells in 2/10 females with minimal hypertrophy of acinar cells in 2/10 females. No adverse effects on development of the offspring from birth to 6 weeks of age were noted, although one pup sacrificed at weaning and one male sacrificed at six weeks of age had congested salivary glands.

Assuming that effects seen in this preliminary study are representative of those that would occur in a comparable multiple generation study, a possible high level for the main study would appear to be less than 30000 ppm but greater than 10000 ppm, with a low level being less than 3000 ppm.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material:

Identification:	Glyphosate technical
Description:	White solid
Lot/Batch #:	206-Jak-25-1
Purity:	98.6%
Stability of test compound:	Stable for at least 5 years
Vehicle:	Plain diet
Test Animals:	
Species:	Rat
Strain:	Sprague-Dawley Crl:CD (SD) BR VAF/Plus strain
Source:	[redacted]
Age:	Approximately 8 - 10 weeks
Sex:	Females
Weight at dosing:	214 - 216 g
Acclimation period:	3 days
Diet/Food:	Labsure LAD No. 2, ad libitum
Water:	Tap water, ad libitum
Housing:	Prior to parturition in groups of five in suspended galvanised metal cages (Bowman ®) equipped with solid sides and back, wire mesh front, floor and top. On Day 20 of pregnancy the rats were re-housed in individual plastic breeding cages (North Kent Plastics, RM-2 type) for the birth and rearing of young. Suitable nesting material was provided. Following weaning, the selected pups were housed by sex, five to a cage. During the first week post weaning plastic cages were used; subsequently, the animals were transferred to metal (Bowman ®) cages.

Environmental conditions:	Temperature:	20 – 23.5 °C
	Humidity:	39 – 62%
	Air changes:	no reported
	Light regime: 12/12 hours	

B. STUDY DESIGN

In life dates: 1989-11-11 to 1990-01-12

Animal assignment and treatment:
In a dose-range-finding study for a subsequent two-generation reproduction study groups of 10 time-mated Sprague-Dawley rats received daily dietary doses of 0, 3000, 10000 and 30000 ppm glyphosate in diet from Day 3 of gestation through gestation and lactation to termination at the end of lactation. These concentrations were equivalent to glyphosate intakes during gestation ranging from 236 - 311, 799 - 1010 and 2515 -2789 mg/kg bw/day in the low, mid and high dose, respectively. Control animals received the base diet alone. All females were allowed to litter and rear their young to weaning, when 10 male and 10 female offspring per group were selected and reared on their respective diets to six weeks of age.

Diet preparation and analyses
The test material as supplied was weighed out and added to a weighed amount of sieved Labsure Laboratory Diet No. 2, and stirred by hand to give a premix of suitable strength. The dietary concentrations required were obtained from this premix by direct dilution with further quantities of diet, homogeneity being achieved by mixing in a rotary double cone blender. Fresh diet was prepared weekly and fed for no more than 7 days from date of preparation and was stored until use in labelled opaque bags.
Diets were not analysed for homogeneity, stability or achieved concentration of the test compound since satisfactory data concerning homogeneity and stability in rodent diet were already available to the Sponsor.

Clinical observations
All animals were regularly handled and observed daily for obvious changes or signs of reaction to treatment.

Body weight
All adult rats were weighed on Days 1, 3, 6, 10, 14, 17 and 20 of gestation and on Days 0, 7, 14 and 21 post partum.

Food consumption and compound intake
For F0 females, food consumption was measured on a cage basis from 'weigh day' to 'weigh day' up to re-housing on Day 20 of pregnancy.
After weaning, food consumption of selected offspring was measured from nominal Week 4 to termination.

Water consumption
Water consumption was measured daily on a cage basis from allocation to treatment groups to Day 20 of pregnancy.

Reproduction parameters
Pregnancy rate
Pregnancy rate was determined as the percentage of surviving females that were pregnant.

Pregnancy and parturition
The duration of gestation was recorded. Parturition was observed wherever possible.

Litter data
As soon as possible after parturition the young were counted, individually identified, sexed, weighed and examined for external abnormalities. Pups were weighed on Days 4, 8, 12 and 21 post-partum keeping nest

disturbance to a minimum, all litters were examined daily for dead and/or abnormal young. After weaning, body weights of selected offspring were recorded on nominal weeks 4, 5 and 6. Dead young were subjected to an autopsy and sexed.

Physical development
Using body weight at Day 21 post partum as a basis for selection, ten male and ten female pups per group from an appropriate spread of litters were retained for a further three weeks on treated diet.

Sacrifice and pathology
Approximately one week after birth of litters, apparently non-pregnant females were sacrificed, subjected to post mortem examination and their uteri immersed in a 10% solution of ammonium sulphide to reveal evidence of implantation.

On, or shortly after Day 21 post partum, parents and excess pups were sacrificed and examined externally and internally for abnormalities. Sex of the pups was confirmed by gonadal inspection. The uterus of each female which gave birth was visually inspected for implantation sites and the number of sites was recorded. The parotid salivary gland of the parent females was preserved and examined histologically for any microscopic changes.

On, or shortly after Week 6, all surviving Fl animals were sacrificed and subjected to macroscopic post mortem examination.

Statistics
Group mean values, except for food and water, were calculated using only the values of animals rearing young to weaning. As F0 animals were group-housed to Day 20, food and water intake were based on all live animals regardless of their pregnancy status.
All derived values (e.g. means, percentage, ratios) were calculated within each litter and the group values derived as a mean of the individual litter values. Statistical analyses were not performed in view of the small group size.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS AND Achieved Test item intake
Analysis of dose formulations was not performed in this dose-range finding study.
F0 females
Group mean achieved intakes of glyphosate during gestation in groups 2 to 4 ranged from 236 - 311, 799 - 1010 and 2515 -2789 mg/kg bw/day, respectively. As expected, as the weight of the animals increased the actual intake of glyphosate (expressed as mg/kg bw/day) generally declined. The 3-fold interval in fixed ppm, between the groups remains established when expressed as mg/kg bw/day.

F1 offspring
Group achieved intakes of glyphosate in Groups 2 to 4, recorded during Weeks 5 and 6, ranged from 368 - 390, 1291 - 1335 and 3918 - 4453 mg/kg/day for males and 355 - 402, 1191 - 1271 and 3961 - 4397 mg/kg/day for females, respectively.

B. MORTALITY
F0 females
There were two mortalities. One female at 3000 ppm was sacrificed on Day 22 post partum due to poor condition. Post mortem examination did not reveal any reason for the apparent dystocia. Since no similar mortalities were seen at higher levels, this death is considered not to be attributable to treatment.
A second animal at 30000 ppm was found dead on Day 21 post partum (Day 43 of study). Post mortem examination or signs prior to sacrifice did not reveal the cause of death. It cannot be finally concluded whether this mortality was due to treatment with the test item or not.

F1 offspring
No mortalities were noted from weaning until scheduled sacrifice at 6 weeks of age.

C. CLINICAL OBSERVATIONS
F0 females
Clinical signs associated with the test item included soft faeces and yellow stained sawdust (considered to be caused by the urine) in both cages of animals at 10000 and 30000 ppm. Onset of these signs was earlier at 30000 ppm than at 10000 ppm (soft faeces occurred immediately after dietary administration commenced at 30000 ppm but not until the third week post partum at 10000 ppm, yellow staining of the sawdust occurred on Day 26 post coitum at 30000 ppm but not until Day 28 post coitum at 10000 ppm), with signs still apparent in both groups at termination. There were no clinical signs at 3000 ppm considered to be attributable to the test item.

F1 offspring
Soft faeces were observed for all animals at 30000 ppm from Week 4 through to sacrifice at Week 6. No other clinical signs were observed at this or lower dosages.

Table 5.6.1-60 Dietary range finding study of glyphosate in pregnant rats and their juvenile offspring ([redacted], 1991): F0 adult performance and clinical signs

Clinical sign	Control (0 ppm)	Low (3000 ppm)	Mid (10000 ppm)	High (30000 ppm)
Performance (no. of females)				
Mated	10	10	10	10
Non-pregnant	1	0	0	1
Sacrificed/deceased	0	1	0	1+
Rearing young to weaning	9	9	10	9+
Clinical signs (no. of females affected)				
Soft faeces	-	-	10	10
Yellow stained sawdust	-	-	10	10
Pale extremities	-	1++	-	-
Hair loss/scabbing on face	2	-	-	-

+ animal died Day 21 post partum, litter reared to weaning
++ animal subsequently sacrificed

D. BODY WEIGHT
F0 females
At 30000 ppm, the rate of body weight gain following the initiation of treatment was reduced to Day 14 of pregnancy; thereafter, the rate of body weight gain to Day 20 of pregnancy was similar to controls, however, absolute parity with controls at Day 20 was not achieved. At both 3000 and 10000 ppm the pattern of body weight gain during pregnancy was essentially similar to controls throughout, although by Day 14 of pregnancy, body weights were slightly lower than controls.
During the first week of lactation the pattern of body weight change in all groups was similar. Thereafter, slight differences in the pattern of change were apparent to weaning at 10000 and 30000 ppm in so much as more weight loss occurred than in the control group. There were no further effects at 3000 ppm.

F1 offspring
For pup body weight development please refer to section H. Litter data. From weaning to termination, males at 30000 ppm had a reduced rate of weight gain. At lower dosages, and at all dosages among females, the rate of weight gain was comparable with controls, however, differences inherent at weaning were still present by Week 6.

Table 5.6.1-61 Dietary range finding study of glyphosate in pregnant rats and their juvenile offspring ([redacted], 1991): F0 females - body weights and body weight changes during gestation (Group mean values)

Dietary concentration (ppm)	No. of animals	Gestation Day					
		1	3	6	10	17	20
Body weights (g)							
0 (Control)	9	187.7	215.2	241.6	280.1	352.4	406.9
3000	9	186.8	216.4	246.0	277.2	343.6	393.9
10000	10	185.4	214.5	241.1	277.8	346.4	404.6
30000	9	187.0	215.8	236.0	266.0	330.0	385.6
		Body weight chance (g) relative to gestation Day 3					
0 (Control)	9	-27.6	0.0	26.3	64.9	137.2	191.7
3000	9	-29.7	0.0	29.6	60.8	127.1	177.4
10000	10	-29.1	0.0	26.6	63.3	131.9	190.1
30000	9	-28.8	0.0.	20.2	50.2	114.2	169.8

Table 5.6.1-62 Dietary range finding study of glyphosate in pregnant rats and their juvenile offspring ([redacted], 1991): F0 females - body weights and body weight changes during lactation (Group mean values)

Dietary concentration (ppm)	No. of animals	Lactation Day			
		0	7	14	21
Body weights (g)					
0 (Control)	9	315.4	339.1	344.6	335.0
3000	9	299.3	329.2	326.7	311.1
10000	10	309.8	338.0	330.1	322.5
30000	8+	292.5	321.0	300.0	286.9
		Body weight chance (g) relative to gestation Day 3			
0 (Control)	9	100.2	123.9	129.3	119.8
3000	9	82.9	112.8	110.2	94.7
10000	10	95.3	123.5	115.6	108.0
30000	8+	72.4	100.4	79.1	62.9

+ excludes one animal found dead Day 21 post partum

Table 5.6.1-63 Dietary range finding study of glyphosate in pregnant rats and their juvenile offspring ([redacted], 1991): F1 offspring - body weights and body weight changes Week 4 –to 6 (Group mean values)

Dietary concentration (ppm)	No. of animals	Nominal age (days)			
		21	28	35	42
Body weights (g)					
Males					
(Control)	10	50	95	156	226
3000	10	47	90	151	218
10000	10	44	86	143	210

Table 5.6.1-63 Dietary range finding study of glyphosate in pregnant rats and their juvenile offspring ([redacted], 1991): F1 offspring - body weights and body weight changes Week 4 – to 6 (Group mean values)

Dietary concentration (ppm)	No. of animals	Nominal age (days)			
		21	28	35	42
30000	10	31	66	115	169
		Females			
0 (Control)	10	48	89	137	175
3000	10	44	83	130	166
10000	10	42	80	129	167
30000	10	31	64	109	148

E. FOOD CONSUMPTION

F0 females

Apart from a slight reduction in food intake during the first three days of treatment at 30000 ppm, food intake of all treated groups was similar to concurrent controls throughout pregnancy.

Table 5.6.1-64 Dietary range finding study of glyphosate in pregnant rats and their juvenile offspring ([redacted], 1991): F0 females- food consumption during gestation (Group mean values)

Dietary concentration (ppm)	No. of animals	Gestation Days				
		3-5	6-9	10-13	14-16	17-19
Food consumption (g/rat/day)						
0 (Control)	10	23	26	27	28	30
3000	10	24	26	27	28	29
10000	10	23	26	26	28	30
30000	10	21	25	27	28	30

F1 offspring

Food consumption at 30000 ppm was lower than controls during Weeks 5 and 6 (males only). There were no other effects considered attributable to treatment for males or females at any dosage. In addition, the food conversion ratio for males at 30000 ppm was slightly greater when compared to controls (Week 6 only), indicating a slightly lower efficiency of food utilisation into body weight gain. There were no other effects on food conversion ratios.

Table 5.6.1-65 Dietary range finding study of glyphosate in pregnant rats and their juvenile offspring ([redacted], 1991): F1 offspring - food consumption in Weeks 5 and 6 (Group mean values)

Dietary concentration (ppm)	No. of animals	Week	
		5	6
Food consumption (g/rat/week)			
Males			
0 (Control)	10	106	168
3000	10	104	168

Table 5.6.1-65 Dietary range finding study of glyphosate in pregnant rats and their juvenile offspring ([redacted], 1991): F1 offspring - food consumption in Weeks 5 and 6 (Group mean values)

Dietary concentration (ppm)	No. of animals	Week	
		5	6
10000	10	104	165
30000	10	83	148
Females			
0 (Control)	10	91	132
3000	10	100	123
10000	10	87	132
30000	10	80	132

F. WATER CONSUMPTION

At 30000 ppm, slight increases in water intake during gestation were apparent towards the end of pregnancy when compared with concurrent control values. At 3000 and 10000 ppm, no adverse effects on water consumption were observed.

Table 5.6.1-66Dietary range finding study of glyphosate in pregnant rats and their juvenile offspring ([redacted], 1991): Water consumption during gestation (Group mean values)

Dietary concentration (ppm)	No. of animals	Gestation Days				
		3-5	6-9	10-13	14-16	17-19
Water consumption (g/rat/day)						
0 (Control)	10	32	35	37	41	44
3000	10	32	33	36	41	45
10000	10	28	31	34	37	42
30000	10	30	34	38	43	49

G. REPRODUCTIVE PARAMETERS

Pregnancy Rate and Duration of Gestation

The pregnancy rate was high, 90, 100 (incl. one ,dam that died on day 21 post partum), 100 and 90% in the control and the low, mid and high dose group, respectively. The duration of pregnancy was similar in all groups and not adversely affected by treatment with glyphosate.

Mating Performance, Fertility and Gestation

There were no treatment-related effects on mating performance, fertility and gestation length for both F0 and F1 generation animals.

Table 5.6.1-67 Dietary range finding study of glyphosate in pregnant rats and their juvenile offspring ([redacted], 1991): performance and gestation parameters

	Control (0 ppm)	Low (3000 ppm)	Mid (10000 ppm)	High (30000 ppm)
Performance (no. of females)				
Mated	10	10	10	10

Table 5.6.1-67 Dietary range finding study of glyphosate in pregnant rats and their juvenile offspring ([redacted], 1991): performance and gestation parameters

	Control (0 ppm)	Low (3000 ppm)	Mid (10000 ppm)	High (30000 ppm)
Non-pregnant	1	-	-	1
Sacrificed/deceased	0	1+	0	1++
Pregnant	9	9	10	9
Rearing young to weaning	9	9	10	9+
Mean duration of gestation (days)	21.3	21.6	21.4	21.3
No. of implantation sites	13.8	15.0	15.0	15.3
Pre-birth loss (%)	3.6	5.3	5.8	5.0

+ sacrificed on Day 22 of pregnancy due to poor condition
++animal died Day 21 post partum, litter reared to weaning

H. LITTER DATA

Size and Viability

A total of 9, 9, 10 and 9 females reared litters to weaning in Groups 1 to 4, respectively (including one female found dead at weaning in Group 4).

The implantation rates in all treated groups were higher than the controls. Since pup losses, both pre-birth and from birth to weaning, were generally similar among the groups, litter size of all treated groups was, as a consequence, generally greater than controls throughout weaning. These findings are not, however, considered to be an adverse effect of treatment. Glyphosate did not selectively affect pups of one sex since, in all groups, sex ratios at birth and weaning were similar. The mean sex ratio did not reveal any treatment-related effect. In the mid dose group, a slightly higher percentage of male pups was noted both at birth and on day 21 post partum (60.5% compared to 46.7% in the control). In the absence of a dose-relationship this finding was considered incidental.

Growth and Development

At 30000 ppm, mean pup weight was reduced at birth and diverged further away from controls through to Day 21 when mean pup weight at this level was only 62% of the controls.

At 3000 and 10000 ppm, mean pup weight was initially (at birth and on day 4 post partum) similar to the controls but diverged below control values to an extent that on Day 21 post partum mean pup weight at 3000 and 10000 ppm was 9% and 13% lower than the corresponding control value. These differences, however, can probably be attributed to the pivotal association between increased litter size and mean pup weight and, at this stage, no conclusive treatment-related effects can be established.

Table 5.6.1-68 Dietary range finding study of glyphosate in pregnant rats and their juvenile offspring ([redacted], 1991): Litter data

	Control (0 ppm)	Low (3000 ppm)	Mid (10000 ppm)	High (30000 ppm)
Mean litter size				
at birth	13.2	14.2	14.1	14.6
at day 4 post partum	12.2	13.8	14.0	14.1
at day 8 post partum	12.1	13.7	13.6	13.8
at day 21 post partum	12.1	13.6	13.5	13.2
Mean pup weight (g)				
at birth	6.6	6.6	6.6	6.3
at day 4 post partum	10.1	9.9	9.9	9.3
at day 8 post partum	17.6	16.3	16.6	14.8

Table 5.6.1-68 Dietary range finding study of glyphosate in pregnant rats and their juvenile offspring ([redacted], 1991): Litter data

	Control (0 ppm)	Low (3000 ppm)	Mid (10000 ppm)	High (30000 ppm)
at day 21 post partum	49.3	45.1	43.1	30.8
Percentage male pups(%)				
at birth	46.7	50.0	60.5	47.4
At day 21 post partum	48.0	50.2	61.5	47.5

I. PATHOLOGY

Necropsy

Watery and/or dark contents in the gastro-intestinal tract were observed in 0, 2, 7 and 8 animals in Groups 1 to 4 respectively. Distended and/or congested stomach was seen in 0, 2, 5 and 4 animals and distended caecum in 0, 0, 0 and 4 animals in Groups 1 to 4 respectively. These findings generally followed the trend noted in the clinical signs observed. Macroscopic changes to the salivary glands (enlarged/firm/congested/swollen) were observed in 0, 2, 6 and 8 animals respectively in Groups 1 to 4. There were no other macroscopic changes considered to be related to treatment.

Table 5.6.1-69 Dietary range finding study of glyphosate in pregnant rats and their juvenile offspring ([redacted], 1991): F0 females - necropsy findings

Clinical sign	Control (0 ppm)	Low (3000 ppm)	Mid (10000 ppm)	High (30000 ppm)
Necropsy findings				
Fur, ventral surface: yellow stained	-	-	1	-
Gasto-intestinal tract: contents watery and/or dark	-	2	7	8
Stomach: distended and/or congested	-	2	5	4
Stomach: contents watery	1	2	-	1
Caecum: distended	-	-	-	4
Caecum: contents watery	1	-	-	1
Salivary glands: enlarged /firm/congested/swollen	-	2	6	8
Lungs: multiple pale subpleural foci	1	3	3	1
Uterus: severe fluid distension	1*	-	-	-

* animal not pregnant

There were no toxicologically significant intergroup differences detected for the brain, spleen or thymus for offspring of either sex from either generation. Furthermore, there were no differences in uterus weights for treated females from either generation when compared to controls.

F1 offspring retained to 6 weeks of age

Post mortem examination revealed swollen/enlarged parotid salivary glands in 5/10 males and 2/10 females at 30000 ppm, and in l/10 males at 3000 ppm. Soft gastro-intestinal contents were noted in 7/10 males and 9/10 females at 30000 ppm; grey/blue contents of the jejunum were noted in 2/10 females at this dosage. Neither the incidence nor the type of other macroscopic changes were considered to be attributable to treatment.

Histopathology
F0 females
A dose-related incidence and degree of granular basophilic cytoplasm of acinar cells of the parotid glands was seen with 0, 2, 0 and 0 animals showing minimal effects, 0, 0, 2 and 0 animals showing moderate effects and 0, 0, 8 and 9 animals showing marked effects in Groups 1 to 4 respectively. This change was associated with hypertrophy of acinar cells with 0, 2, 2 and 0 animals with minimal hypertrophy and 0, 0, 8 and 9 animals in Groups 1 to 4 respectively with moderate hypertrophy of the acinar cells. Prominent mitoses were also seen in 2 animals at 30000 ppm, but not at lower treatment levels or the controls.

Table 5.6.1-70 Dietary range finding study of glyphosate in pregnant rats and their juvenile offspring ([redacted], 1991): F0 females - microscopic findings

Microscopic findings	Control (0 ppm)	Low (3000 ppm)	Mid (10000 ppm)	High (30000 ppm)
No. of animals examined	9	9	10	9
Parotid salivary glands				
Granular basophilic cytoplasm of acinar cells:				
Minimal	-	2	-	-
Moderate	-	-	2	-
Marked	-	-	8	9
Hypertrophy of acinar cells:				
Minimal	-	2	2	-
Moderate	-	-	8	9
Prominent mitosis in acinar cells	-	-	-	2

III. CONCLUSIONS

Study conclusion:
Assuming that effects seen in this preliminary study are representative of those that would occur in a comparable multiple generation study, a possible high level for the main study would appear to be less than 30000 ppm but greater than 10000 ppm, with a low level being less than 3000 ppm.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Dietary administration of glyphosate to pregnant dams at 30000 ppm resulted in a single death, clinical signs and body weight effects; at 10000 ppm resulted in clinical signs and body weight effects and at 3000 ppm slightly lower body weight gain by day 14 of pregnancy. Findings in F1 pups at 30000 ppm resulted in clinical signs, reduced food intake and body weight effects. While dose dependent changes were observed in the parotid glands of the parents and offspring these findings are considered to be an adaptive response due to oral irritation from the ingestion of glyphosate, an organic acid, in the diet and as such not considered to be toxicologically relevant. The doses selected for the definitive study were 0, 1000, 3000 and 10000 ppm.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	**CA 5.6.1/010**
Report author	[redacted]
Report year	1990
Report title	Two Generation Reproduction Feeding Study with Glyphosate in Sprague-Dawley Rats
Report No	[redacted]-10387
Document No	M-643937-01-1
Guidelines followed in study	Not stated, but in general accordance with OECD 416 (1983)
Deviations from current test guideline (OECD 416, 2001)	No data on food efficiency; no details on fertility indices, number of live births and post-implantation loss, number of pups with grossly visible abnormalities, no sperm analysis, no determination of oestrus cycle length, no determination of physical or sexual development landmarks, most parental animal organ weights missing, several offspring organ weights missing
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

30 Sprague-Dawley rats sex/dose group (F0 and F1a generation) were fed daily with glyphosate at concentrations of 2000, 10000 and 30000 ppm (corresponding to 132-140, 666-711, 1983-2230 mg/kg bw/day for males and 160-163, 777-804, 2322-2536 mg/kg bw/day for females (calculated from F0 and F1a adults)) through two generations for approximately 11 (F0-generation) and 14 weeks (F1a-generation), respectively. Animals of the F1a-generation were mated twice to produce the F2a and F2b-generations.
Pairing of animals within each dose group was undertaken on a 1:1 basis. At weaning of offspring from the F0 mating phase, groups of 30 male and 30 female offspring from each dose group were selected to form the F1a generation. The remaining surviving F0 females and unselected offspring were terminated at Day 21 post partum. Males were sacrificed after completion of mating phase. The offspring selected for the F1a generation were dosed for approximately 14 weeks and then paired within each dose group to produce the F2a and F2b litters. At weaning of the F2 litters all surviving adults and their offspring were killed, whereas F1 males were sacrificed after completion of mating phase.

Clinical observations for mortality and moribundity were performed twice daily, and detailed observations for signs of toxicity once weekly, when diets were prepared. Body weights were weekly determined for adults and on day 0, 4 (pre- and post-culling), 14 and 21 of lactation for offspring. Food consumption was determined weekly for adults and on days 0-7, 7-4 and 14-21 of gestation and lactation.
All animals at termination and which died, or were sacrificed moribund, were subjected to a gross necropsy. Histopathological evaluation of selected tissues was performed for control and highest dose level animals.

No significant changes concerning mortality, mating, fertility, organ weights, gross pathology and histopathology were observed. In the high dose group at 30000 ppm, soft stool in adults were observed in both sexes, being accompanied by reduced body weights of adult animals (about 8%) and pups (about 10%) when compared to controls. This effect was assumed to be treatment-related. Furthermore, decreased pup weights were observed when pups began supplementing their milk with the glyphosate-containing food. In

the 10000 ppm dose group, decreased pup body weights were observed, too, but the effect was less pronounced and occurred not in both sexes of all generations.
At the highest dose level of 30000 ppm a slightly, and statistically not significant, reduced average litter size was observed in the F0 and to a lesser degree in the F1 dams. A reduction was not noted when F1 animals were re-mated and treatment-relation was considered to be equivocal.

I. MATERIALS AND METHODS

A. Materials

Test Material:

Identification:	Glyphosate
Description:	White powder
Lot/Batch #:	XLI-203
Purity:	97.67%
Stability of test compound:	Not reported
Vehicle:	Plain diet
Test Animals:	
Species:	Albino Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	Approximately 7 weeks (F0 adults)
Sex:	Males and females
Weight at study start (F0):	Males: 165 – 207.6 g; Females: 135.6 – 162.7 g
Acclimation period:	No data
Diet/Food:	Purina Mills Certified RODENT CHOW No. 5002, ad libitum
Water:	St. Louis public water, ad libitum
Housing:	Housing for premating and gestation (day 0 through 13): individual suspended stainless steel cages over paper bedding; during mating females were housed in the male´s cages Housing for gestation and lactation (from day 14 of gestation through lactation): females housed in double wide cages with solid bottoms and wood shavings for bedding
Environmental conditions:	Temperature: 18 - 26°C Humidity: 40 - 70% 12 hours light/dark cycle

B. STUDY DESIGN

Animal assignment and treatment:

In a two-generation reproduction study, groups of 30 Sprague-Dawley rats per sex of the F0 generation received daily dietary doses of 2000, 10000 and 30000 ppm (corresponding to 132-140, 666-711, 1983-2230 mg/kg bw/day for males and 160-163, 777-804, 2322-2536 mg/kg bw/day for females (calculated from F0 and F1a adults)) glyphosate in the diet. After 11 weeks of treatment pairing of animals within each dose group was undertaken on a one male: one female basis in a male´s cage for 7 days, to produce the F1a litters. If there was no evidence of mating after 7 days (copulatory plug, or vaginal smear), the female was co-housed with a male having recorded copulatory activity for additional 7 days, or until copulatory evidence was found. For F0 and F1 generation, gestation day 0 was set on the day on which copulatory evidence was found and lactation day 0 the day on which delivery of pups was completed.

At weaning of offspring from the F0 mating phase, groups of 30 males and 30 females offspring from each dose group were selected to form the F1 generation and the mating procedure for F1a adults was conducted in the same way except modifications to exclude sibling matings. The remaining surviving F0 females and unselected offspring were terminated at Day 21 post partum. F0 males were killed at completion of mating phase. The offspring selected for the F1a generation were dosed for approximately 14 weeks and then mated to produce the F2a and F2b litters (a second mating of the F1 generation was performed due to reduced litter sizes in pups from F0 of the 30000 ppm dose group). At weaning of the F2 litters all surviving adults and their offspring were killed, whereas F1 males were sacrificed after completion of mating phase.

Diet preparation and analyses
Approximately each week (except in one week when diets were prepared twice the same week and not during the following week) a known amount of glyphosate was mixed with the diet for 10 minutes in a HOBART HCM-450 mixing machine to achieve a batch size of 18 kilograms at each dose level.
The stability and homogeneity of the test substance in the diet were determined by liquid chromatography of duplicate samples from top, middle and bottom of mixer from the lowest and highest dietary levels stored in an open container at ambient temperature for 6 and 14 days or when frozen in a closed container for 35 days.

Clinical observations
A detailed observation for signs of toxicity was performed once weekly for the adult animals and for the offspring on days of weight measurement.

Body weight
Adult male animals of the F0 and F1a generation were individually weighted once weekly. The same was done for the female animals until copulation was confirmed, then females were weighted on days 0, 7, 14, and 21 of gestation and lactation.
Offspring was weighted on days 0, 4 (pre- and post-culling), 14 and 21 of lactation (except F1a males approximately two weeks prior to sacrifice and F1a females for approximately three weeks prior to mating for the F2b generation).

Food consumption and compound intake
Food consumption was recorded weekly for F0 and F1a adult males, except during mating, and also weekly for adult F0 and F1a female animals until mating. After confirmed copulation, the maternal food consumption was monitored for days 0-7, 7-14 and 14-21 of gestation and lactation, but it was not determined for females approximately three weeks prior to mating for the F2b generation and generally not for female animals that did not became pregnant.
Food conversion efficiency was not calculated.

Water consumption
No data on water consumption was given in the report.

Pregnancy and parturition
Data on total paired females, females with confirmed copulation/total paired, pregnant/total paired, pregnant/ confirmed copulation was monitored as well as precoital (for pregnant animals) and gestational length in days. For males, the following items of interest were given: males with confirmed copulation/total paired, males impregnating females/total paired and males impregnating females/confirmed copulation.

Litter data
The following litter data were recorded: Litter size, dead pups/litter, mean pup weight (on day 0, 4 (pre-/post-cull), 14, 21) and survival (%).

Physical and sexual development
No details on physical and sexual development of the offspring was reported.

Sacrifice and pathology
All adult animals, which died or were sacrificed in moribund condition were subjected to a gross necropsy and selected tissues were sampled. Pups found dead or culled pups also underwent gross pathology, but no tissues were saved. No organ weights were determined.

All F1a weanlings, that were not selected for mating, F2a and F2b weanling pups as well as females which had littered on or after 21 of lactation were sacrificed as scheduled. Non-pregnant adult females were killed at least 5 days after last expected parturition date and adult males after completion of the mating phase.

External and internal cavities of the dead animals were opened and the organs were examined in place and then removed. Hollow organs were opened and examined. The following organs of F0 and F1a males and females from each dose group that were sacrificed at the end of the study sampled, were weighed: ovaries and testes with epididymides. When present, the following organs from the F0 and F1a adults (unscheduled deaths and scheduled sacrifice) were retained: kidneys, ovaries, prostate, seminal vesicle, skin/mammary gland, testes, epididymis, uterus/vagina and gross lesions (pituitary retained for F1a adults only). Tissues from the F1a weanlings were saved at the discretion of the necropsist. From the F2a and F2b weanlings, which where sacrificed at schedule, the kidneys of 1 pup per sex and litter were saved.

A histopathological examination was performed on all sampled tissues from all F0 and F1 control and high-dose animals, and on one F2b weanling/sex/litter (selected at random) as well as on all retained tissues from unscheduled adult deaths. For preparation, fixed tissues were washed, dehydrated, embedded in paraffin, sectioned, stained with haematoxylin and eosin and examinated under light microscopy.

Statistics
Dunnett´s multiple comparison test (two-tailed) was used to detect statistically significant differences in adult body weights and food consumption between treated animals and their respective control.

Terminal body weights, maternal body weights and food consumption during gestation and lactation, pup weights, precoital length, gestational length, litter size, dead pups/litter, pup survival, absolute organ weights and organ/body weight ratios were evaluated by decision-tree statistical analyses procedures which, depending on the results of tests for normality and homogeneity of variance [Bartlett's Test], were chosed either parametric [Dunnett's Test and Linear Regression] or nonparametric [Kruskal-Wallis, Donckheere's and/or Mann-Whitney Tests] routines to detect differences and analysed for trend.

The uncorrected Chi-Square test was used to examine fertility indices, e.g. females/males with confirmed copulation/total paired, pregnant/confirmed copulation (females) and males impregnating females/total paired as well as males impregnating females/confirmed copulation.

Fisher´s Exact test with Bonferroni Inequality Procedure was used for statistical analysis of microscopic lesions.

Other statistical routines used for some data included: Bartlett's Test to evaluate homogeneity of variances, Analysis of Variance to determine if the sample (group) means could be considered as an estimate of a common population, and Grubb's Test to detect outliers.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS
The analysis of the test substance stability conducted over the time span of the study indicated that the test material was stable in the diet and homogeneity was adequate for study use. The stability of the test material in the diet was demonstrated at the low and high dose level, stored in an open container at ambient temperature for 6 and 14 days, or when frozen in a closed container for 35 days.
Analysis for achieved concentrations, demonstrated that the test substance-levels in the prepared diet were

in the range of 95 to 96.7% of the nominal concentration.

B.TEST COMPOUND INTAKE
The group mean achieved dosages are summarised in the table below.

Table 5.6.1-71 Glyphosate technical: Two generation reproduction feeding study with glyphosate in Sprague-Dawley rats ([redacted], 1990): Group mean compound intake levels during pre-mating periods of F0 and F1

Dose group	Dietary concentration (ppm)	Mean daily test substance intake (mg/kg bw/day)*			
		F0		F1	
		Males	Females	Males	Females
control	0	0	0	0	0
low	2000	132	160	140	163
mid	10000	666	777	711	804
high	30000	1983	2322	2320	2536

* based on actual food intake and body weight data; values were calculated in the report

C. MORTALITY
There were no treatment-related mortalities.
One female of the F0 generation at 10000 ppm died early in the study. This animal was never mated and at necropsy changes in bladder in kidneys were observed. One male animal in each of the 2000 and 30000 ppm dose groups (F1 generation) died. Necropsy of these animals noted thymus and respiratory changes. One female animal of the F1 generation (2000 ppm) was sacrificed in extremis and another female (same generation, same dose group) died. Kidney changes and retained foetus; pups in uterus and stomach changes, respectively, were observed in these two females.
Concerning the offspring, dead pup counts at day 0 and survival of all F1a, F2a and F2b treated pups were not adversely affected when compared to the controls.

D. CLINICAL OBSERVATIONS
The only clinical signs that were related to the test substance were soft stool in the animals of the 30000 ppm dose group. Other clinical signs, such as red ocular discharge/laboured respiration/overgrown teeth/piloerection/abrasions/emaciated and dehydrated appearance/misuse of limbs/focal loss of hair/swollen feet, occurred sporadically and were not considered to be treatment-related.

E. BODY WEIGHT
At the highest exposure level of 30000 ppm, reduced body weights were observed in both sexes and in the F0 and F1 generation. In the F0 generation, body weights gains gradually decreased over time to approximately 8% less than controls prior to mating. F0/F1 weaning animals were lighter in weight than their corresponding controls and maintained that weight difference (approx. 10% less than control) until the end of the study
No test-substance related body weight effects were observed in the adult animals of the 2000 and 10000 ppm dose groups prior to mating.
During gestation and lactation, maternal body weights in the highest dose group tended to remain lower than in controls, but the animals showed a rather greater body weight gain than the controls during gestation and lactation so that by the end of lactation, body weights were approximately the same as those of the controls.
Terminal body weights were significantly decreased for both sexes at the highest exposure level.

Table 5.6.1-72 Glyphosate technical: Two generation reproduction feeding study with glyphosate in Sprague-Dawley rats ([redacted], 1990): Selected mean group body weights

Dietary concentration (ppm)	No. of animals		Mean group body weight (g) at Day					
			0	72	T#	0	72	T#
			F0 Generation					
			Males			Females		
0 (Control)	30	mean	187.9	494.6	549.56	150.5	276.7	296.31
		SD	11.65	34.86	46.76	6.86	23.85	23.63
2000	30	mean	188.1	497.6	550.19	150.5	272.6	290.64
		SD	11.35	49.87	80.72	7.03	22.86	19.50
10000	30	mean	188.1	484.4	539	150.2	273	290.71
		SD	11.57	42.13	58.13	7.04	27.92	25.35
30000	30	mean	188	455.8**	503.51**	150.3	253.8**	265.91
		SD	11.56	46.46	45.66	7.06	18.46	15.44
			F1 Generation					
			129	219	T#	128	219	T#
0 (Control)	30	mean	118.3	534.7	625.04	99.8	285.8	316.21
		SD	26.11	38.84	53.11	17.44	27.63	37.37
2000	30	mean	115.2	540.3	632.14	96.7	282.1	313.74
		SD	16.2	44.9	74.57	11.47	24.5	30.53
10000	30	mean	114.8	514.1	590.98	97.1	275.9	312.36
		SD	17.42	58.31	70.06	14.18	20.55	26.71
30000	30	mean	104.9*	483.4**	543.40**	88.8*	253.7**	284.72**
		SD	19.79	41.32	58.12	16.32	19.56	18.04

*: Dunnett´s test (two-tailed) indicates statistically significant difference ($p<0.05$)
**: Dunnett´s test (two-tailed) indicates statistically significant difference ($p<0.01$)
#T: Termination

Table 5.6.1-73Glyphosate technical: Two generation reproduction feeding study with glyphosate in Sprague-Dawley rats ([redacted], 1990): Mean maternal body weights during gestation

Dietary concentration (ppm)	No. of animals		Mean group body weight (g) at Day (Gestation)			
			0	7	14	21
			F0 Generation			
0 (Control)	24	mean	274	301.83	324.41	398.26
		SD	24.26	24.58	22.85	26.12
2000	29	mean	272.72	297.33	319.90	392.86
		SD	20.52	21.71	19.84	24.28
10000	28	mean	271.80	299.22	323.43	395.08
		SD	24.12	26.40	28.44	25.87
30000	28	mean	255.05**	282.44**	305.83**	375**
		SD	16.49	16.27	17.44	24.70
			F1 Generation (First Mating)			
			0	7	14	21
0 (Control)	24	mean	285.29	308.95	328.70	392.56
		SD	25.48	26.58	29.18	36.19
2000	29	mean	278.65	304.40	324.15	383.45
		SD	23.42	23.48	25.06	28.18
10000	28	mean	268.89*	297.23	319.08	382.71
		SD	19.24	18.81	19.27	21.77
30000	28	mean	251.30**	276.28**	299.48**	360.46**
		SD	17.42	18.92	19.29	33.31
			F1 Generation (Second Mating)			
			0	7	14	21

Table 5.6.1-73Glyphosate technical: Two generation reproduction feeding study with glyphosate in Sprague-Dawley rats ([redacted], 1990): Mean maternal body weights during gestation

Dietary concentration (ppm)	No. of animals		Mean group body weight (g) at Day (Gestation) 0	7	14	21
0 (Control)	24	mean	324.22	340.99	363.44	428.99
		SD	23.11	27.81	27.98	36.87
2000	29	mean	315.21	338.27	360.35	426.88
		SD	26.06	28.67	28.39	33.67
10000	28	mean	305.27*	333.66	357.50	428.51
		SD	20.26	22.45	24.49	26.17
30000	28	mean	281.46**	308.92**	330.95**	393.67**
		SD	17.79	22.19	22.36	34.88

*: Dunnett´s test (two-tailed) indicates statistically significant difference (p<0.05)
**: Dunnett´s test (two-tailed) indicates statistically significant difference (p<0.01)

Table 5.6.1-74Glyphosate technical: Two generation reproduction feeding study with glyphosate in Sprague-Dawley rats ([redacted], 1990): Mean maternal body weights during lactation

Dietary concentration (ppm)	No. of animals		Mean group body weight (g) at Day (Lactation) 0	7	14	21
			F0 Generation			
0 (Control)	24	mean	299.96	319.59	317.33	313.39
		SD	23.21	23.58	28.96	20.01
2000	29	mean	297.48	317.91	314.53	313.96
		SD	21.10	18.66	25.22	16.63
10000	28	mean	298.78	315.15	312.41	319.10
		SD	20.81	22.04	22.94	18.61
30000	28	mean	285.84*	307.64	304.75	316.68
		SD	13.91	12.48	20.68	15.43
			F1 Generation (First Mating)			
			0	7	14	21
0 (Control)	24	mean	299.29	313.60	337.68	313.49
		SD	27.02	26.12	25.31	21.38
2000	29	mean	295.16	308.28	332.10	314.69
		SD	23.58	22.56	23.92	23.95
10000	28	mean	296.63	310.80	328.29	313.14
		SD	19.01	18.64	18.33	14.06
30000	28	mean	277.91**	289.88**	315.88**	306.15
		SD	17.89	17.23	17.47	20.18
			F1 Generation (Second Mating)			
			0	7	14	21
0 (Control)	24	mean	342.78	343.21	353.34	337.16
		SD	32.46	27.11	21.15	17.22
2000	29	mean	340.16	336.62	348.40	331.96
		SD	28.54	16.11	25.89	20.67
10000	28	mean	333.80	342.41	352.70	334.56
		SD	23.35	26.93	20.43	13.82
30000	28	mean	312.39**	324.09*	337.08	329.95
		SD	23.73	20.50	19.09	18.41

*: Dunnett´s test (two-tailed) indicates statistically significant difference (p<0.05)
**: Dunnett´s test (two-tailed) indicates statistically significant difference (p<0.01)

F. FOOD CONSUMPTION

Overall, food intake was not notably affected during the study.
All animals of the 30000 ppm dose group consumed about 1 to 2 grams/day less than controls. This effect was most pronounced in the first week of exposure and also observed in the F0 dams. Subsequent dams (F1 first and second matings) tended to eat similar or larger amounts of the diet than controls.
No effects on food consumption were observed in the animals of the 2000 and 10000 ppm dose groups.

G. REPRODUCTIVE PARAMETERS

Mating Performance, Fertility, Gestation and Lactation

No effects on mating and fertility rates were observed in the F0 and F1 dams when compared to controls and no effects were observed on precoital length or length of gestation at any treatment level.

Table 5.6.1-75Glyphosate technical: Two generation reproduction feeding study with glyphosate in Sprague-Dawley rats ([redacted], 1990): Mating, fertility and gestation parameters (F0).

	Dietary concentration (ppm)			
	0	**2000**	**10000**	**30000**
# of females	30	30	29	30
Females with confirmed copulation /Total paired	96.7%	100%	100%	100%
Pregnant total/Paired	80%	98.7%	96.6%	93.3%
Mean precoital time, days (% of control)	3.58	3.41 (95%)	3.07 (86%)	3.74 (104%)
# of pregnant animals/total number	24/30	27/30	28/29	27/30
Males with confirmed copulation/total paired	86.7%	93.3%	93.1%	90.0%
Males impregnating females/total paired	70.0%	90.0%	89.7%	83.3%
Gestational length (days) (% of control)	22.3	22.2 (100%)	22.5 (101%)	22.3 (100%)

Table 5.6.1-76Glyphosate technical: Two generation reproduction feeding study with glyphosate in Sprague-Dawley rats ([redacted], 1990): Mating, fertility and gestation parameters (F1A First Mating).

	Dietary concentration (ppm)			
	0	**2000**	**10000**	**30000**
# of females	30	30	30	30
Females with confirmed copulation /Total paired	100%	93.3%	98.7%	98.7%
Pregnant total/Paired	93.3%	80.0%	80.0%	86.7%
Mean precoital time, days (% of control)	2.84	3.13 (110%)	3.61 (127%)	3.17 (112%)
# of pregnant animals/total number (% of control)	25/30	24/30	23/30	24/30
Males with confirmed copulation/total paired	93.3%	86.7%	83.3%	83.3%
Males impregnating femals/total paired	90.0%	73.3%	76.7%	80.0%
Gestational length (days) (% of control)	22.4	22.6 (101%)	22.6 (101%)	22.6 (101%)

Table 5.6.1-77Glyphosate technical: Two generation reproduction feeding study with glyphosate in Sprague-Dawley rats ([redacted], 1990): Mating, fertility and gestation parameters (F1B Second Mating).

	Dietary concentration (ppm)			
	0	2000	10000	30000
# of females	**30**	**30**	**30**	**30**
Females with confirmed copulation /Total paired	83.3%	83.3%	80.0%	86.7%
Pregnant total/Paired	53.3%	70.0%	83.3%	83.3%
Mean precoital time, days (% of control)	3.69	3.20 (87%)	3.05 (83%)	2.45 (67%)
# of pregnant animals/total number (% of control)	16/30	20/30	19/30	22/30
Males with confirmed copulation/total paired	70.0%	69.0%	70.0%	80.0%
Males impregnating femals/total paired	46.7%	58.8%	60.0%	76.7%
Gestational length (days) (% of control)	22.4	22.6 (101%)	22.4 (100%)	22.5 (100%)

H. LITTER DATA

Size and Viability

Day 0 dead pup counts among treated groups were comparable to the control group for all three litters of pups (F1a, F2a and F2b generation).
A slight reduction in the average litter size was observed in the F0 dams of the 30000 ppm dose group. This effect was less pronounced in animals after the first F1 mating. Although the difference was not statistically significant and not accompanied by an increase in dead pups/litter, a treatment-related effect could not be excluded. Therefore a second mating of the F1a adults was performed. In the resulting F2b generation, no dose-related decrease in litter size was observed.

Growth and Development

Birth weights and initial growth rate for pups from the treated dams compared well to the ones of the control, except the pups of the 30000 ppm dose group had reduced body weight gains on day 21 of lactation (more than 10% difference to controls). The effect was present earlier in the F1 matings (day 14). This effect was explained by the titrated uptake of the test substance-containing diet at the end of lactation. In the mid dose group, slight and transient decreases in pups body weight gains were observed compared with the control. These effects were not evident in both sexes from all generations and therefore regarded of questionable toxicological significance. Pup survival during the postnatal period was not affected.

Table 5.6.1-78 Glyphosate technical Two generation reproduction feeding study with glyphosate in Sprague-Dawley rats ([redacted], 1990): Mean pup weights

Dietary concentration (ppm)	No. of litters^c		Mean group body weight (g) at Day			
			0	21	0	21
			Males		Females	
			F0 Generation			
0 (Control)	24	mean	6.28	53.39	6.96	50.80
		SD	0.49	3.90	0.52	4.39
2000	29	mean	6.27	51.82	6.91	49.47
		SD	0.48	5.26	0.48	5.05
10000	28	mean	6.43	50.42*	6.15	49.16

		SD	0.47	3.66	0.50	3.12
30000	28	mean	6.47	46.30**	6.12	44.99**
		SD	0.62	4.09	0.59	4.34
			F1A Generation (First Mating)			
0 (Control)	28	mean	6.33	55.11	5.95	51.93
		SD	0.60	5.64	0.55	5.07
2000	23	mean	6.20	52.47	5.90	51.42
		SD	0.76	9.15	0.70	4.08
10000	22	mean	6.32	51.53*	5.98	48.49*
		SD	0.74	7.35	0.64	5.93
30000	26	mean	6.50	47.29**	6.05	44.41**
		SD	0.84	4.62	0.74	4.90
			F1B Generation (Second Mating)			
0 (Control)	16	mean	6.48	55.03	6.04	49.35
		SD	0.75	6.38	0.63	10.96
2000	18	mean	6.17	52.74	5.86	50.73
		SD	0.74	6.12	0.83	5.91
10000	17	mean	6.36	52.29	5.92	49.48
		SD	0.52	3.35	0.47	2.52
30000	24	mean	6.51	44.43**	6.04	43.10**
		SD	0.63	6.86	0.55	3.81

c: Combined sexes

Table 5.6.1-79 Glyphosate technical: Two generation reproduction feeding study with glyphosate in Sprague-Dawley rats ([redacted], 1990): Pup survival data

Dietary concentration (ppm)	No. of litters		Combined (m/f) survival rates (%) for postnatal days		
			0-4	4-14	4-21
			F0 Generation		
0 (Control)	24	mean	96.4	99.0	99.0
		SD	9.64	3.53	3.53
2000	29	mean	97.6	99.6	99.6
		SD	10.41	2.32	2.32
10000	28	mean	99.4*	99.6	99.6
		SD	3.34	2.36	2.36
30000	28	mean	100.0**	98.4	98.4
		SD	0.00	6.65	6.65
			F1A Generation (First Mating)		
0 (Control)	28	mean	96.7	100.0	100.0
		SD	5.77	0.00	0.00
2000	23	mean	90.9	96.7	96.7
		SD	22.62	15.64	15.64
10000	24	mean	96.2	99.5	99.5
		SD	7.20	2.55	2.55
30000	26	mean	99.4	100.0	99.5
		SD	2.18	0.00	2.45
			F1 Generation (Second Mating)		
0 (Control)	18	mean	98.8	100.0	100.0
		SD	2.60	0.00	0.00
2000	19	mean	91.1	99.3	99.3
		SD	24.19	2.95	2.95
10000	19	mean	96.5	94.1	94.1
		SD	12.16	22.96	22.96
30000	23	mean	96.9	100.0	100.0

Table 5.6.1-79 Glyphosate technical: Two generation reproduction feeding study with glyphosate in Sprague-Dawley rats ([redacted], 1990): Pup survival data

Dietary concentration (ppm)	No. of litters		Combined (m/f) survival rates (%) for postnatal days		
			0-4	4-14	4-21
		SD	11.67	0.00	0.00

Clinical signs
No clinical signs were observed in the offspring of treated animals.

I. PATHOLOGY
Necropsy
There were no toxicologically significant macroscopic gross lesions attributed to the test chemical administration.

Organ weights
There were no statistically significant organ weight changes, except a slight increase in testes to body weight ratios in F1a adults of the 30000 ppm dose group. This effect was attributed to their lower terminal body weight.

Histopathology
No treatment-related changes were detected.

III. CONCLUSIONS

Study conclusion:
The oral administration of glyphosate to rats via diet at a dose levels of 2000, 10000 and 30000 ppm for two successive generations resulted in possible treatment-related changes at the maximum dose of 30000 ppm. A high incidence of soft stools in adults was accompanied by consistent reduction of body weight gains of adults and pups at this dose level. Decreases in body weight gains of the pups obviously occurred at the end of lactation, with the beginning of consuming the test substance-containing diet. Furthermore, slightly but not statistically significant reduced average litter size was noted in F0 dams of the 30000 ppm dose group at first mating. This effect was not observed in the litters of the second mating. Therefore the NOAEL was considered to be 10000 ppm for adult toxicity for both the F0 and F1 generations (corresponding to 666-711 mg/kg bw/day for males and 777-804 mg/kg bw/day for females).

The NOAEL for reproductive toxicity, for both generations and offspring was considered to be 30000 ppm. The NOAEL for developmental toxicity, for both generations and offspring was considered to be 10000 ppm.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The NOAEL was considered to be 10000 ppm for adult toxicity for both the F0 and F1 generations (corresponding to 666-711 mg/kg bw/day for males and 777-804 mg/kg bw/day for females). The NOAEL for reproductive toxicity, for both generations and offspring was considered to be 30000 ppm. The NOAEL for developmental toxicity, for both generations and offspring was considered to be 10000 ppm.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	**CA 5.6.1/011**
Report author	[redacted]
Report year	1988
Report title	Report on effect of glyphosate technical of Excel Industries Ltd. Bombay, on fertility and general reproductive performance (Segment I)
Report No	None stated
Document No	42-90619
Guidelines followed in study	No guideline cited in the report and not in accordance with OECD 415 (1983)
Deviations from current test guideline (OECD 415, 2019)	Test substance purity not given; group size too small; missing endpoints: body weights or food consumption for parental males, body weights for females during premating period and until Day14 of gestation, estrous cycle monitoring, pre-coital interval, duration of gestation, sperm analysis, organ weights, histopathology, monitoring of physical and sexual offspring development, dose levels tested too low
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory when the study was performed
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Three groups of rats were administered glyphosate technical orally, by gavage, at dose levels of 0, 5 and 10 mg/kg bw/day. Controls received the vehicle (corn oil), only. Male rats were treated for 60 days before mating while treatment of female rats started 14 days before mating and was continued during gestation and lactation. The animals were mated in a sex ratio of 1 male : 3 females. Male rats were sacrificed after mating and testes were examined hitsopathologically. Half of the females were sacrificed on Day 13 of gestation and the uteri were examined for living foetuses, early and late deaths and the ovaries for the number of corpora lutea. The remaining females were allowed to litter and rear their offspring until day 21 post partum. Litter size, growth rate and survival rates of pups were recorded. Body weight and food consumption of females were recorded periodically. Under the condition of this study, glyphosate technical did not produce any toxic effects on fertility and general reproduction performance in rats.

I. MATERIALS AND METHODS

A. Materials

Test Material:

Identification:	Glyphosate technical
Description:	Not reported
Batch #:	Not reported
Purity:	Not reported

Expiry date:	Not reported
Stability of test compound:	Not reported
Vehicle:	Corn oil
Test Animals:	
Species:	Rat
Strain:	Wistar rats
Source:	Not reported
Number of animals:	120 (30 males and 90 females)
Age at start of treatment:	Not reported
Sex:	Males and females
Initial body weights:	70-130 g (no further information)
Acclimation period:	Not reported
Diet/Food:	Pelleted food from Lipton India
Water:	Not reported
Housing:	Polypropylene rat cages with paddy as bedding; single housing during pregnancy, dams were housed with their litters until weaning
Environmental conditions:	Temperature: 19-25°C Humidity: 50-70% Air changes: Not reported 12 hours light/dark cycle

B. STUDY DESIGN
In life dates: not reported

Animal assignment and treatment:
In this reproduction toxicity study groups of 10 male and 30 female Wistar rats received daily doses of 0, 5 and 10 mg/kg bw glyphosate technical per gastric gavage. After approximately 60 days of treatment for males and 14 days of treatment for females pairing of animals within each dose group was undertaken on a one male: three female basis. Males were sacrificed after mating. Pregnant females were either sacrificed on Day 13 of gestation or were allowed to litter and raise their offspring until weaning on Day 21 post partum.

Clinical observations
All animals were observed daily for any signs of toxicity.

Body weight
Parental female body weights were recorded periodically during gestation and lactation.

Food consumption
Parental female food consumption was recorded periodically during gestation and lactation.

Mating procedure and vaginal smears
After the scheduled period of treatment (approx. 60 days for males and 14 days for females), pairing of animals within each dose group was undertaken on a one male: three female basis. Succesful mating (Day 0 of pregnancy) was confirmed by presence of vaginal plugs.

Pre-coital interval

Not reported.

Duration of gestation
Not reported.

Reproduction parameters
Half of the females were sacrificed on Day 13 of gestation and the uteri were examined for living foetuses, early and late deaths and the ovaries for the number of corpora lutea. The remaining females were allowed to litter and rear their offspring until day 21 post partum.

Litter data
Litter size, growth rate and survival rates of pups were recorded.

Assessment of development and reproductive performance of progeny
Not reported.

Sacrifice and pathology
Males were sacrificed after mating. Pregnant females were either sacrificed on Day 13 of gestation or at weaning on Day 21 post partum.

Statistics
Not reported.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS
Not reported.

B. MORTALITY
There were no mortalities in parental animals.

C. CLINICAL OBSERVATIONS
No clinical signs of toxicity were noted throughout the study

D. BODY WEIGHT
There were no effects on body weights in female rats up to and including 10 mg glyphosate/kg bw/day.

Table 5.6.1-80 Glyphosate technical: Report on effect of glyphosate technical of Excel Industries Ltd. Bombay, on fertility and general reproductive performance (Segment I) ([redacted], 1988a): Body weights (group mean) of females

Body weight (g) ± SE	Dose level (mg/kg bw/day)		
Day	0 (control)	5	10
Post coitum			
15	210.5 ± 5.65	194.2 ±4.47	200.7 ± 4.06
20	225.4 ± 5.07	211.3 ±4.81	217.2 ± 4.17
Post partum			
0	172.6 ± 5.33	174.7 ± 5.10	166.2 ± 4.25
6	170.6 ± 4.90	179.2 ± 5.10	168.5 ± 4.31
12	172.9 ± 5.00	179.3 ± 4.99	171.2 ± 4.02
18	174.8 ± 4.80	180.3 ± 4.62	171.5 ± 3.87

21	176.5 ± 5.16	180.7 ± 4.51	172.2 ± 3.77

E. FOOD CONSUMPTION

There were no effects on food consumption in female rats up to and including 10 mg glyphosate/kg bw/day.

F. REPRODUCTIVE PARAMETERS

When the animals were sacrificed on Day 13 of gestation, the number of corpora lutea, total implantations, early and late deaths and living implantations were similar in test item-treated and control animals.
When allowed to give birth, the litter size, growth and survival rate of test item-treated and control animals were similar.

Table 5.6.1-81 Glyphosate technical: Report on effect of glyphosate technical of Excel Industries Ltd. Bombay, on fertility and general reproductive performance (Segment I) ([redacted], 1988a): Reproduction data Day 13 of gestation

Parameter	Dose level (mg/kg bw/day)		
	0 (control)	5	10
No. of dams in group	13	12	12
Total No. of corpora lutea	113	107	121
Total No. of implantations	108	104	117
Total No. of early deaths	3	1	1
Total No. of late deaths	2	1	2
Total No. of living foetuses	103	102	114

G. LITTER DATA

Number of pups delivered
There was no relevant difference in litter size between the three groups.

Viability index

A small number of dead pups was noted between post partum days 0 and 4 in all dose groups including the control. The incidence of dead pups was not affected by treatment with the test item. No pup mortalitiy was noted in any group from Day 4 post partum until weaning on Day 21 post partum.

Table 5.6.1-82 Glyphosate technical: Report on effect of glyphosate technical of Excel Industries Ltd. Bombay, on fertility and general reproductive performance (Segment I) ([redacted], 1988a): Litter size and survival rates (group means)

Parameter	Dose level (mg/kg bw/day)		
	0 (control)	5	10
Mean litter size ± SE	8.92 ± 0.73	8.92 ± 0.54	8.58 ± 0.66
Survival rates (%)			
Day 0	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00
Day 4	97.3 ±1.51	98.4 ± 1.03	99.2 ± 0.76
Day 14	97.3 ± 1.51	98.4 ± 1.03	99.2 ± 0.76
Day 21	97.3 ± 1.51	98.4 ± 1.03	99.2 ± 0.76

Body weights

Pup weights generally developed similar in the test item-treated groups and the control group.

Table 5.6.1-83Glyphosate technical: Report on effect of glyphosate technical of Excel Industries Ltd. Bombay, on fertility and general reproductive performance (Segment I) ([redacted], 1988a): Pup body weights (group means)

Body weight (g) ± SE	Dose level (mg/kg bw/day)		
Day	0 (control)	5	10
Post partum			
0	5.77 ± 0.10	5.42 ± 0.14	5.70 ± 0.19
4	10.09 ± 0.24	9.41 ± 0.28	9.97 ± 0.41
14	18.87 ± 0.59	17.96 ± 0.48	19.76 ± 1.02
21	29.56 ± 0.69	29.25 ± 0.55	30.37 ± 0.62

H. Histopathology
No histopatholical findings were noted in the testes of any animal on study.

Study conclusion:
Glyphosate technical supplied by Excel Ltd., Bombay, did not produce any toxic effect on fertility and general reproductive performance up to the high dose of 10 mg/kg bw/day.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Under the conditions of this study, glyphosate technical did not produce any effects on fertility and general reproductive performance up to the high dose of 10 mg/kg bw/day.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	**CA 5.6.1/012**
Report author	[redacted]
Report year	1988
Report title	Report on effect of pesticides on reproductive process - Segment IV - Three generation reproduction study with alblino rats using glyphosate technical of Excel Industries Limited, Bombay
Report No	Not reported
Document No	Not reported
Guidelines followed in study	No guideline cited in the report and not in accordance with OECD 416 (1983 or 2001)
Deviations from current test guideline (OECD 416, 2001)	Test substance purity not given; Group size too small; no justification for dose levels; no information on pre-mating dosing period; missing endpoints: estrous cycle monitoring, pre-coital interval, duration of gestation, body weights during gestation, sperm analysis, organ

	weights, monitoring of physical and sexual offspring development
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities1,2	No, GLP was not compulsory when the study was performed
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary
Glyphosate technical was given via the diet at dose levels of 0, 75, 150 or 300 ppm to Wistar rats over three generations. In the F0 generation, the groups consisted of 8 male and 16 female animals from which similar groups were produced by selective mating for the F1, F2 and F3 generations. Two litters per generation were delivered. The rats were observed for changes in body weight and food consumption, pregnancy rate, litter size, number of live and stillborn pups and any abnormalities. Testes of F0 males and selected organs and tissues of parental animals in the F1 and F2 generations were examined histopathologically.
There were no adverse effects of treatment observed in any of the parameters investigated.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material:

Identification:	Glyphosate technical
Description:	White powder
Batch #:	Not reported
Purity:	Not reported
Expiry date:	Not reported
Stability of test compound:	Not reported
Vehicle:	Plain diet
Test Animals:	
Species:	Rat
Strain:	Wistar rats
Source:	Not reported
Age at start of treatment (F0):	100 days (F0 generation)
Sex:	Males and females
Mean body weight at initiation of dosing:	Males: 148 - 150 g; females: 137 - 144 g
Acclimation period:	Not reported
Diet/Food:	Pelleted food from Lipton India, Bangalore
Water:	ad libitum
Housing:	Polypropylene rat cages with paddy as bedding; single housing during pregnancy, dams were housed with their litters until weaning

Environmental conditions:	Temperature: 19-22 °C Humidity: 30-70 % Air changes: Not reported 12 hours light/dark cycle

B. STUDY DESIGN
In life dates: not reported

Animal assignment and treatment:
In this three-generation reproduction study groups of 8 male and 16 female Wistar rats of the F0 generation received daily dietary doses of 0, 75, 150 and 300 ppm glyphosate technical in diet. Animals were kept on this diet until weaning of the F3b generation.
After 60 days and 14 days of treatment for males and females, respectively, pairing of animals mating within each dose group was undertaken on a one male: two female basis, to produce the F1 litters. Pregnant females were allowed to deliver and rear their litters (F1a) until weaning on Day 21 post partum. After a rest of 10-15 days parental females were mated again with the same male in a similar manner to deliver and rear the F1b litter. Eight males and 16 females were chosen from the F1b offspring as parents for the F2 generation. The mating of siblings was avoided. The procedure described above was repeated to produce the F3 offspring. Standardisation of litter size: on day 4 post partum litter size was adjusted to 4 males and 4 females, where possible.

Diet preparation and analyses
The test item was mixed with food to achieve concentrations of 75, 150 and 300 ppm. The control group received plain food.

Clinical observations
A gross general observation was made for each animal once a day.

Body weight
Body weights of F0 animals, and for F1 and F2 animals selected for rearing were recorded weekly. Body weights of the F0 animals were recorded during mating and weaning period only. Offspring not selected to produce a next generation was weighed on days 0, 4, 14 and 21 post partum. F1 offspring (males) selected to produce the F2 generation were weighed up to and during mating. F2 offspring (males) selected to produce the F3 generation were weighed up to and during mating for the F3b generation. F1 offspring (females) selected to produce the F2 generation were weighed up to weaning of the F2b generation. F2 offspring (females) selected to produce the F3 generation were weighed up to weaning of the F3b generation.

Food consumption and compound intake
The amount of food consumed was determined once weekly for parental animals of all three generations. However, reported values do not include data for all animals in every group.

Mating procedure and vaginal smears
After the scheduled period of treatment (60 days for males and 14 days for females), females were paired on a one- to-two basis with males from the same treatrnent group for a maximum of one week.

Pre-coital interval
Not reported.

Duration of gestation
Not reported.

Reproduction parameters

For each generation and mating phase, the number of dams mated, pregnant and littering were recorded.

Litter data
For each generation and mating phase, the number of live and dead pups per dam were recorded.

Assessment of development and reproductive performance of progeny
Not reported.

Sacrifice and pathology
All F1a offspring was sacrificed after weaning. F0 males were sacrificed after mating for the F1b generation. Necropsy was performed and testes were preserved for histopathological examination.
F1b offspring not selected for rearing were sacrificed after weaning. All F2a offspring were sacrificed after weaning. F1b parental males were sacrificed 1 week after mating for the F2b generation. Necropsy was performed and a range of organs (see below) including testes were preserved for histopathological examination. F2b offspring not selected for rearing was sacrificed after weaning. F1b and F2b parental females were sacrificed after weaning of the F2b and F3b generation, respectively. Necropsy was performed and the following tissues were preserved for histopathological examination: adrenals, aorta, brain, colon, duodenum, eyes, heart, ileum, kidney, liver, lung, oesophagus, ovaries, pancreas, pituitary, seminal vesicles, spleen, stomach, testes, thyroid, urinary bladder, uterus and salivary glands. All offspring of the F3a generation was sacrificed after weaning. F2b parental males were sacrificed 1 week after mating for the F3b generation. Necropsy was performed and the tissue listed above were preserved for histopathological examination. All F3b offspring was sacrificed after weaning.

Statistics
Not reported.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE CONCENTRATIONS IN THE DIET
Not reported.

B. TEST COMPOUND INTAKE
Not reported.

C. MORTALITY
There were no mortalities in animals after weaning. A low incidence of pup mortality was noted in all groups without any test item effects.

D. CLINICAL OBSERVATIONS
No clinical signs of toxicity were noted throughout the study.

E. BODY WEIGHT
There was no substantial difference in the body weight gain of test item-treated animals at any dose level compared to the respective control animals.

F. FOOD CONSUMPTION
No significant differences in food consumption were observed between test item-treated and control animals in any of the three generations.

G. REPRODUCTIVE PARAMETERS
Pregnancy rate
The number of pregnant females was not significantly different between test item-treated and control groups.

Table 5.6.1-84Glyphosate technical: Three generation reproduction study with albino rats using glyphosate technical of Excel Industries Limited, Bombay ([redacted], 1988): Reproduction data F0 animals

	Group 1 - control 0 ppm		Group 2 75 ppm		Group 3 150 ppm		Group 4 300 ppm	
	F1a	F1b	F1a	F1b	F1a	F1b	F1a	F1b
Number of dams in group	16	12	16	13	16	12	16	12
Number of dams mated	16	12	16	13	16	12	16	12
Number of dams pregnant	12	9	13	9	12	9	12	9
Number of dams littered	12	9	13	9	12	9	12	9
Mean litter size ± SE	9.0 ± 0.28	8.9 ± 0.35	8.6 ± 0.29	9.0 ± 0.29	8.8 ± 0.37	8.7 ± 0.33	8.8 ± 0.33	9.0 ± 0.29
Mean number of live pups/litter ± SE	8.8 ± 0.25	8.6 ± 0.29	8.4 ± 0.24	8.7 ± 0.24	8.4 ± 0.29	8.3 ± 0.29	8.4 ± 0.29	8.7 ± 0.33
Mean number of dead pups/litter ± SE	0.25 ± 0.13	0.33 ± 0.17	0.23 ± 0.12	0.33 ± 0.17	0.33 ± 0.14	0.33 ± 0.17	0.33 ± 0.14	0.33 ± 0.17

Table 5.6.1-85 Glyphosate technical: Three generation reproduction study with albino rats using glyphosate technical of Excel Industries Limited, Bombay ([redacted], 1988): Reproduction data F1 animals

	Group 1 - control 0 ppm		Group 2 75 ppm		Group 3 150 ppm		Group 4 300 ppm	
	F2a	F2b	F2a	F2b	F2a	F2b	F2a	F2b
Number of dams in group	16	12	16	12	16	13	16	12
Number of dams mated	16	12	16	12	16	13	16	12
Number of dams pregnant	12	9	12	10	13	10	12	9
Number of dams littered	12	9	12	10	13	10	12	9
Mean litter size ± SE	8.7 ± 0.31	8.7 ± 0.41	8.8 ± 0.30	8.4 ± 0.34	8.4 ± 0.24	8.9 ± 0.23	8.4 ± 0.29	8.7 ± 0.24
Mean number of live pups/litter ± SE	8.4 ± 0.31	8.3 ± 0.33	8.4 ± 0.29	8.1 ± 0.23	8.1 ± 0.21	8.6 ± 0.22	8.2 ± 0.21	8.3 ± 0.24
Mean number of dead pups/litter ± SE	0.25 ± 0.13	0.33 ± 0.24	0.33 ± 0.14	0.30 ± 0.15	0.31 ± 0.17	0.30 ± 0.15	0.25 ± 0.18	0.30 ± 0.17

Table 5.6.1-86Glyphosate technical: Three generation reproduction study with albino rats using glyphosate technical of Excel Industries Limited, Bombay ([redacted], 1988): Reproduction data F2 animals

	Group 1 - control 0 ppm		Group 2 75 ppm		Group 3 150 ppm		Group 4 300 ppm	
	F3a	F3b	F3a	F3b	F3a	F3b	F3a	F3b
Number of dams in group	16	13	16	12	16	13	16	13
Number of dams	16	13	16	12	16	13	16	13

mated								
Number of dams pregnant	13	10	12	9	13	10	13	10
Number of dams littered	13	10	12	9	13	10	13	10
Mean litter size ± SE	8.2 ± 0.30	8.5 ± 0.34	8.4 ± 0.31	8.8 ±0.28	8.3 ± 0.29	8.8 ± 0.25	8.5 ± 0.32	8.7 ± 0.37
Mean number of live pups/litter ± SE	7.9 ± 0.19	8.2 ± 0.25	8.2 ± 0.24	8.4 ± 0.24	8.1 ± 0.27	8.5 ± 0.27	8.2 ± 0.27	8.4 ± 0.34
Mean number of dead pups/litter ± SE	0.31 ± 0.18	0.30 ± 0.15	0.25 ± 0.13	0.33 ± 017	0.23 ± 0.12	0.30 ± 0.15	0.31 ± 0.13	0.30 ± 0.15

H. LITTER DATA

Number of pups delivered

Litter size varied from 7 to 10 in all four groups in all three generations and there was no significant difference in litter size.

Viability index

The number of dead pups per litter varied from 0 to 2 and the rate was less than 3.3 pups per litter. There was no significant difference in the number of dead pups in the test item-treated groups when compared to their respective control group.

Body weights

Pup weights generally developed similar in the test item-treated groups and the respective control groups.

I. Histopathology

Histopathological examination of various tissues from animals at all dose levels did not reveal any structural changes attributable to treatment with the test item.

Study conclusion:

There were no adverse effects of treatment observed in any of the parameters investigated. Thus, a dose level of 300 ppm was considered the NOAEL for both parental and reproduction toxicity. Using the usual conversion factor of 20, this concentration would correspond to an approximate daily intake of 15 mg/kg bw/day.

3. Assessment and conclusion

Assessment and conclusion by applicant:

supportiveThere were no adverse effects of treatment observed in any of the parameters investigated in this study, thus a dose level of 300 ppm was considered the NOEL for both parental and reproduction toxicity. Using the usual conversion factor of 20, this concentration would correspond to an approximate daily intake of 15 mg/kg bw/day.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	**CA 5.6.1/013**
Report author	[redacted]
Report year	1985

Report title	Three-generation reproduction study in rats with the oral administration of glyphosate
Report No	Not reported
Document No	Not reported
Guidelines followed in study	None (pre-guideline)
GLP	No (GLP was not compulsory at the time the study was performed)
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	Glyphosate (purity not indicated) was fed to Wistar rats for three consecutive generations at dietary levels of 0, 200, 1000 and 5000 ppm. Rats were administered the test substance in their diet for a 12–week period before first mating. Males and females were mated in a sex ratio of 1:1. In the F1 and F2 generations, there were two matings. In contrast, three litters were produced in the F3 generation. The number of paired animals varied between the generations. In the Fa generation, 6 males and 6 females were used. The respective numbers were 12 and 24 for the F1B and F2B generations. These animals were sacrificed on day 28 after parturition. Glyphosate administration was continued until this day. In the F3C generation, 10 animals per sex and dose group were selected by randomisation and examined following an 8–week dosing period and a subsequent 4–week recovery phase. All the rats were daily observed for signs of toxicity. Body weight and food consumption were determined regularly. Litter parameters were recorded. Prior to sacrifice of adult rats, blood was taken for haematological and clinical chemistry investigations. Following sacrifice, the animals were necropsied and organ weights were determined. A limited number of rats (3 or 5 per dose group and generation) was subjected to histopathology.
Short description of results:	No adverse effects of treatment were observed neither in adult rats nor in the pups in any of the groups up to the highest dose of 5000 ppm. There were occasional differences in the parameters indicative of reproductive performance among the groups, however, no consistent and dose-related trend throughout the generations was found.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	Study was deleted due to major deficiencies and/or because dose levels were much too low
Reasons why the study report is not available for submission	The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL.
Category study in AIR 5 dossier (L docs)	Category 4b

1. Information on the study

Data point:	**CA 5.6.1/014**
Report author	[redacted]

Report year	1981
Report title	A three generation reproduction study in rats with glyphosate
Report No	77-2063
Document No	M-644052-01-1
Guidelines followed in study	None (pre-guideline)
Deviations from current test guideline	Not applicable
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities1,2	No (pre-GLP)
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

Rats were administered glyphosate oral via diet continuously for three successive generations. Each generation (F0, F1 and F2) consisted of 12 male and 24 female Charles River CD® rats. Dietary concentrations were adjusted weekly during growth, and between mating rest periods, to achieve dose levels of 3, 10, and 30 mg/kg bw/day. A concurrent control group (plain diet) was included in the study. Each parent generation was mated to produce two litters. Offspring from the second litters of the F0 and F1 parents (F1b and F2b litters, respectively) were selected to be parents for subsequent generations. Offspring not included in the selection procedure and offspring from the first litter intervals of each generation (F1a, F2a, and F3a) were sacrificed at weaning and given a gross post mortem examination. Pathological and histopathological examinations were conducted from control and high-dose parent generations (F0, F1 and F2) and from control and high dose F3b offspring at weaning (10/sex/group). Parameters evaluated for each generation included: mortality, body weight, food consumption, clinical observation, maternal body weights (gestation/lactation), reproduction-fertility indices (mating, pregnancy and fertility indices), litter data at parturition and organ weight data. Offspring from each litter interval were evaluated during a 21–day lactation period for growth, survival, sex distribution data and gross post mortem observations including organ weight data (F3b offspring only).

No treatment-related effect was evident in adult mortality data, body weight and food consumption data (growth and rest periods), and clinical observation data throughout the study (F0, F1 and F2 generation). Considerable variability was seen in the mating-fertility indices during this study in both, the control and treated groups, in particular during the F1 and F2 generations. In the control group, mating indices were low for both mating intervals of the F1 generation and in the F2 generation these indices were higher than normally encountered. Similar fluctuations were seen in mating indices for some of the treated groups during these same intervals. Throughout the study no consistent effect was seen in mating indices, fertility indices (male) or pregnancy rate data to suggest an adverse effect of treatment.

No adverse effects of treatment were evident in maternal weight data, gestation length, parturition data (number of live/dead pups at birth) or litter survival indices throughout the study. Concerning the offspring, no treatment-related effects were indicated in sex distribution data, body weights, survival or gross post mortem findings. Likewise, no effect of treatment was evident in mean organ weight data (absolute and relative to body or brain weight) for randomly selected Day 21 F3b offspring.

No adverse effect of treatment was evident in organ weight data for the F0, F1 generations and F2 adult males. In the F2 treated females, a non-dose related, albeit statistically significant, decrease in mean liver/body weight ratio was evident; liver/brain weight ratios for these females showed a similar reduction; however, these differences from control were not statistically significant.

Gross post mortem evaluations of the adult generations and histological evaluations of tissues from randomly selected F0, F1 and F2 high-dose animals and F3b high-dose offspring did not indicate a

treatment-related effect.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material:

Identification:	Glyphosate
Description:	Fine white powder
Lot #:	XHJ-64
Purity:	considered 100% active ingredient for dosing preparations
Stability of test compound:	Not reported
Vehicle:	Plain diet
Test Animals:	
Species:	Rat
Strain:	CD® (Sprague-Dawley derived)
Source:	[redacted]
Age at treatment initiation:	43 days
Sex:	Males and females
Mean weight at initiation of dosing:	Males: 139.9 - 144.3 g; females: 118.0 - 119.2 g
Acclimation period:	7 days
Diet/Food:	Standard laboratory diet (Purina Lab Chow® 5001), ad libitum
Water:	Automated watering system (Elizabethtown Water Company), ad libitum
Housing:	Individually (except during mating and lactation), in elevated stainless steel wire mesh cages; nesting material: Litter Kleen® hardwood shavings added to cages on Day 19 of gestation and changed when wet or soiled through Day 14 of lactation
Environmental conditions:	12 hours light/dark cycle No details on temperature and humidity reported

B. STUDY DESIGN

In life dates: 1978-06-14 - 1980-04-09

Animal assignment and treatment:
In a three generation reproduction study groups of 12 male and 24 female CD rats received beginning 63 days prior to mating of the F0 generation daily dietary doses of 0, 3, 10 and 30 mg glyphosate/kg bw in diet. Diet samples were taken at four week intervals for analysis of achieved test substance concentrations.

Mating: One male and two females of equivalent dose levels were caged together nightly until a sign of mating (sperm and/or copulation plug in the vagina) was observed or until 15 days had elapsed with no evidence of mating. The day on which evidence of mating was observed was defined as Day 0 of gestation.

In this study, the first litters (F1a, F2a and F3a) from each mating were raised to weaning and discarded. Rats produced by the second matings (F1b and F2b) were selected to become parents of succeeding generations or to be subjected to complete gross necropsy (F3b).

Table 5.6.1-87 Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat ([redacted], 1981): Study design

Group	Dose level (mg/kg bw/day)	No. of adults initially assigned to mate F0, F1, F2		No. of matings per generation F0, F1, F2	Gross post mortem examination	Histopathology of F0, F1 and F2 parents, F3b weanlings	
		Males	Females			Male	Female
1 Control (plain diet)	0	12	24	2	All	10	10
2	3	12	24	2		none	none
3	10	12	24	2		none	none
4	30	12	24	2		10	10

Diet preparation and analyses
Diets were prepared weekly during the study and were adjusted on the basis of body weight and food consumption.

Clinical observations
A check for clinical signs of toxicity and mortality was made twice daily. A detailed physical examination was performed on adult generations at weekly intervals throughout the study.

Body weight
Body weights of all animals were determined weekly during growth and rest periods of all generations. Pregnant females were weighed on Days 0, 6, 15 and 20 of gestation and lactating females were weighed on Days 0, 4, 14 and 21 of lactation.

Food consumption and compound intake
Food consumption was recorded weekly during growth and rest periods of all generations. Test substance intake was calculated from individual body weight and food consumption data and reported as a group mean value for weekly intervals during the growth and rest periods of all generations.

Reproduction parameters
The day on which evidence of mating was observed was designated as Day 0 of gestation; the day of delivery was designated as Day 0 of lactation.
Mating indices, pregnancy rates, length of gestation and male fertility indices were recorded.

Litter data
Pups of all generations were examined daily for general appearance and mortality. On Days 0, 4, 14, and 21 they were counted to record the number of live and dead pups. Body weights were determined on Days 0, 4, 14, and 21 as a litter and on Day 21 individually.
Total number of live and dead pups, and the number of males and females per litter were determined on Day 0 of lactation. The sex ratio was calculated for each group on Days 0 and 21 of lactation. Viability indices, were determined for each litter on Lactation Days 0, 4 and 21.

Sacrifice and pathology
Animals of all generations that died, were found dead or were killed moribund during the study period were necropsied as soon as possible. All adult males and females were sacrificed after pup selection of the last Fb litter (F0, F1) and after last F3b litter weaned (F2) by lethal exposure to ether. Pups that were found dead or stillborn pups were weighed and given a gross post mortem examination including internal sex determination, presence of milk in stomach. F1a, F2a, F3a and F3b animals were sacrificed at weaning,

given a gross post mortem examination and abnormal tissues were saved. F1b and F2b animals which were not selected as future parents were sacrificed after ensuing selection of parental animals, given a gross post mortem examination and abnormal tissues were saved.

The following organs and tissues were preserved from all parents (F0, F1, F2) and from 10/sex/group of the F3b weanlings: adrenals, aorta, bone and bone marrow (sternal), brain, colon, duodenum, eyes with optic nerve and Harderian gland, gonads (ovaries and testes), heart, ileum, kidney (2), liver (2 sections), lung with main stem bronchi, lymph nodes (mesenteric), mammary gland (right inguinal), pancreas, pituitary, salivary gland, skeletal muscle (biceps femoris with right sciatic nerve), skin, spinal cord, spleen, stomach, thyroid/parathyroid, urinary bladder, uterus/prostate, gross lesions, tissue masses, thymus.
Microscopic examination of histological sections of these tissues were done for 10 male and 10 female animals from control and high-dose groups of F0, F1 and F2 parents and of F3b offspring.

The following organs were weighed from all parents sacrificed after weaning of the second litters and from eighty F3b weanlings (10 males and 10 females per group): adrenals, gonads, kidneys, brain, spleen, liver, heart and pituitary.

All pups of the second litter of the F2 parents (F3b) were necropsied at weaning and specified tissues were preserved for selected animals in each group.

F
Body weights, body weight gain, maternal body weights, food consumption, number of offspring, offspring body weights, terminal body weights and organ weight data (absolute and relative), offspring survival, litter survival, pup viability index at birth, mating indices, pregnancy rates and male fertility indices data were compared to the control. Statistically significant differences were evaluated using several methods including Dunnett's test, ANOVA, Barlett's test, Kruskal-Wallis test and Fisher Exact Test.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS
Not reported.

B. MORTALITY
F0 adults (2 dead females in mid-dose group)
In the F0 generation, no unscheduled mortality occurred in the control, low- or high-dose groups. One female of the mid-dose group died during on Lactation Day 20 of first litter having 13 live pups at time of death. A second female of the mid-dose group died on Lactation Day 7 of second litter; this female delivered eight pups - seven live and one dead - and all pups were dead at time of death. No mid-dose F0 male died.

F1 adults (1 dead female in mid-dose group, 1 dead female in high-dose group)
In the F1 generation, no unscheduled mortality occurred in the control or low-dose groups. In the mid dose group one female was killed in a moribund condition during the post-mating period for the second litter. This female had mated during the first mating but did not deliver a litter; during the second mating this female had not mated. No other mortality occurred in the mid-dose group. In the high dose group one female died due to an accident (animal was caught in the feeder jar). A second high-dose female died on Day 21 of gestation for the second litter; the uterus of this female contained 15 term foetuses. No other mortality occurred in the high-dose group.

F2 adults (1 dead female in low-dose group, 1 dead male in mid-dose group)
In the F2 generation, no unscheduled mortality occurred in the control or high-dose groups. In the low-dose group one female died during the F3a lactation period. This female delivered a litter containing only dead pups (13 pups) and died the day after parturition. No other mortality occurred in the low-dose group. In the mid-dose group one male was killed in a moribund condition during the period between mating of the first

and second litters. This male had mated and impregnated both females during the first mating period. No other mortality occurred in the mid dose group.

C. CLINICAL OBSERVATIONS
Clinical observation data were similar between the control and treated groups for each generation interval throughout the study. No adverse treatment effects were indicated.

D. BODY WEIGHT
Mean body weight data during the growth and rest periods were comparable between the control and treated groups for each generation, throughout the study. Likewise, mean weight gain during the growth periods were comparable between these same groups for both sexes throughout all generations. No treatment effect on body weight data during the growth and rest periods was evident.

Maternal body weights
Mean body weight data during the gestation and lactation intervals and mean weight change during these same periods were comparable between the control and treated group for each pregnancy interval from each generation throughout the study. No treatment effect was indicated in gestation - lactation body weight data throughout the study.

Offspring body weights
Mean pup body weight data during each litter interval for each generation were comparable between the control and treated groups. No adverse effects of treatment on pup weight data was evident.

Adult animals (F0, F1 and F2)
Mean terminal body weight data were comparable between the control and treated groups for both males and females throughout the study.

E. FOOD CONSUMPTION AND TEST COMPOUND INTAKE
Mean food consumption data were considered comparable between the control and treated groups (both sexes) during the growth and rest periods for each generation, throughout the study. No adverse effect of treatment on food consumption was evident throughout the study. Mean weekly test substance intake values ranged from 2.8 to 3.3 mg/kg bw/day for the low-dose group, from 9.5 to 11.2 mg/kg bw/day for the mid-dose group and from 27.7 to 33.1 mg/kg bw/day for the high dose group for all generations including both genders.

F. REPRODUCTIVE PARAMETERS
Male and female mating indices and male fertility indices during both mating intervals of the F0 generation were considered comparable between the control and treated groups. During the second mating interval of the F0, pregnancy rates were lower than control in each of the treated groups; however, no indication of a dose-relationship was evident as the lowest pregnancy rate was seen in the mid-dose group. This reduction in pregnancy rate for the mid-dose group was not statistically significant. In the absence of a dose-response relationship the reduction in pregnancy rate during this mating interval (F1b) in the treated groups was not considered treatment-related.

In the F1 generation, mating indices (males and females) for both litter intervals were comparable between the control and treated groups. It is note-worthy that for both mating intervals of this generation, mating indices for control and some treated groups were lower than normally encountered in multi-generation studies. The reason for the poorer mating performance in this generation was unclear but no treatment effect was indicated since mating indices were lowest in the control group. Pregnancy and male fertility indices for the first mating interval of the F1 were comparable between the control and treated groups. During the second litter interval, pregnancy rates were lower than those seen for the first interval in control and treated groups. The lowest pregnancy rate was seen in the high-dose group; however, this difference from the control value was not statistically significant. Pregnancy rates for the low- and mid-dose groups, during the second mating interval, were considered comparable to control. Male fertility indices for this same mating

interval were considered comparable between the control and treated groups.

In the F2 generation mating indices for the treated groups were lower than control for each mating interval. During the first mating interval of the F2 generation, the female mating indices were lower than control in each of the treated groups; however, only in the high-dose group was this difference from control statistically significant. The female mating index for the control group at this interval was 100% which is higher than normally encountered. The female mating indices observed for the control group in this study have shown considerable variability ranging from 70.9 to 100% The poor mating performance for the treated groups during the first mating interval is attributed to two males in each treatment group that did not mate either female in their mating unit (each mating unit was comprised of one male and two females).

During the second mating interval of the F2 generation, male mating performance improved in the mid- and high-dose groups as both mid-dose males and one of two high-dose males that did not mate during the first mating interval, mated and impregnated at least one female. Male mating indices for the low-dose group remained unchanged as the same two males that did not mate during the first interval, failed to mate during the second interval. Pregnancy and fertility indices for the treated groups were comparable to control for both litter intervals of the F2 generation.

Mean gestation length was comparable between the control and treated groups for each pregnancy interval in each generation. Over the entire study no consistent, dose-related effect was seen in mating, fertility or pregnancy indices to indicate an adverse effect of treatment.

G. LITTER DATA

Litter size

Mean litter size data on Day 21 of lactation (weaning) was comparable between the control and treated groups for each litter interval throughout the study.

Sex ratio

Pup sex distributions ratios at Day 0 and 21 were generally comparable between the control and treated groups for each litter interval for each generation. No adverse treatment effect on sex distribution data was evident.

Viability index

The mean numbers of live, dead and total pups at birth and pup viability at birth for each pregnancy interval, were comparable between the control and treated groups for each generation. The litter survival indices were comparable between the control and treated groups for each lactation interval in the F0, F1 and F2 generation. In the F0 generation, postnatal survival indices for Days 0-4 and 4-21 were comparable between the control and treated groups for the first lactation interval (F1a). For the second litter interval of the F0, postnatal survival indices for the Day 0-4 interval were comparable between the control and treated groups. During the Day 4-21 interval, survival indices were significantly lower than control in each treatment group. The increase in pup mortality during this interval (i.e. Days 4-21) was attributed to high pup mortality within one or more litters at each treatment level. In the low-dose group the lower pup survival was attributed to one female that experienced complete litter mortality (litter contained 14 live pups at Day 4). In the mid-dose group, one female died on Day 7 of lactation and all seven pups in her litter died during the Day 4-7 lactation interval. Additionally, three mid-dose litters lost five or more pups from their litters during the Day 4-21 lactation interval. In the high-dose group, one female lost nine of l2 pups during the Day 4-21 lactation interval.

In the F1 and F2 generations postnatal survival indices for Days 0-4 and 4-21 during both litter intervals were considered comparable between the control and treated groups. Some statistically significant differences in these indices were observed between the control and treated groups; however, no trend was evident through successive generations to indicate an adverse effect of treatment.

I. PATHOLOGY

Necropsy
F0, F1 and F2 generations
Gross necropsy of parental animals of both sexes did not indicate any adverse effect of treatment.

F1, F2 and F3 offspring
Gross post mortem observations of offspring at weaning (F1a, F2a, F3a, F3b) or post-weaning (F1b, F2b) did not demonstrate an adverse effect of treatment. Likewise, evaluation of dead pups recovered at birth and during the 21–day lactation period did not note a treatment-related effect.

Organ weights
F0, F1 and F2 generations
Mean organ weight data (absolute and relative to body weights or brain weights) were comparable between the control and treated groups for both males and females from the F0 and F1 generations. Some statistically significant differences were noted between control and treated groups both in mean organ weight data and in the relative weight data; however, no trends were evident within dose levels or through these generations.

In the F2 generation, mean organ weight data (absolute and relative) for the males were comparable between the control and treated groups. In the F2 female group, mean liver/body weight ratios were significantly lower than control in each of the treated groups; however, no clear dose-relationship was apparent. Mean liver/brain weight ratios for the treated F2 females were lower than control; however, these differences from control values were not statistically significant. Mean spleen weights (absolute and relative to brain and body weights) were significantly higher than the control value in the F2 mid-dose female group; however, mean spleen weight data for the low- and high-dose F2 females were comparable to control values. In the absence of an effect on spleen weight in the high-dose Fg female group, the change seen in spleen weight data for the mid-dose females was considered spurious and not biologically meaningful. Other mean organ weight data (absolute and relative to body weight or brain weight) for the treated F2 female groups were considered comparable to control data.

F3b offspring
Mean organ weight data (absolute and relative to body weights or brain weights) were comparable between the control and treated groups for both males and females. No treatment-related effect was evident in organ weight data for the F3b offspring.

F
In total 160 male and female rats (40 adults of each generation F0, F1 and F2 and 40 weanlings of F3b) were examined microscopically. No microscopic findings were considered treatment related. Proliferative tissue changes diagnosed as neoplasms were few. The microscopic tissue alterations, neoplastic and non-neoplastic, were indicative of common incidental histological findings.

III. CONCLUSIONS

Study conclusion:
The oral administration of glyphosate to rats by dietary admixture at a maximum dose level of 30 mg/kg bw/day for three successive generations of CD rats resulted in no treatment-related signs of toxicity in parental animals. The NOAEL for reproduction is 30 mg/kg bw/day, since the reproductive performance was not affected in any dose group. The NOAEL for offspring is 30 mg/kg bw/day, since no adverse effects on offspring were observed.

3. Assessment and conclusion

<u>Assessment and conclusion by applicant</u>:

The oral administration of glyphosate to rats by dietary admixture at a maximum dose level of 30 mg/kg

bw/day for three successive generations of CD rats resulted in no treatment-related signs of toxicity in parental animals or offspring. Therefore the NOAEL for reproduction and offspring is 30 mg/kg bw/day, corresponding to the highest dose tested.

Assessment and conclusion by RMS:

Publications on Reproductive Toxicity Studies

A literature search for the active substance glyphosate was performed in accordance to the provisions of the EFSA Guidance "Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) 1107/2009" and updated Appendix to this Guidance document. The following publications were found relevant and reliable for this section and the summaries are thus presented below and are part of the general discussion at the beginning of the section.

In some studies the effects on glyphosate and Round-up formulations have been investigated. In the following, only the effects after glyphosate treatment are discussed.

In the most recent study, a direct effect of glyphosate on Sertolli cell function was investigated under in vitro conditions by Gorga *et al.* (2020) (CA 5.6.1/015). Glyphosate did not modify the expression of the androgen receptor or intercellular junction proteins (claudin11, occludin and ZO-1). Further, intracellular signalling via P-p38-MAPK and P-ERK1/2 pathways were not affected. Lactate production, glucose uptake, GLUT1, FA oxidation, or *FAT/CD36* and *CPT1* expression was unaffected by glyphosate. Thus, the conducted molecular and cell biological investigations indicate that glyphosate does not influence testicular function due to disturbances of nutritional function or metabolism in Sertolli cells. *In vitro* exposure to glyphosate at non-cytotoxic concentrations (10 - 100 ppm) altered Sertoli cell junction barrier permeability and decreased testosterone-stimulated TER. Further, redistribution of claudin11 at the zone of contact between cells was detected after glyphosate stimulation. If the effects observed on Sertolli cell junction barrier permeability may contribute to a postulated effect on male reproductive function cannot be finally assessed. In general, an evaluation of such highly specific cell biological endpoints for hazard assessment is rather difficult as they cannot be transferred easily to intact organisms. As the biological *in vivo* relevance of such "isolated" *in vitro/ex vivo* findings is not validated for intact organisms, an interpretation of the obtained results on the redistribution of one special protein and cell junction barrier permeability is difficlut and not considered sufficiently conclusive.

Manservisi *et al.* (2019) (CA 5.6.1/016) investigated effects of glyphosate in rats exposed to a single dose of 1.75 mg glyphosate acid /kg bw/day (equivalent to the US Acceptable Daily Intake) during pregnancy and lactation and their offspring until adulthood. The study was performed similar to a pilot study according to OECD 443. Reproductive parameters remained to be unaffected by glyphosate exposure. The only significant finding related to glyphosate treatment was increased anogenital distance in pups at PND4 pointing to a delay in male sexual development in a statistically significant manner. In contrast, vaginal opening was not affected by treatment with glyphosate. In male offspring, exposure to glyphosate did not affect BPS or sperm parameters. On hormonal basis, a statistically significant increase in TSH levels was detected in glyphosate treated male rats. Overall, a delay in male sexual development indicated by increased anogenital distance combined with increased TSH level might point to possible androgenic properties of glyphosate. However, number and blood sample collection was limited to the final sacrifice of animals, which is considered as confounding factor. Further, another source of uncertainty is the timing of blood sampling during the necropsy session (9.00 am – 3.00 pm) which does not allow to exclude a circadian-dependent modulation of circulating hormones. The rather high standard deviations in different hormone concentrations might support the uncertainty in the conducted hormone measurements. Furthermore, the biological significance of the altered anogenital distance in pups remains unclear as no similar finding is reported in any available reproductive toxicology studies, including multi-generational studies. In one study performed by [redacted] (2007) (CA 5.6.1/001 – 003) a delay in preputial separation was observed in

male offspring at high doses which indicated a delay in male sexual development. However, the number of animals used in the study performed by Manservisi *et al.* (2019) (CA 5.6.1/016) is considered to be too low to allow a conclusive decision on the biological significance of the observed effects on male sexual development and androgenic properties in male rats. Likewise, the significance of increased TSH levels determined in the performed study is questionable as no histological finding and/or physiological disturbance in the affected animals is reported. For the assessment of possible endocrine actions please refer to section 5.8.3.

The effect of glyphosate on spermatogenesis was tested by Pham *et al.* (2019) (CA 5.6.1/017) in the mouse. Effects on the male reproductive system were analysed in male offspring of glyphosate-exposed pregnant mice treated to 0.5, 5 and 50 mg/kg bw/day. Male offspring derived from treated mice were sacrificed at 5, 20, and 35 days old (d.o.) and 8 months old (m.o.) for analysis. Various parameters were evaluated at different time points, including number of spermatogonia and gene expression in 5–day old mice, testicular histopathology in 20–day old mice, relative weight of testes, epididymis and seminal vesicles, epididymal sperm count, serum testosterone levels, GATA1 positive cell count and ZBTB16 positive cell count in 35–day old mice, and relative weight of testes, epididymis and seminal vesicles and serum testosterone levels in 8-month old mice. No statistically significant change was found for the number of spermatogonia in 5–day old mice. The only genes of which the expression was statistically significantly changed in a dose-related fashion were Bcl2 and Kit. In 20–day old mice, sperm depleted seminiferous tubules were noted at 5 mg/kg bw/day but not at 0.5 and 50 mg/kg bw glyphosate, thereby indictaing a rather incidental finding than a treatment-related effect. In 35–day old mice there was no statistically significant change in the relative weight of the epididymis and the seminal vesicles, epididymal sperm count and GATA1 positive cell count. No dose-effect relationship could be established for relative weight of testes, serum testosterone levels and ZBTB16 positive cell count. In 8-month old mice no statistically significant change was observed for relative weight of epididymis and seminal vesicles and serum testosterone levels. No dose effect relationship could be established for the decrease in relative testes weights. Due to missing dose-responses, the biological significance of the observed findings on seminiferous tubules depletion, relative testes weight, serum testosterone levels and ZBTB16 positive cell count remains questionable. The overall quality of the obtained data is not sufficiently conclusive to indicate test substance-specific adverse effects on testicular function and development when male mice were exposed during early phases in life to up to 50 mg/kg bw/day glyphosate, which has been corroborated by available reproduction toxicity studies with rats at much higher dose levels. In contrast, the data might indicate that glyphosate exposure does not result in long-lasting effects on spermatogenesis after substance withdrawal.

In a further in vivo study, toxic effects on prenatal exposure to glyphosate on lipid metabolism in offspring was investigated Ren *et al.* (2019) (CA 5.6.1/018). A significant decrease in body weight and obvious hepatic steatosis with excessive lipid droplet formation was observed in the offspring. Moreover, the concentrations of lipids such as triglycerides (TGs), total cholesterol (T-CHO), and low-density lipoprotein cholesterols (LDL-C) increased to a significant extent in serum and liver. Furthermore, significant differences in expression levels of genes related to lipid biosynthesis or catabolism in the liver was detected, incl. SREBP1C, SREBP2, Fasn, Hmgcr, Hmgcs and PPARa. Although the authors concluded that there were treatment related effects on foetal and offspring body weight, there is no evidence that glyphosate exposure had any impact on foetal development or pup development postnatally. There was no effect on average birth weight of pups. Further, group mean body weight of pups by sex showed no statistical differences from control. Considering the small group size, the large inter animal variability, the lack of consistency between the same parameter across the sexes, timepoints or sampling matrices, and the reported deficiencies listed in CA 6.6.1/018, it is not possible to clearly attribute any of the observed differences to glyphosate exposure. Therefore, the current study provides no conclusive evidence that glyphosate exposure causes lipid metabolism disruption in offspring following prenatal exposure.

The effects of glyphosate on maturation of isolated mouse oocytes was investigated under in vitro conditions by Zhang *et al.* (2019) (CA 5.6.1/019). Reduced rates of germinal vesicle breakdown (GVBD) and first polar body extrusion (PBE) after treatment with 500 µM glyphosate were reported. Changes in the mRNA expression of related antioxidant enzyme genes (*cat, sod2, gpx*) were interpreted to correlate to

increased formation of intracellular reactive oxygen species (ROS) in mouse oocytes exposed to glyphosate. After 14 h of exposure to glyphosate, metaphase II (MII) mouse oocytes displayed an abnormal spindle morphology and DNA double-strand breaks (DNA-DSBs). Simultaneously, mitochondria showed an aggregated distribution and decreased membrane potential in mouse oocytes exposed to glyphosate. The protein expression levels of apoptosis factors (Bax, Bcl-2) and the mRNA expression levels of apoptosis-related genes (*bax, bcl-2, caspase3*) were measured by Western blot and qRT-PCR, respectively. Meanwhile, the expression levels of autophagy-related genes (*lc3, atg14, mtor*) and proteins (LC3, Atg12) were significantly decreased in the glyphosate treatment group compared with the control group.

Based on these results, the authors hypothesised that glyphosate negatively affected oocyte development and might influence intracellular pathways involved in the cell death machinery.
Although good scientific standards have been applied during conduct of the study, meaning that all experiments have been performed in three biological replicates and at least 50 oocytes have been statistically evaluated using appropriate statistical methods, a sufficiently conclusive assessment on the biological significance of the observed effects is difficult. No distinction between spontaneous or treatment-related causes is possible as only one concentration was applied and hence, an evaluation of a dose-repsonse relationship is not possible which might prove a plausible biological link. Further, it remains unclear why a different number of donor animals was used for different approaches. This narrow source of oocytes limits the robustness of certain conclusions. Furthermore, there are insufficient details reported in the methods to establish whether mice were of the same age before oocyte harvesting.

The effects of glyphosate on testes obtained from rats after exposure to glyphosate over a time period of 14 weeks to 2.5 and 25 mg/kg bw/day (5x and 50x Acceptable Daily Intake, ADI, respectively) was investigated by Johansson *et al.* (2008) (CA 5.6.1/020). No effects were found on the testicular parameters tested, including various endpoints like intra-testicular testosterone levels, expression of Leydig cell specific genes (Cyp11a1, Cyp17a1, Insl3, Hsd3β1 and Star) and expression of somatic marker gene Ar or germ cell marker gene Ddx4, expression of Leydig cell-specific steroidogenesis factors CYP11A1 and STAR. Further, testicular histopathology and apoptosis measurements did not reveal glyphosate induced alterations compared to controls. Thus, glyphosate did not exhibit adverse effects in testes after subchronic exposure.

First results of a subchronic NTP study performed similar to a modified one-generation study are published by Panzacchi *et al.* (2018) (CA 5.6.1/021), describing the effects of glyphosate treatment at the US ADI of 1.75 mg/kg bw/day in rats. Mortality, body weight and body weight gain of dams and offspring was unremarkable. Water and feed consumption during gestation and lactation were not different across groups. Litter sizes were fully comparable among groups. Post weaning water and feed consumption were not affected by treatment. There was no clinical evidence of alterations in activity or behavior, reflexes, eyes or skin, respiratory, gastrointestinal, genitourinary and cardiovascular systems. Thus, no evidence of adverse effects on pregnancy and early life stages due to glyphosate exposure was observed.

A potential effect of glyphosate on ovarian function was investigated by Perego *et al.* (2017) CA 5.6.1/022) in bovine granulosa cells and theca cells under in vitro conditions. Statistically significant effects have been observed at 0.5 and 5 µg/mL (but not at lower test concentrations (0.01 and 0.3 µg/mL)) on cell proliferation in FSH/IGF1 stimulated granulosa cells. The effect was more pronounced at 0.5 µg/mL. Further, estradiol production was reduced only at 5 µg/mL after stimulation with FSH and IGF-1, but not at any other dose. Progesterone production and theca cell proliferation was not affected at any dose. Overall, with the exception of slight, non-dose-related alterations in granulosa cell proliferation under different test conditions and estradiol production at the highest dose tested, this study showed no effects of glyphosate on ovarian function of bovine granulosa and theca cells. Due to the isolated occurrence of the observed effects on proliferation and estradiol production without any dose-response relationship, the biological significance of those findings is rather limited and not considered to indicate hazardous properties of glyphosate on overall ovarion function.

Dai *et al.* (2016) (CA 5.6.1/023) investigated potential toxicity of glyphosate on the male reproductive system in the rat after oral treatment with glyphosate for 5 weeks at dose levels up to 500 mg/kg bw/day. No adverse effects were observed on body weight, food intake, daily weight gain, absolute and relative reproductive organ weight, serum hormone levels, oxidative stress parameters, testicular histopathology and expression of the androgen receptor. A significant decrease in absolute (but not relative) weight of the seminal vesicle and coagulating gland and a decrease in sperm count was determined at the highest dose tested. Likewise, a significant decrease in homogenisation-resistant spermatid count in F0 males was observed at ca 1000 mg/kg bw/day in the study performed by [redacted] (2007) (CA 5.6.1/001 – 003).

In a further study on steroidogenesis, glyphosate was not found to induce any effects on testosterone production (Forgacs *et al.* (2012) (CA 5.6.1/024*)*, thereby indicating that glyphosate did not possess intrinsic properties to influence the steroidogenesis pathway in the applied *in vitro* system.

1. Information on the study

Data point:	**CA 5.6.1/015**
Report author	Gorga, A. *et al.*
Report year	2020
Report title	In vitro effects of glyphosate and Roundup on Sertoli cell physiology
Document No	doi.org/10.1016/j.tiv.2019.104682 E-ISSN: 1879-3177
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	None
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary of the study according to OECD format

The aim of the present study was to analyse whether glyphosate (G) and Roundup (R) are able to affect Sertoli cell functions, such as energy metabolism and blood-testis barrier (BTB) integrity. Sertoli cell cultures from 20–day-old rats were exposed to 10 and 100 ppm of G or R, doses which do not decrease cell viability. Neither G nor R caused impairment in lactate production or fatty acid oxidation. G and R decreased Transepithelial Electrical Resistance, which indicates the establishment of a Sertoli cell junction barrier. However, neither G nor R modified the expression of claudin11, ZO1 and occludin, proteins that constitute the BTB. Analysis of cellular distribution of claudin11 by immunofluorescence showed that G and R induced a delocalisation of the signal from membrane to the cytoplasm.

Materials and methods

Materials: [2,6-3H]-2-deoxy-D-glucose (2-DOG) and [9,10(n)-3H] palmitic acid were purchased from NEN (Boston, MA, USA). Culture media, glyphosate and all other drugs and reagents were purchased from SigmaAldrich (St Louis, MO, USA). The glyphosate formulation used in this work was the formulation available on the market called Roundup Full II (Monsanto Argentina S.A.I.C.), which contains 54 % w/v acid glyphosate.

Sertoli cell (SC) isolation and culture: Twenty–day-old Sprague-Dawley rats (Rattus norvegicus) were obtained from the Animal Care Laboratory, Facultad de Ciencias Veterinarias, Buenos Aires, Argentina. Animals were killed by CO2 asphyxiation according to protocols for animal laboratory use following the

principles and procedures outlined in the National Institute of Health Guide for Care and Use of Laboratory Animals. The protocol was approved by the Comité Institucional de Cuidado y Uso de Animales de Laboratorio (CICUAL) from the Hospital de Niños "Dr. Ricardo Gutiérrez". SC were isolated as previously described. Decapsulated testes were digested with 0.1 % w/v collagenase and 0.006 % w/v soybean trypsin inhibitor in Hanks' balanced salt solution (HBSS) for 5 min at room temperature by manual agitation. The enzymatic action was stopped by dilution with four volumes of HBSS. Seminiferous tubules were collected by sedimentation and washed twice with HBSS. Then seminiferous tubules were cut with a razor and submitted to 1M glycine-2mM EDTA (pH 7.4) treatment for 10 min to remove peritubular cells. At the end of the incubation period, nine volumes of HBSS were added and a 30 min sedimentation was performed. The washed tubular pellet was then digested again with 0.1 % w/v collagenase and 0.006 % w/v soybean trypsin inhibitor in HBSS for 10 min at room temperature by continuous pipetting. The enzymatic action was stopped by dilution with four volumes of HBSS. The cell suspension was collected by centrifugation at 200 x g for 3 min. The cell suspension was diluted with HBSS and submitted to a 10 min sedimentation to remove germ cells. The pellet containing SC was filtered through a nylon mesh and SC were recovered by centrifugation at 200 x g for 3 min. SC were resuspended in culture medium which consisted of a 1:1 mixture of Ham's F-12 and Dulbecco's modified Eagle's medium, supplemented with 10mM HEPES, 100 IU/mL penicillin, 2.5 µg/mL amphotericin B, 1.2 mg/mL sodium bicarbonate, 10 µg/mL transferrin, 5 µg/mL insulin, 5 µg/mL vitamin E and 4 ng/mL hydrocortisone. SC were cultured on 6-, 24- or 96-multiwell plates (5 µg DNA/cm^2), on Matrigel-coated cell culture inserts (15 µg DNA/cm^2) placed on 24-multiwell plates or on glass coverslips coated with laminin at 34 °C in a mixture of 5 % CO2:95 % v/v air. No myoid cell contamination was revealed in the cultures when an immunoperoxidase technique was applied to SC cultures using a specific antiserum to smooth muscle α actin. Remaining cell contaminants were of germ cell origin and this contamination was below 5 % after 48 h in culture as examined by phase contrast microscopy.

Culture conditions: SC were allowed to attach for 48 h in the presence of insulin and medium was replaced at this time with fresh medium without insulin. Treatment with glyphosate (G) and Roundup (R) was performed with variable doses and for variable periods of time. Cells incubated for 48 h with 10, 100 and 1000 ppm of G and R harvested on day five were used to evaluate cell viability and LDH leakage. The cells treated for 48 h with 100 ppm of G and R were harvested on day five and used to evaluate GLUT1, FAT/CD36 and CPT1 mRNA levels, glucose uptake and fatty acid (FA) oxidation and the 48 h-conditioned media were utilised to evaluate lactate production. For Western blot studies, cells cultured for 4 days under basal conditions and pretreated for 30 min with 10 and 100 ppm of G or R were used. To quantify Transepithelial Electrical Resistance (TER), SC were cultured at high cell density (15 µg DNA/cm^2, corresponding to 1.2 × ^{106} cells/cm^2) on Matrigel-coated (1,6 dilution with F12/DMEM v/v) cell culture inserts (Millicell HA inserts) (Millipore, Billerica, MA, USA) placed on 24-multiwell plates. On day 3 in culture, testosterone was added and TER across the SC monolayer was recorded every 24 h in culture. On day 5, when the tight junction barrier had been formed, different doses of G or R were added and TER was recorded until day 8. To study the distribution and localisation of claudin11, the cells were cultured on glass coverslips coated with laminin and treated with 100 ppm G or R in the presence or absence of testosterone for 48 h and harvested on day 5.

Evaluation of Sertoli cell energetic metabolism: Energetic metabolism in SC has been considered to have features of its own. Lactate, produced by SC, provides the energetic substrate to germ cells in the adluminal compartment. Consequently, it has been postulated that SC utilizes FA as their energy source. In this context, lactate production, glucose uptake, FA oxidation and the expression of genes involved in these processes were evaluated.

- *Lactate determination:* Conditioned media obtained from cells cultured in 24-multiwell plates were used to determine lactate production. Lactate was measured by a standard method involving conversion of NAD+ to NADH.
- *Measurement of 2-deoxyglucose (2-DOG) uptake:* Glucose transport was studied using the uptake of the labelled non-metabolizable glucose analogue 2-DOG on cells cultured in 24-multiwell plates as previously described.

- *Fatty acid oxidation assay:* FA oxidation was performed measuring the release of ^3H_2O to the incubation medium from [^{3}H]-palmitate on SC cultured in 24-multiwell as previously described.

Evaluation of BTB function: The main component of the BTB is the presence of tight junctions between neighboring SC. In order to evaluate BTB function, Transepithelial Electrical Resistance (TER), claudin11 cellular distribution and the expression of proteins that participate in tight junction assembly were evaluated.

a) *Transepithelial Electrical Resistance (TER) measurement:* The establishment of the SC junction barrier was assessed daily from day 3 to day 8 by measurement of TER across the SC monolayer by a Millicell electrical resistance system (Millipore), as described previously. Briefly, a short (~2 s) 20-µA pulse of current was passed through the epithelial monolayer between 2 silver-silver chloride electrodes and electrical resistance was measured. Electrical resistance was then multiplied by the surface area of the insert to yield the area of resistance in ohms.cm^2. The net value of electrical resistance was then computed by subtracting the background, which was determined by Matrigel-coated cell-free inserts. Each time point had quadruplicate bicameral units. This experiment was run four times on different batches of cells.
b) *Immunofluorescent (IF) detection of claudin11 protein:* Monolayers were fixed with methanol for 10 min at -20 °C. After washes with PBS, cells were permeabilised with 0.1 % Triton X-100 in PBS for 30 min at room temperature. After 3 washes with PBS for 1 min each, the cells were blocked with 5 % bovine serum albumin (BSA). Then, the coverslips were incubated with a 1:50 dilution of polyclonal antibody against claudin11 in PBS overnight at 4 °C. After 3 washes with PBS for 1 min each, coverslips were incubated with an anti-rabbit IgG fluorescein isothiocyanate (FITC)-conjugated (1,25; Vector Laboratories, Burlingame, CA, USA). For negative controls, primary antibodies were replaced by PBS. Finally, the coverslips were washed 3 times with PBS for 1 min each, mounted in buffered glycerine and observed using an Axiophot fluorescent microscope with epi-illumination (Carl Zeiss Inc., Oberkochen, Germany).

RT-Real-time PCR (RT-qPCR): The expression of genes that participate in energetic metabolism (GLUT1, FAT/CD36 and CPT1) and in BTB organisation (occludin, claudin11 and ZO-1) was evaluated by RT-qPCR. Total RNA was isolated from SC cultured in 6-multiwell plates with TRI Reagent (Sigma-Aldrich). The amount of RNA was estimated by spectrophotometry at 260 nm. Amplification was carried out as recommended by the manufacturer: 25 µL reaction mixture containing 12.5 µL of SYBR Green PCR Master mix (Applied Biosystems), the appropriate primer concentration and 1 µL of cDNA. The relative cDNA concentrations were established by a standard curve using sequential dilutions of a cDNA sample. The data were normalised to HPRT1. The amplification program included the initial denaturation step at 95 °C for 10 min, 40 cycles of denaturation at 95 °C for 15 s, and annealing and extension at 60 °C for 1 min. Fluorescence was measured at the end of each extension step. After amplification, melting curves were acquired and used to determine the specificity of PCR products. The comparative ΔΔCt method was used to calculate relative gene expression.

Western blot analysis: Cells cultured in 6-multiwell plates were washed once with PBS at room temperature. Then, 200 µL of PBS containing 2 µL of protease inhibitor cocktail (P-8340; Sigma-Aldrich), 1 mM NaF, 1 mM EGTA, 1 mM EDTA, 50 nM okadaic acid and 2 mM PMSF was added to each well. Cells were then placed on ice and disrupted by ultrasonic irradiation. Western blot analysis was performed as previously described. Membranes were probed with antibodies that allow specific recognition of total Akt and mTOR, phosphorylated p38-MAPK and ERK1/2 (Cell Signaling Technology, Inc., Danvers, MA, USA), claudin11 (Zymed Lab. Inc.), androgen receptor and GAPDH (Santa Cruz Biotechnology, Inc., USA). A 1:1000 dilution of primary antibodies, as indicated by the manufacturer, was used. For chemiluminescent detection of the blots, a commercial kit from Cell Signaling Technology was used. The intensities of the autoradiographic bands were estimated by densitometry scanning using NIH Image Software (Scion Corporation). Levels of the corresponding total Akt, mTOR and GAPDH served as loading controls.

Cytotoxicity: A cell viability test (MTT assay) was performed in cells cultured in 96-multiwell using a commercial kit (CellTiter 96® AQueous NonRadioactive Cell Proliferation Assay; Promega Corporation). Cell cytotoxicity was determined by measuring the activity of LDH enzyme leaked from the cytosol of damaged cells into the medium as previously described. Results were expressed as the percentage of activity detected in the media over the sum of the activities in the media and in cells.
Statistical analysis: All experiments were run in triplicates and repeated 3–4 times. One way ANOVA and post hoc analysis using Tukey-Krämer's multiple comparisons test were performed using InfoStat versión 2016 (Grupo InfoStat, FCA, UNC, Argentina). P values <0.05 were considered statistically significant.

Results
Effects of glyphosate and Roundup on SC cell viability: SC cultures were exposed for 48 h to glyphosate (G) and Roundup (R) at concentrations ranging from 10 to 1000 ppm, corresponding to 0.01 to 1 g/L respectively. Cell viability was analysed by MTT assay and by measuring LDH leaked from the cytosol of damaged cells into the medium. The highest dose tested for R (1000 ppm) caused a cell death (Fig. 1). Therefore, in the present investigation destined to analyse G and R effects on SC functions only doses of 10 and 100 ppm were utilised.

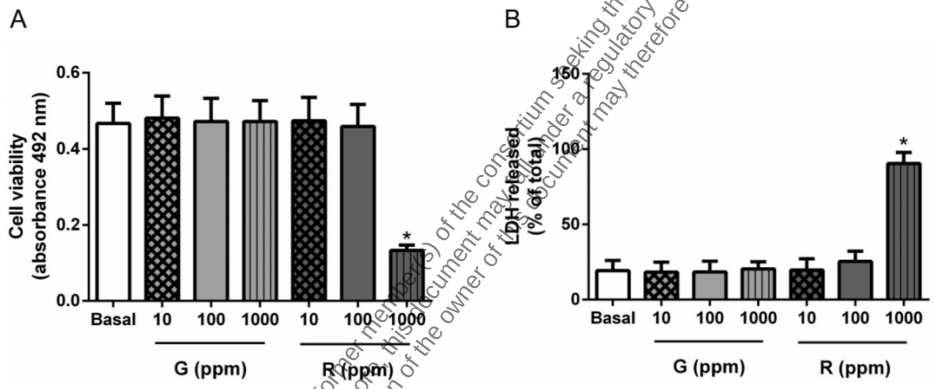


Fig. 1. Effect of G and R on SC cytotoxicity.
SC were maintained under Basal conditions or incubated with 10, 100 or 1000 ppm of G or R for 48 h. (A) Cell viability was determined by MTT assay. (B) LDH activity was determined in SC monolayer and in the culture medium. Values represent mean ± S.D. of one representative experiment out of three. *p < 0.05 versus Basal.

Effects of glyphosate and Roundup on SC energetic metabolism: SC cultures were exposed for 48 h to 100 ppm of G and R. Fig. 2 shows the results obtained for lactate production, glucose uptake and GLUT1 mRNA levels. The exposure to G or R did not modify lactate production neither glucose uptake nor GLUT1 expression.

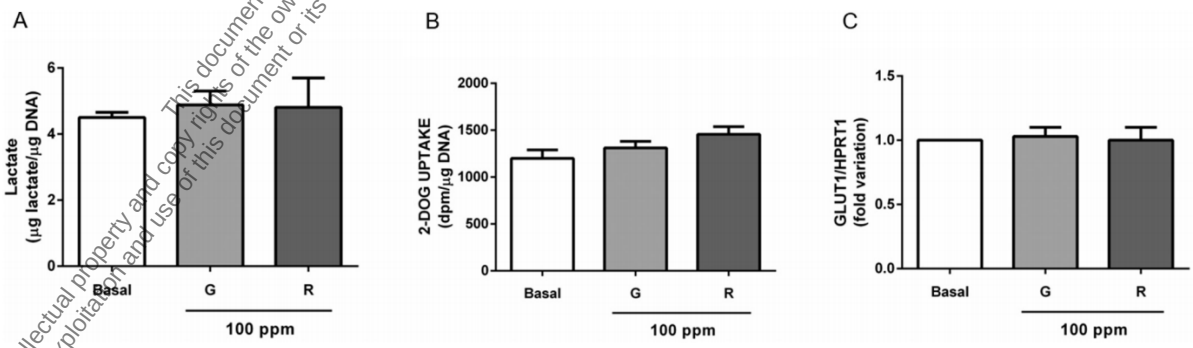


Fig. 2. Effect of G and R on lactate production, glucose uptake and on *Glut1* mRNA levels in SC.
SC were maintained under Basal conditions or incubated with 100 ppm of G or R for 48 h. (A) Lactate levels were determined in the conditioned media. (B) Glucose uptake assay (2-DOG uptake) was performed after the 48 h incubation period. (C) Total RNA was extracted and RT-qPCR was performed to detect *Glut1* mRNA levels. The comparative ΔΔCt method was used to calculate relative gene expression. Graphics show pooled data from four independent experiments performed indicating fold variation in mRNA levels relative to Basal.

Effects of glyphosate and Roundup on blood-testis barrier integrity: SC supply germ cells with a microenvironment preserved by the BTB. The main component of the BTB is the presence of tight junctions

between neighboring SC. The establishment of these junctions between SC in culture was assessed daily from day 3 to day 8 by measuring TER across the SC monolayer. When SC were plated, junctions begin to assemble and an increase in TER was observed. On day 5, when SC completed barrier assembly, G or R were added to the culture medium. Compared with Control (92.3±3.1), a significant decline in TER was produced by the addition of 100 ppm of G (58.0±1.5) or 10 and 100 ppm of R (67.8±2.8 or 62.2±4.9, respectively) (Fig. 4 and Table 2).

Table 2
Effect of 48-h treatment with G and R on TER.

	TER ($\Omega \cdot cm^2$)
Control	92,3 ± 3,1
G 10 ppm	85,5 ± 4,5
G 100 ppm	58,0 ± 1,5*
R 10 ppm	67,8 ± 2,8*
R 100 ppm	62,2 ± 4,9*

SC monolayers were maintained under Basal conditions or treated with 10 or 100 ppm of G or R on day 5 for 48 h. Results are presented as means ± SD of four independent experiments ($*p < 0.05$).

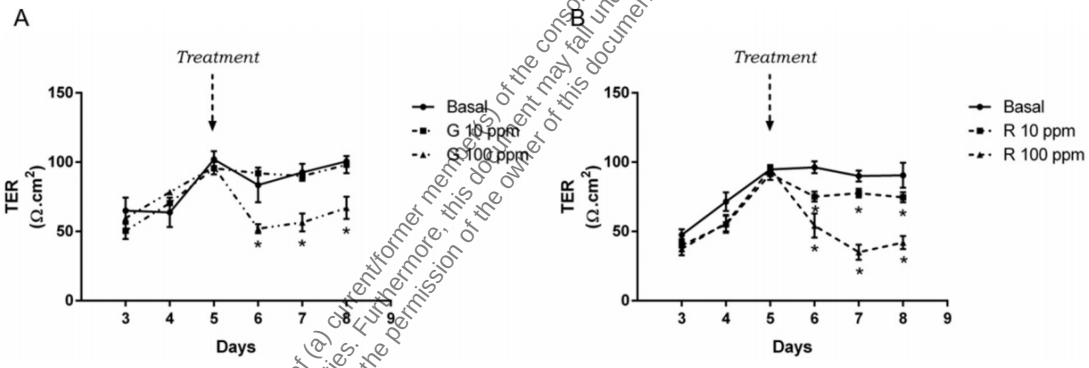


Fig. 4. Effect of G and R on TER across SC.
SC monolayers were maintained under Basal conditions or treated with 10 or 100 ppm of G (A) or R (B) on day 5. TER across SC monolayer was measured from day 3 to 8. Values represent mean ± S.D. of triplicate wells from the same culture of one representative experiment out of four. Asterisks indicate significant differences from basal cultures for each particular day, $p < 0.05$.

Considering that it had been demonstrated that p38-MAPK and ERK1/2 signaling pathways were involved in the disruption of BTB integrity by xenobiotics, the possible alteration of these pathways by G or R treatment was evaluated. R increased P-p38-MAPK and P-ERK1/2 levels. G did not modify P-p38-MAPK and P-ERK1/2 levels at any dose tested. 100 ppm G or R treatment did not modify *claudin11*, neither *occludin* nor *ZO-1* mRNA levels. Claudin11 protein levels were not modify by G or R treatment. In control conditions, claudin11 was detected at the zone of contact between adjacent cells, in a linear and continuous pattern that delineated cell borders in basal conditions. Addition of 100 ppm G or R induced redistribution of claudin11 since immunofluorescence became discontinuous and was redistributed from the cell surface into the cytoplasm.

Effects of glyphosate and Roundup on testosterone regulation of bloodtestis barrier integrity: It is well known that testosterone is the main regulator of BTB function, and that G or R can act as endocrine disruptors. In order to elucidate a possible mechanism responsible for adverse effects of G or R, we decided to evaluate whether herbicides can interfere with androgen action in BTB. Fig. 8A and B shows that 100 ppm G or R treatment did not modify androgen receptor mRNA or protein levels. Fig. 9 shows that, similar to what was observed under basal conditions, 100 ppm G or R treatment induced a redistribution of claudin11 from cell membrane to cytoplasm under the presence of testosterone.

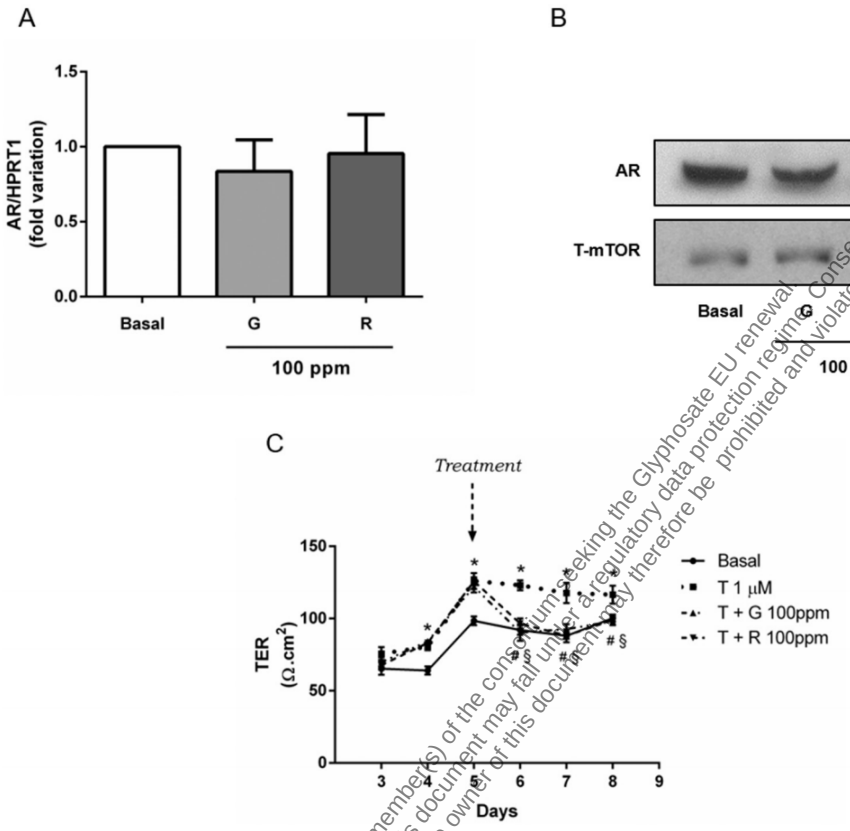


Fig. 8. Effect of G and R on androgen receptor (AR) expression and testosterone regulation of TER across SC.
SC were maintained under Basal conditions or incubated with 100 ppm of G or R for 48 h. A) Total RNA was extracted and RT-qPCR was performed to detect AR mRNA levels. The comparative ΔΔCt method was used to calculate relative gene expression. Graphics show pooled data from four independent experiments performed indicating fold variation in mRNA levels relative to Basal. B) Western blot analysis was performed utilizing antibodies for AR or total Akt (T-Akt). Results are representative of three independent experiments performed/treatment group. C) SC monolayers were maintained under Basal conditions or stimulated with testosterone (T) since day 3. On day 5, SC monolayers were treated with 100 ppm of G or R. TER across SC monolayer was measured from day 3 to 8. Values represent mean ± S.D. of triplicate wells from the same culture of one representative experiment out of three. Symbols indicate significant differences for each particular day: *p < 0.05 T vs Basal; #p < 0.05 T vs T + G; §p < 0.05 T vs T + R.

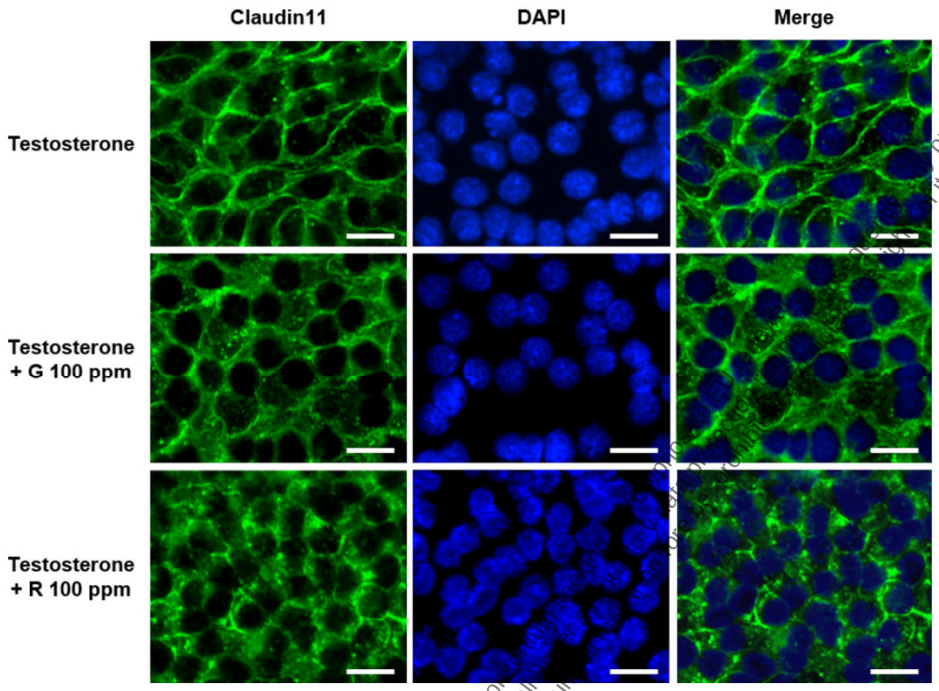


Fig. 9. Effect of G and R on claudin11 localization in the presence of testosterone in SC.
SC were incubated with 100 ppm of G or R in the presence of testosterone for 48 h. Claudin11 was revealed by IF. Bars: 50 µm.

Discussion

Over the last 60 years a progressive decrease in male reproductive function has been observed. Epidemiological and experimental studies suggest that one of the main causes is exposure to environmental toxicants. As previously stated, several studies have shown that G or R can potentially cause adverse effects in male reproduction. Despite the compelling documented evidence proving the existence of adverse effects on testis function, little is known about direct effects on Sertoli cell function and the possible mechanisms involved. Primary cultures of Sertoli cells are a good and reliable model to assess the direct effects of xenobiotic exposure on this cell type. Regarding G and its commercial formulation R, initial studies on Sertoli cell cultures utilizing doses from 1 to 10,000 ppm of G and R were performed. In these experiments, treatment with 1000 ppm R produced Sertoli cell apoptosis with a maximal effect in 24 h-incubation period. However, in the same study G did not induce apoptosis in isolated Sertoli cells. Other studies, which measured apoptosis and/or cell viability, have also demonstrated that several human cell lines are more sensitive to R than to G. Coincidentally, the results presented herein show that 1000 ppm R decreases cell viability while G does not. These differences on the actions of both agents can be interpreted by the presence of various adjuvants in R. These adjuvants change human cell permeability and amplify toxicity by G, which may explain the differences observed between R and G effects on Sertoli cell viability. As we were focused on analyzing possible effects on Sertoli cell function, in this study we utilised 10 and 100 ppm doses which do not decrease cell viability. As mentioned in the introduction, two essential functions of mature Sertoli cells are the provision of nutrients to germ cells and the maintenance of a favorable microenvironment for spermatogenesis. As for Sertoli cell nutritional function, it has been well documented that Sertoli cell glycolysis provides lactate to satisfy germ cell energy demands while FA oxidation supplies energy to fulfill Sertoli cell energetic demands. Consequently, it can be predicted that an alteration in Sertoli cell metabolism can lead to a perturbation in normal germ cell development. The results obtained in the present investigation show that neither G nor R modify lactate production, glucose uptake and GLUT1 expression in Sertoli cells. Additionally, the results show that G and R do not modify FA oxidation and FAT/CD36 and CPT1 expression, proteins that are essential for FA utilisation. Altogether, the results presented herein lead us to conclude that G and R effects on testicular function are not mediated by impairment of Sertoli cell metabolism. Noticeably, other reproductive toxicants, including phthalate esters, nitro-benzene, gossypol and certain heavy metal ions, alter Sertoli cell lactate production and show that

toxicant effects strongly depend on the chemical nature of the toxicant. Regarding lipid metabolism, it has been demonstrated that 24 h exposure to 5000 ppm G or R induce lipid droplet accumulation in the Sertoli cell line TM4 cytoplasm. This increase in lipid droplet accumulation was interpreted as storage of potentially deleterious lipophilic formulants in the cytoplasm of TM4 cells and was considered a sign of the cytotoxic effect. An alternative explanation for the above results would be a modification in lipid metabolism, however, the authors did not explore this possibility. We have explored the latter possibility and the results presented herein show that 100 ppm G and R, doses that do not affect Sertoli cell viability, do not alter FA oxidation. It is worth mentioning that Sertoli cells, in addition to the nourishment provided to germinal epithelia, supply germ cells with a suitable microenvironment for successful meiosis and completion of spermatogenesis. This microenvironment is sustained by the BTB, whose main components are the tight junctions between neighboring Sertoli cells. The BTB is highly dynamic and is regulated by an array of intriguingly coordinated signaling pathways and molecules. Several studies have shown that many environmental toxicants, such as cadmium, bisphenol A, fluoride and sulfur dioxide exert their effects by targeting Sertoli and germ cell junctional proteins, as well as the permeability of the BTB. TER is a widely accepted quantitative technique that measures the integrity and permeability of BTB in vitro. The results presented in this investigation show that 100 ppm G and 10 and 100 ppm R decrease TER after 24 and 48 h-treatment. Notable, the disrupting effect of G on the permeability of other barriers had already been observed. It was shown that exposure to 10 mg/mL (10,000 ppm) G reduces TER and increases permeability to mannitol in Caco-2 and IEC-18 intestinal cell lines. In addition, 1 and 10 µM G treatment (0.16 and 1.6 ppm) decreases TER and increases permeability to fluorescein in iPSC-derived brain microvascular endothelial cells cell line (BMECs). Results from other authors and our own let us postulate that one of the mechanisms by which G exerts toxic effects is related to disruption of barrier properties in different important organs such as intestine, brain and testis. It has been shown that p38-MAPK and ERK1/2 pathways play central roles in the dynamics of BTB. For example, TGFb3-induced physiological effect on Sertoli cell BTB dynamics is mediated via the p38-MAPK pathway. As for toxicant effects, it has been demonstrated that the polychlorinated biphenyls (PCBs), such as congener Aroclor1254, and the perfluorooctane sulfonate (PFOS) can disrupt the BTB integrity by activating the p38-MAPK pathway. The present investigation shows that R increases P-p38-MAPK and PERK1/2 levels. Therefore, it might be suggested that R decreases TER through a p38-MAPK and ERK1/2 dependent pathways. However, the same reasoning cannot be applied to the effects of G considering that it lowers TER while it does not modify P-p38-MAPK and P-ERK1/2 levels. Hence, it seems that there is no direct relationship between P-p38-MAPK and P-ERK1/2 levels and TER levels, at least as a consequence of G and R exposure. We then decided to look for further mechanisms that may be involved in the disruption of BTB integrity by exposure to G and R. The next set of experiments was devoted to analyse possible alterations in the expression of some tight junction proteins, such as occludin, claudin11 and ZO-1, in response to G or R. An increased expression of these proteins, at the time when the junctions are assembled as manifested by a stable TER across the Sertoli cell epithelia, was demonstrated. It is worth mentioning that certain agents that perturb BTB permeability, such as cytokines or toxicants, alter the expression of cell junction proteins. The results obtained in the present investigation show that neither occludin nor claudin11 or ZO-1 expression was modified by G or R treatment. Finally, we decided to investigate whether the cellular localisation of a tight junction protein such as claudin11 could shed some light on the mechanism underlying the decrease in TER. Claudin11 is a key molecule that provides functional integrity to the BTB. It is located in functional Sertoli cell tight junctions and its intracellular distribution pattern changes when gonadotropins are suppressed, coincident with a dysfunctional barrier. Claudin11 null mice are sterile highlighting the importance of this protein for BTB integrity. It is worth mentioning that in men with testicular disorders such as intraepithelial neoplasia, hypospermatogenesis, spermatogenic arrest, and Sertoli cell only testes, claudin11 is located in the cytoplasm, away from the tight junctions reinforcing the idea that localisation of claudin11 in cytoplasm can be considered a sign of BTB dysfunction. In in vitro studies, a direct relationship between TER and localisation of claudin11 in membrane junctions has been observed. The results presented herein show that although G and R treatment does not modify claudin11 protein levels, a redistribution of claudin11 from membrane to cytoplasm is observed. This alteration in the distribution of claudin11 may be interpreted as the result of an increase in membrane protein recycling from cell surface to cytoplasm. This last result may explain, at least in part, the effects of G and R in the integrity of BTB and the deleterious effect of these toxicants at testicular level. Disruption of the Sertoli cell permeability

barrier by loss of usual distribution patterns of other junctional proteins such as occludin, ZO-1, and Cx43 induced by other toxicants has also been demonstrated and these results indicate that redistribution of these proteins can also contribute to alter barrier permeability. Further studies will be necessary to definitively assign a role to this phenomenon in the alteration of BTB dynamics by G or R. Several lines of evidence obtained from in vivo and in vitro approaches highlight the importance of testosterone in the regulation of BTB assembly and function. In vivo studies showed that reestablishment of testicular androgen levels by hCG or testosterone treatment leads to a restoration of claudin11 localisation and BTB function. In vitro studies showed that Sertoli cell permeability barrier formation and claudin11 localisation are promoted by androgen treatment. Furthermore, numerous studies describe G or R as endocrine disruptors. On the one hand, it was demonstrated that in vivo treatment of drakes with R decreases the expression of the androgen receptor (AR) in Sertoli cells while in rats, G treatment does not modify AR expression in the same cell type. On the other hand, it was shown that in vivo treatment of rats decreases serum testosterone levels, and furthermore, it was demonstrated that the effect on testosterone levels may be attributed to a decrease in the expression of STAR in Leydig cells. Therefore, it is tempting to speculate that G or R effects on BTB integrity can be partially attributed to the interference with androgen action. The results presented herein show that neither G nor R treatment modified androgen receptor mRNA and protein levels. They also show that the effects of the herbicides on TER and on claudin11 localisation in the presence of testosterone were similar to those observed under basal conditions. Thus, G and R are able to disrupt BTB function in the presence of testosterone. Further in vivo experiments will be necessary to determine a possible role of G or R as endocrine disruptors.

Conclusion

In summary, this investigation shows that G and R alter the Sertoli cell junction barrier permeability. This study also shows that, at least in part, the loss of location of claudin11 at the interface between neighboring Sertoli cells might be responsible for the disassemble of the barrier. We postulate that BTB integrity is a sensitive target for the adverse effects of G or R on male reproductive function.

7. Assessment and conclusion

Assessment and conclusion by applicant:

This *in vitro* investigation showed that exposure to G and R at 100 ppm alters Sertoli cell (SC) junction barrier permeability, measured by decreased TER, and also decreased testosterone-stimulated TER. This study also showed that, at least in part, the loss of location of claudin11 at the interface between neighboring Sertoli cells might be responsible for the disassembly of the barrier. G or R did not modify androgen receptor mRNA or protein levels, nor did G modify P-p38-MAPK and P-ERK1/2 signalling pathways involved with BTB integrity at any doses tested, or affect the expression of intercellular junction proteins (claudin11, occludin and ZO-1). However, G and R induced redistribution of claudin11 at the zone of contact between cells. Neither G nor R modified lactate production, glucose uptake, GLUT1, FA oxidation, or *FAT/CD36* and *CPT1* expression in SC, thus indicating no effect of G or R on SC nutritional function or metabolism.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate used was not sufficiently characterised, there was no positive control and most of the endpoints were tested at only 2 concentrations preventing any dose-response evaluations, with the highest concentration exceeding a physiologically relevant dose.

Reliability criteria for *in vitro* toxicology studies

Publication: Gorga *et al*., 2020	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y?	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	N	Purity not reported. Source: Sigma-Aldrich, St Louis, USA.
Only glyphosate acid or one of its salts is the tested substance	N	Also formulation was tested: Roundup Full II (Monsanto, Argentina)
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	Y	
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described		
Test concentrations in physiologically acceptable range (< 1 mM)	Y	2 test concentrations used of which the highest was > 1 mM (1000 µg/mL).
Cytotoxicity tests reported	Y	
Positive and negative controls	N	No positive control reported.
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported		Explored but not found (only 2 concentration levels tested for most endpoints)
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate used was not sufficiently characterised and most of the endpoints were tested at only 2 concentrations.		

1. Information on the study

Data point:	**CA 5.6.1/016**
Report author	Manservisi, F. *et al.*
Report year	2019
Report title	The Ramazzini Institute 13–week pilot study glyphosate-

	based herbicides administered at human-equivalent dose to Sprague Dawley rats: effects on development and endocrine system
Document No	doi.org/10.1186/s12940-019-0453-y E-ISSN: 1476-069X
Guidelines followed in study	Pilot study based on OECD guideline 443: Extended one-generation reproductive toxicity study.
Deviations from current test guideline	Yes, only one dose level for each test item and insufficient number of animals per dose level used.
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary of the study according to OECD format

The present pilot study examines whether exposure to glyphosate-based herbicides (GBHs) at the dose of glyphosate considered to be "safe" (the US Acceptable Daily Intake - ADI - of 1.75 mg/kg bw/day), starting from in utero life, affect the development and endocrine system across different life stages in Sprague Dawley (SD) rats. In pups, anogenital distance (AGD) at PND 4 was statistically significantly increased both in Roundup-treated males and females and in glyphosate-treated males. Age at first estrous (FE) was significantly delayed in the Roundup-exposed group and serum testosterone concentration significantly increased in Roundup-treated female offspring from the 13–week cohort compared to control animals. A statistically significant increase in plasma TSH concentration was observed in glyphosate-treated males compared with control animals as well as a statistically significant decrease in DHT and increase in BDNF in Roundup-treated males. Hormonal status imbalances were more pronounced in Roundup-treated rats after prolonged exposure.

Materials and methods

Chemicals - Glyphosate (purity of > 99.5 %), Pestanal™ analytical standard purchased from Sigma-Aldrich (Milan, Italy). The representative formulated product, Roundup Bioflow (MON 52276, containing 360 g/L of glyphosate acid in the form of 480 g/L isopropylamine salt of glyphosate (41.5 %), water (42.5 %) and surfactant (16 %)) was purchased from Consorzio Agrario dell'Emilia, Bologna, Italy.

Animals and experimental design - Each of the 24 virgin female SD rats (17 weeks old, 270–315 g) was mated outbred with one breeder male rat of the same age and strain. Every day, the females were examined for the presence of sperm. After evidence of mating, females were housed separately during gestation and delivery. Newborns were housed with their mothers until weaning. Weaned offspring were co-housed, by sex and treatment group, not more than 3 per cage. Cages were identified by a card indicating study protocol code, experimental and pedigree numbers, and dosage group. The cages were placed inside a single room at 22 °C ± 3 °C and at 50 ± 20 % relative humidity. A light/dark cycle of 12 hours was maintained. Two groups of dams and their pups were treated with either glyphosate or MON 52276 diluted in drinking water to achieve the desired glyphosate dose of 1.75 mg/kg bw/day. The F0 female breeders received the test item from gestation day (GD) 6 to the end of lactation, while the offspring (F1) continued to be exposed after weaning for an additional 6 or 13 weeks. Glyphosate or MON 52276 solutions were freshly prepared on a daily basis taking into account body weight and water consumption. During pregnancy and lactation, embryos and offspring (F1) were all retained in the litter and received the test compounds mainly through their dams (F0). The day of birth was designated post-natal day 1 (PND 1) for pups and lactation day 1 (LD 1) for dams. After weaning, the offspring (F1) were treated via the drinking water until sacrifice. On PND 28, offspring were randomly distributed in two cohorts: 8/sex/group of the 6–week cohort and 10/sex/group of the 13–week cohort. Altogether, 108 rats (54 males and 54 females) were enrolled in the post-weaning treatment phase.

Measurements in F0 dams and litters prior to weaning - Mean gestational length was calculated as the number of days from detection of a positive vaginal smear (GD 0) to birth of a litter. Pregnancy was

confirmed by the occurrence of parturition. The body weight of the dams was recorded on GD 0, 3, 6 and then daily during gestation until parturition. During lactation, the body weight of the dams was recorded at LD 1, 4, 7, 10, 13, 16, 19, 21 and 25 (last measurement before weaning). Body weight of the pups by sex and litter was determined on PND 1, 4, 7, 10, 13, 16, 19, 21 and 25. Feed and water consumption of the dams was recorded twice weekly during gestation on GD 0, 3, 6, 9, 12, 15, 18, and 21, and twice weekly during lactation on LD 1, 4, 7, 10, 13, 16, 19, 21 and 25. Dead pups were removed when found and sexed when possible. Sex was determined on PND 1. The mean litter size was assessed on PND 0 (within 24 hours from delivery), 1, 4, 7, 10, 13, 16, 19, 21, and 25. Litter size included dead as well as live offspring. Dead pups were visually examined by floating the lungs in saline, to distinguish if they were stillborn (died in utero) or died shortly after birth. Live-birth index was determined on PND 0 as (number of pups born alive / total number of pups born) × 100. Survival index, calculated as (total number of live pups at designated time point / number of live pups born) × 100, was measured on PND 1, 4, 7, 10, 13, 16, 19, 21 and 25. For all the pups, anogenital distance (AGD) was measured and body weight recorded on PND 4.

Post weaning endpoints up to adulthood - After weaning, body weight was measured twice a week, until PND 73 ± 2, then weekly until PND 125 ± 2 and before terminal sacrifice, the means of individual body weights were calculated for each group and sex. Daily water and feed consumption per cage were measured twice a week, until PND 73 ± 2, then weekly until PND 125 ± 2. Time to vaginal opening (VO) was determined by daily inspection of all female pups starting on PND 28. The body weight of each female was recorded on the day VO was observed. Time to balano-preputial separation (BPS) was determined by daily inspection of all males beginning on PND 35. The body weight of each male was recorded on the day BPS was observed. The female rats belonging to the developmental (6–week) cohort were also monitored for the time to first estrous (FE).

Estrous cycle characterisation - Starting on approximately PND 95 and for the duration of 3 weeks, daily vaginal lavage was performed on female rats of the 13–week cohort. To reduce variability, vaginal cytology samples were collected by vaginal lavage at the same time of the day over the course of the experiment, in the mid-morning, between 10:00 and 13:00 h. Collection, processing and vaginal smear evaluation was performed as described previously.

Necropsy - Five days after weaning, dams were sacrificed and following tissues were collected and fixed in alcohol: mammary glands (4 sites: axillary and inguinal, right and left), adrenal glands, uterus (including cervix), ovaries, and vagina. The adrenal glands, uterus and ovaries were also weighed. For the determination of serum testosterone, blood was collected and serum prepared by centrifugation and stored at -80 °C pending analysis. All male and female pups belonging to both cohorts were sacrificed on PND 73 ± 2 for the 6–week cohort and PND 125 ± 2 for the 13–week cohort. Following tissues were collected for histopathology and fixed in alcohol: mammary gland (4 sites: axillary and inguinal, right and left), thyroid and parathyroid, adrenal glands, bladder and prostate, seminal vesicles and coagulating gland, left and right testis with epididymis (half of the right testis and the whole right epididymis were frozen in liquid nitrogen and stored at -80 °C until evaluation), uterus (including cervix), ovaries and vagina. During necropsy, all tissues with gross lesions were removed for histopathology. Adrenal glands, bladder and prostate, seminal vesicles and coagulating gland, left testis, left epididymis, uterus (including cervix) and ovaries were weighed. In case of paired organs, both organs were preserved. Organ weight was expressed as absolute and relative organ weight. Rats were sacrificed randomly across the 4 stages of the estrous cycle. In order to determine and allow correlation with histopathology in reproductive organs and hormone analysis, the stage of estrous cycle was determined by histological appearance of the various components of the reproductive tract for F1 females of the 6–week cohort or by a vaginal smear examined on the day of necropsy for F1 females of the 13–week cohort.

Sperm analysis - Sperm analyses were performed on each male from both cohorts, at scheduled necropsies on PND 73± 2 and PND 125 ± 2. At necropsy, half of the right testis and the whole right epididymis were frozen in liquid nitrogen and stored at -80 °C until evaluation. Spermatids resistant to homogenisation and spermatozoa present in the caput/corpus and cauda epididymis were counted. The tunica albuginea was removed from the (half) testicle, and a sample of the parenchyma was weighed and homogenised in 5 mL

saline-TritonX-100 at 0.05 %. The samples were then diluted 10-20 times in saline, and the mature spermatids resistant to homogenisation (step 17-19 spermatids) were counted using a Thoma chamber. Four fields per animal were recorded, and the numbers of spermatids per gram of testis were calculated. To calculate the daily sperm production (DSP) these values were subsequently divided by 6.1, which is the number of days step 17-19 spermatids are present in the seminiferous epithelium. Similarly, the segments of the epididymis (caput, corpus and cauda) were cut with a scissor, weighed, homogenised, diluted and counted as described for the testes. The number of spermatozoa in each homogenate was determined and the total number of spermatozoa for each segment of the epididymis calculated. The epididymal sperm transit time through the epididymal caput/corpus and cauda was calculated by dividing the number of spermatozoa present in each portion of the epididymis by the DSP of the associated testis. To assess the percentage of morphologically abnormal sperm half of left cauda epididymis of each rat was transferred to a Petri dish containing 2.5 mL (for 70 day old animals) or 3.5 mL (for 120 day old animals) of Dulbecco's PBS at 37 °C, cut in 2-3 pieces and incubated for approximately 3 minutes at 37 °C with gently swirling to facilitate release of sperm cells from the cauda. Dried smears of epididymal spermatozoa were stained with 1 % Eosin Y for 30 minutes and evaluated at 400 x magnification. Five hundred spermatozoa per rat were evaluated and scored as morphologically normal or abnormal according to the presence or absence of head or tail defects.

Histopathology - After fixation, samples were trimmed, processed, embedded in paraffin wax, sectioned to a thickness of 4-5 µm and then processed in an alcohol-xylene series and stained with hematoxylin and eosin for microscopic evaluation. Histopathology evaluation was performed blind by at least two pathologists. At least one senior pathologist peer reviewed all lesions of oncological interest as well as any lesion of dubious interpretation. All the pathologists used the same evaluation criteria and the same classification based on international standard criteria (INHAND, NTP) described in the specific Standard Operating Procedures and long adopted at the CMCRC/RI.

Hormone analysis - Serum concentration of free (fT) and total testosterone (TT), 5α-dihydrotestosterone (DHT), 17β-estradiol (E2) and Sex Hormone Binding Globulin (SHBG) were measured in duplicates by solid phase enzyme-linked immunosorbent assays (ELISAs): "Estradiol rat ELISA" (#DEV9999), manufactured by Demeditec Diagnostics GmbH, "Rat Free Testosterone (F-TESTO) ELISA" (#CSB-E0597r), "Rat Testosterone, T ELISA" (#CSB-E05100R); "Rat dihydrotestosterone (DHT) ELISA" (#CSB-E07879r), and "Rat sex hormone-binding globulin (SHBG) ELISA" (#CSB-E12118r), manufactured by Cusabio Biotech Co. Ltd. The detection range and the Lower Limit of Detection (LLD) of each ELISA kit was 2.5-1280 pg/mL and 2.5 pg/mL for E2, 0.3-60 pg/mL and 0.15 pg/mL for fT, 0.13-25.6 ng/mL and 0.06 ng/mL for TT, 10-2000 pg/mL and 5 pg/mL for DHT, 375-6000 ng/mL and 375 ng/mL for SHBG. Each kit was used following the manufacturer's instructions and absorbance was measured at 450 nm using a 96-well plate reader. Plasma pituitary hormones were measured in duplicate using the "Rat Pituitary Magnetic Bead Panel", a Luminex® bead-based immunoassay, following manufacturers' instructions. Seven plasma pituitary hormones were measured in plasma samples from 40 pups (20 females and 20 males) randomly selected from the 6–week cohort (N = 48 total): adrenocorticotropic hormone (ACTH), brain-derived neurotrophic factor (BDNF), follicle stimulating hormone (FSH), growth hormone (GH), luteinizing hormone (LH), prolactin (PRL) and thyroid stimulating hormone (TSH). FSH and LH were also assessed in 40 pups (20 females and 20 males) randomly selected from the 13–week cohort ($N = 60$ total). BDNF and TSH results from the 6–week cohort showed marginal differences by exposure groups in male pups, it was therefore attempted to validate these results by measuring BDNF and TSH in all male pups ($N = 30$) from the 13–week cohort. Plasma TT was measured in duplicates in all dams ($N = 24$) using an ELISA kit, the "Testosterone Parameter Assay Kit", following manufacturers' instructions.

Statistical methods - Where data on a particular endpoint were collected from both sexes, analyses were conducted separately. All statistical tests were made using a significance level of $\alpha = 0.05$. For continuous data including body weight, weight gain and organ weights, which are most often normally distributed, one-way ANOVA, followed by a Dunnett's test was used to compare treatment versus control groups. For hormone data, which are usually non-normally distributed and have high inter-individual variability, a

screening for outliers was made, based on a Box and Whisker Plot procedure and considering as outliers the values that were outside the box boundaries by more than 3 times the size of the box itself. In the case of hormone ratios, the same outliers of the single evaluation were considered. Nonparametric Kruskal-Wallis' tests, using beta approximation, were used in cases where data were not normally distributed (all hormones). Counting data, not normally distributed, were also analysed with appropriate regression models. Where the observations were grouped (such as for litter data), fixed and mixed effect models were estimated (litter as random effect) and both reported. For bio-logical parameters related to the body weight (such as the AGD), statistical analyses were always performed including the body weight of each pup in the regression model. The incidence of pathological lesions, reported as the numbers of animals bearing lesions, were compared using a two-tail Fisher exact test. The statistical analysis was performed using Stata/IC 10.1 (for all regressions) and Statisti× 10 (for all the other tests); graphs were obtained using Microsoft Excel and Statisti× 10.

Results

No statistically significant differences were observed between the control and the glyphosate and MON 52276 groups for gestational index, mean gestational length, relative weight gain during pregnancy, relative weight gain during lactation, total number of pups delivered at PND 0, litter size, sex ratio, mean life birth index, number of dams with reported stillbirths, number of still born pups, and survival index on PND 1 and PND 21. Also no treatment related effects were observed for water or feed consumption during gestation or lactation. In pups, AGD on PND 4 was statistically significantly increased both in MON 52276 treated males and females and in glyphosate treated males. Results were still significant after running multilevel linear regression models adjusted for body weight and litter as a random effect. Post-weaning body weights as well as water and feed consumption showed no difference in both female and male offspring. In female offspring, age and body weight at vaginal opening (VO) was similar across treatment groups, however, age at FE was statistically significantly delayed in the MON 52276 exposed group. Female offspring in the control and glyphosate treated groups presented the FE within 6 days from the VO, while in the MON 52276 treated group 2/10 females presented a more than doubled interval (12 and 14 days) between VO and FE. In female pups followed up to 13–weeks (N = 30), the percent of time spent in each stage of the estrous cycle did not differ between glyphosate and MON 52276 treated animals and controls. In male offspring, exposure to glyphosate or MON 52276 did not affect BPS or sperm parameters (number of mature spermatids in the testis, daily sperm production, number and sperm transit time through caput/corpus and cauda epididymis and morphology). There were no treatment-related gross lesions in F0 and F1 reproductive and endocrine organs in either sex and there was no statistically significant effect on absolute and relative organ weight of adrenal glands, uterus and ovaries in the dams, adrenal glands, testis, epididymis, bladder/prostate and seminal vesicles/coagulating glands in male offspring (with the exception of a decrease in absolute epididymal weight in the 13–week cohort), and adrenal glands, uterus and ovaries in female offspring.

Most pituitary hormones were unaffected by exposure to glyphosate or MON 52276 in males, with the exception of a statistically significant increase in plasma TSH in the glyphosate group of the 6–week cohort and the MON 52276 group of the 13–week cohort and a statistically significant increase in BDNF in the MON 52276 group of the 6–week cohort. Apart from a statistically significant decrease in DHT in MON 52276 treated males of the 13–week cohort and a statistically significant increase in total testosterone in MON 52276 treated females of the 13–week cohort no effects were found on sex hormones. Hormone ratios were calculated as indicators of the general balance between hormones and sex steroid hormone bioavailability. The TT/SHBG ratio was statistically significantly increased in MON 52276 treated females of the 13–week cohort. The E2/SHBG ratio was statistically significantly increased in MON 52276 treated males of the 6–week cohort. The fT/TT ratio was statistically significantly decreased in glyphosate treated males of the 6–week cohort and in MON 52276 treated males of the 13–week cohort. Male and female MON 52276 treated rats of the 13–week cohort showed a marked and statistically significant decrease in DHT/TT ratio. No such differences were observed for DHT/TT ratio in males and females of the 6–week cohort and no statistically significant differences were observed for the E2/TT ratio in males and females of the glyphosate and MON 52276 treated groups of both cohorts.

Discussion

MON 52276, when administered to rats from in utero through adulthood at a dose level corresponding to the glyphosate RfD defined by the US EPA (1.75 mg/kg bw/day), elicited subtle but potentially adverse effects on reproductive development and hormone concentrations. Overall, these effects indicate an impact on pre- and peri-pubertal sexual maturation. The effects of treatment with glyphosate were essentially limited to increased AGD and TSH concentration, and both changes were specific to males. MON 52276 seemed to affect both females and males, resulting in a statistically significant increase of AGD and sexual hormones imbalances in both cohorts. Statistically significant differences in apical endpoints (AGD and FE) together with changes in hormonal activity detected in both treatment groups should be taken into account suggesting evidence for reproductive toxicity via an endocrine disruption mechanism. A longer AGD at birth in both sexes and an increased age at FE, together with the increased TT in female offspring, are considered endpoints for androgen-mediated activity. The significant increase in AGD and the delay in the appearance of the first estrous cycle observed in MON 52276-treated female rats is consistent with increased developmental androgenisation. The first ovulation is the true endpoint of a series of morphological and functional changes at different levels of the hypothalamic–pituitary–gonadal (HPG) axis, hence, it constitutes the unequivocal sign that puberty has been achieved. No difference in the achievement of vaginal opening (VO) was observed among the groups tested. In males, a prolonged, albeit of low-intensity, androgenizing effect could eventually evoke a counteracting feed-back response from the HPG axis. As apical endpoint, an increased AGD was observed in both treatment groups. Hormone profiling in males, revealed a decreased serum DHT in the MON 52276 group of the 13–week cohort, suggesting an effect on TT metabolism after a prolonged exposure. The lower conversion of TT to DHT might indicate a possible reduction in 5α-reductase enzyme activity. However, this effect was not observed in females of the 6–week cohort. It is of note that males treated with MON 52276 showed normal seminiferous tubules and sperm production which is consistent with the absence of any effect on testosterone and FSH. A statistically significant increase in TSH was observed in glyphosate treated males of the 6–week cohort and MON 52276 treated males of the 13–week cohort. Since no histological changes were observed in the thyroid gland, the increased plasma concentration of TSH can be considered as not indicative of a thyroid related effect. BDNF is a neurotrophin playing a fundamental role in survival and differentiation of selected neuronal populations during development, and in the maintenance and plasticity of neuronal networks during adulthood. A statistically significant increase in BDNF was observed in MON 52276 treated males of the 6–week cohort but not in males of the 13–week cohort. BDNF is an explorative and new endpoint for neurodevelopment and the utility of neurotrophins as potential biomarkers is not completely understood.

The present study has some limitations. First, this is a pilot study performed on a limited number of animals where only one dose was used. However, the dose was selected specifically for its relevance to human health risk assessment, as it is the chronic current RfD defined by the USEPA (1.75 mg/kg bw/day) and therefore a dose level that is expected to be “safe”. Furthermore, the number and timing of blood sample collection was limited to the final sacrifice of animals, considering that this was a pilot study and that *in vivo* blood sampling could lead to maternal and pups stress. Another source of uncertainty, which is currently difficult to assess, is the timing of blood sampling during the necropsy session (9.00 am – 3.00 pm), a circadian-dependent modulation of circulating hormones cannot be completely ruled out. Standard errors in different hormone concentrations were large, in relation to the relatively small group sizes and the physiological variability of hormone concentrations. In females, the estrous cycle status at the time of necropsy is another important source of variability when analyzing sexual hormone profiles. However, even if sacrificing animals on a specific day of the cycle might improve the ability to observe changes in the baseline hormone concentrations, the issue of sacrificing animals in the same cycling period (e.g estrous) is still controversial. The updated OECD Test Guidelines on reproductive-developmental toxicity do not require the sacrifice of females in the same stage of estrous, only the examination of the estrous cycle on the day of necropsy is recommend to allow correlation with the histopathology in reproductive organs. Finally, the adjuvant(s) present in Roundup Bioflow (corresponding to 16% of the formulation) could not be studied since the nature of the co-formulants is a trade secret. These are supposed to be surfactants, diluents or adjuvants stabilizing glyphosate and allowing its penetration in plants. The majority of significant changes observed in hormonal status emerged in the 13–week cohort (animals sacrificed at adulthood) compared to the 6–week cohort (animals sacrificed after puberty) suggesting that more

prolonged exposures were more effective in producing imbalances in the hormone concentrations. In this experiment, MON 52276 was shown to be definitely more potent than glyphosate alone.

Conclusions
The present study demonstrates that exposure to MON 52276 at a dose level equivalent to 1.75 mg glyphosate acid/kg bw/day, from the prenatal period to adulthood, induced endocrine effects and altered reproductive developmental parameters in male and female SD rats. MON 52276 exposure was associated with androgen-like effects, in particular in females, including a statistically significant increase of ano-genital distance in both males and females, a delay of first estrous and increased testosterone in females. MON 52276 exposure was also associated with altered testosterone metabolism in both males and females, where a statistically significant decrease in DHT/TT ratio was observed in the longest treated group (13–week cohort). Overall, MON 52276 elicited more pronounced effects than glyphosate, which only increased anogenital distance and TSH concentration in male rats in the peripubertal window (6–week cohort).

3. Assessment and conclusion

Assessment and conclusion by applicant:

In this pilot study the effect of glyphosate and its reference formulation Roundup Bioflow (MON 52276) at a dose of 1.75 mg glyphosate acid eq./kg bw/day on endocrine modulation was investigated in female rats during pregnancy and lactation, and in male and female rats during lactation, the peripubertal period and adulthood. The endpoints analysed were body weight, water and food consumption, gestational parameters, litter parameters, landmarks of sexual development, estrous cyclicity, gross and histopathology of reproductive and endocrine tissues, sperm parameters and serum and plasma hormone levels. MON 52276 exposure was associated with statistically significant increase of ano-genital distance in males and females, a delay of first estrous and increased serum testosterone in females and altered testosterone metabolism in both males and females. MON 52276 elicited more pronounced effects than glyphosate, which only increased statistically significantly anogenital distance during the peripubertal period. The statistically significant increase in TSH levels in glyphosate and MON 52276 treated rats was not associated with histopathological changes in the thyroid and thus of minor toxicological significance. The effect of glyphosate on ano-genital distance is not corroborated by any reproductive toxicity study where rats were exposed to much higher doses of glyphosate (> 1,000 mg/kg bw/day).

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because of the limited number of animals used per dose level and only one dose level tested.

Reliability criteria for *in vivo* toxicology studies

Publication: Manservisi *et al.,* 2019	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	Pilot study based on OECD guideline 443 but with deviations.
Study performed according to GLP	N	
Study completely described and conducted following	Y	

scientifically acceptable standards		
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity of > 99.5 % as Pestanal™ . Source: Sigma-Aldrich, Milan, Italy.
Only glyphosate acid or one of its salts is the tested substance	N	Also representative formulated product tested. Roundup Bioflow (MON 52276, containing 360 g/L of glyphosate acid in the form of 480 g/L isopropylamine salt of glyphosate (41.5 %), water (42.5 %) and surfactant (16 %)). Source: Consorzio Agrario dell'Emilia, Bologna, Italy.
AMPA is the tested substance	N	
Study		
Test species clearly and completely described	Y	
Test conditions clearly and completely described	Y	
Route and mode of administration described	Y	Oral via the drinking water.
Dose levels reported	Y	1.75 mg glyphosate acid eq./bw/day.
Number of animals used per dose level reported	Y	Dams: 8/group. Offspring: 8 M + 8F/group (6–week cohort); 10 M + 10F/group (13–week cohort).
Method of analysis described for analysis test media	N	
Validation of the analytical method	N	
Analytical verifications of test media	N	
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical control data of the laboratory reported	N	
Dose-effect relationship reported	Y	Not possible, only comparison between glyphosate and MON 52276.
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Reliability not assignable		
Not reliable		
This publication is considered as relevant for the risk assessment of glyphosate but reliable with restrictions because of the limited number of animals used per dose level and only one dose level was tested.		

1. Information on the study

Data point:	**CA 5.6.1/017**
Report author	Pham, T.H. *et al.*
Report year	2019
Report title	Perinatal Exposure to Glyphosate and a Glyphosate-Based Herbicide Affect Spermatogenesis in Mice

Document No	doi: 10.1093/toxsci/kfz039 E-ISSN: 1096-0929
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	No
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary of the study according to OECD format

This study analysed the effects of glyphosate, alone or in formulation, on the male reproductive system. Pregnant mice were treated from E10.5 to 20 days postpartum by adding glyphosate or a GBH (Roundup 3 Plus) to their drinking water at 0.5 (the acceptable daily intake, ADI dose), 5 and 50 mg/kg/day. Male offspring derived from treated mice were sacrificed at 5, 20, and 35 days old (d.o.) and 8 months old (m.o.) for analysis.

Materials and methods

Chemicals - Glyphosate (purity 99.2 %) was purchased from Sigma-Aldrich, St Louis, USA.

Animals – Four-month-old outbred Swiss adult mice were exposed to glyphosate via the drinking water at concentrations corresponding with 0.5, 5 and 50 mg/kg bw/day from the day of vaginal plug detection (embryonic day 0.5) to 20 days post-partum (dpp). The control group received water alone. Control and treated mice of 5, 20, 35 days old and 8 months old were euthanised and the reproductive organs excised. At least 5 animals derived from at least 3 to 4 different litters in each group were used for this test.

Testosterone quantification - Serum was collected from ketamine/xylazine-anaesthetised adult animals by terminal cardiac exsanguination and aliquots were stored at -20 °C. Testosterone levels in the serum were assayed in duplicate using a commercial radioimmunoassay based on competitive binding with I125-labeled testosterone according to the manufacturer's recommendations.

Epididymal sperm count - The mice were euthanised and the epididymis excised, rapidly frozen in liquid nitrogen and stored at -80°C pending sperm count. The tissue was first cut in pieces and homogenised in 6 mL of 0.15 M NaCl containing 0.005 % (v/v) Triton X-100. After homogenisation by sonication, an aliquot of the cell suspension was loaded onto a Malassez hemocytometer, and spermatozoa heads were counted. The average sperm count was calculated from at least 6 controls or treated animals.

Histology and numbers of germ cells and Sertoli cells - Testis samples were fixed in Bouin's solution and embedded in paraffin. Histological sections (5 µm thick) were stained with hematoxylin and eosin (H&E). For immunohistochemistry (IHC), only the adult animals were perfused and the testes were fixed for 24 hours in 4 % (w/v) paraformaldehyde and then embedded in paraffin. Testis sections (5 µm thick) were incubated overnight at 4°C with a primary rabbit polyclonal antibody DDX4 and then with secondary Alexa-Chicken anti-rabbit 488 antibody during 1 hour to check the number of undifferentiated spermatogonia in testes of mice of 5 days old. For IHC on testes of mice of 35 days old, testis sections (5 µm thick) were incubated overnight at 4 °C with goat anti-ZBTB16 and rat anti-GATA1 and then with secondary Alexa-chicken anti-goat 488 antibody and secondary Alexa-donkey anti-rat 594 during 1 hour. The sections were all counterstained with 0.001 % (v/v) 4,6-diamidino-2-phenylindole dihydrochloride and mounted in Vectashield before microscopic analysis. To quantify the number of Sertoli cells (GATA1-positive cells) and undifferentiated spermatogonia (ZBTB16-positive cells), the cells were manually counted in 30 sections on average of seminiferous epithelium at stage VII in controls and glyphosate treated groups. Cells in 3 different areas of the testis were analysed for each biological replicate

RNA extraction and quantitative PCR - Total RNA was extracted from testes of animals of 5 days old using the RNeasy plus mini kit according the protocol of the manufacturer and reverse transcription was

performed with 1 mg of RNA using the iScript cDNA Synthesis Kit according to the manufacturer's instructions. The resulting cDNA was diluted 5 times and used for quantitative PCR. QPCR was performed using the iTaq Universal SYBR Green Supermix according to the manufacturer's instructions on a CFX384 Touch Real-Time PCR Detection system. The PCR amplification of the coding regions of Actb and Rplp0 was used for normalisation. The data from at least 6 samples were analysed, compared, plotted, and expressed as a fold change in treated samples compared with controls.

Statistical analysis - Statistical tests were carried out using R software. For each experiment, the results were separated and compared by modality. First, the data were tested for normality by the Shapiro test and homoscedasticity by the Bartlett test. If the data distribution for each modality followed a normal distribution and if the variances were equal, the ANOVA test was performed, a parametric comparison test. If the data of at least 1 of the groups were not normally distributed or if the data of the groups were distributed normally but the variances were not equal, the Kruskal-Wallis test was carried out, a nonparametric comparison test of average. The differences were considered statistically significant when $p < 0.05$. In this case, the Tukey test was performed after the ANOVA test and the Mann-Whitney test after the Kruskal-Wallis test. In animals of 5 and 20 days old and 8 months old, data were collected by randomising in view of origin litter and pups were used as the experimental unit for statistical analysis. In animals of 35 days old, corresponding to the first complete wave of spermatogenesis, litters were used as the experimental unit for statistical analysis.

Results

Male reproductive parameters of prepubertal mice of 5 days old – No statistically significant change has been found in the number of spermatogonia of mice exposed to glyphosate at 0.5, 5 and 50 mg/kg bw/day. No statistically significant change has been found in RNA expression of Cyp11a1, Cyp19a1 and Ret at all dose levels tested. Statistically significant changes were observed in RNA expression of Bax (increase at 0.5 mg/kg bw only), Bcl2 (increase at all dose levels), Dazl (increase at 0.5 mg/kg bw/day only), Kit (decrease at 50 mg/kg bw/day only), Sall4 (decrease at all dose levels), Nanos3 (increase at 0.5 and 50 mg/kg bw/day but not at 5 mg/kg bw/day) and Foxo1 (increase at 0.5 mg/kg bw/day only).

Male reproductive parameters of mice of 20 days old - The histopathological analysis of testis sections revealed that glyphosate causes an adverse effect on testis morphology when compared with the control. An increase in vacuoles in the seminiferous epithelium was observed at all dose levels. Empty seminiferous tubules were seen at 5 mg/kg bw/day but not at 0.5 and 50 mg/kg bw/day glyphosate.

Male reproductive parameters of mice of 35 days old – No statistically significant change has been found in relative epididymis weight, relative seminal vesicles weight, epididymal sperm count and GATA1 positive cells. A decrease in relative testes weight was recorded at 0.5 mg/kg bw/day glyphosate but not at the other dose levels. Serum testosterone was found to be statistically significantly decreased at 0.5 and 50 mg/kg bw/day but not at 5 mg/kg bw/day glyphosate. The number of ZBTB16 positive cells were statistically significantly decreased at 5 mg/kg bw/day glyphosate but not at 0.5 and 50 mg/kg bw/day.

Male reproductive parameters of mice of 8 months old – No statistically significant changes were noted for relative epididymis weight, relative seminal vesicles weight and serum testosterone. A statistically significant decrease has been observed for relative testes weight of mice exposed to glyphosate at 0.5 mg/kg bw/day but not at the higher dose levels.

Discussion

Glyphosate did not have any effect on the body weight of animals tested in this study. The decrease in relative testicular weight observed at 0.5 (statistically significant) and 5 mg/kg bw/day (not statistically significant) in mice of at 35 days old and at 0.5 mg/kg bw/day (statistically significant) in mice of 8 months old indicates that glyphosate, even at low doses, can significantly impair spermatogenesis, as testicular weight is a very reliable indicator of the normal function of male reproductive system. This fact was confirmed by measuring the level of testosterone in plasma after glyphosate exposure. In this study, serum testosterone levels were statistically significantly decreased in mice of 35 days old when exposed to

glyphosate at 0.5 and 50 mg/kg bw/day and in mice of 8 months old when exposed to glyphosate at 0.5 mg/kg bw/day but not at higher dose levels. The treatment with different doses of glyphosate did not produce monotonic dose-responses. This is the case for relative weight of the epididymis and seminal vehicles, testosterone levels and sperm counts in mice of 35 days old. Glyphosate exposure led to a non-statistically significant decrease in epididymal sperm count in mice of 35 days old at 0.5 and 5 mg/kg bw/day leading to a non-statistically significant decrease in relative epididymis weight. This decrease in sperm count suggests that exposure to glyphosate could reduce male fertility.

The increase in the expression of key apoptosis genes (Bax, Bcl2) in mice of 5 days old exposed to glyphosate could influence the balance between Sertoli cells and spermatogonia. At 5 mg/kg bw a statistically significant decrease in spermatogonia (ZBTB16 positive cells) number was observed in animals of 35 days old. As the cell count of the pool of undifferentiated spermatogonia in the testes of mice of 5 days old confirmed that there were no changes in the number of germ cells in the glyphosate treatment groups, the decrease in spermatogonia number in mice of 35 days old may be the result of a modification of key genes involved in germ cell differentiation. In this study, there was a decrease in Sall4 and Kit mRNA expression in 5–day old mice exposed to glyphosate. Both factors are part of the retinoic acid (RA)-dependent signalling pathway involved in the differentiation of Aal spermatogonia into A1 which are irreversibly committed in the differentiation process. This result suggests that glyphosate is able to alter spermatogonial differentiation and maturation and this explains, at least in part, the molecular mechanism responsible for the morphological changes observed in 20–day old animals and the decrease in sperm count observed in adults.

Conclusion

This study shows that glyphosate at the ADI dose of 0.5 mg/kg bw/day could have endocrine disrupting effects which could impair the male reproductive system in mice.

8. Assessment and conclusion

Assessment and conclusion by applicant:

The effect of glyphosate exposure from the day of vaginal plug detection to 20 days post partum via the drinking water at concentrations corresponding with 0.5, 5 and 50 mg/kg bw/day on male reproductive parameters in mice of 5, 20 and 35 days old and 8 months old was investigated. The parameters measured were the number of spermatogonia and expression of genes important to testicular function in 5–day old mice, testicular histopathology in 20–day old mice, relative weight of testes, epididymis and seminal vesicles, epididymal sperm count, serum testosterone levels, GATA1 positive cell count and ZBTB16 positive cell count in 35–day old mice, and relative weight of testes, epididymis and seminal vesicles and serum testosterone levels in 8-month old mice. No statistically significant change was found for the number of spermatogonia in 5–day old mice. The only genes of which the expression was statistically significantly changed in a dose-related fashion were Bcl2 and Kit. In 20–day old mice, sperm depleted seminiferous tubules were noted at 5 mg/kg bw/day but not at 0.5 and 50 mg/kg bw/day glyphosate. In 35–day old mice there was no statistically significant change in the relative weight of the epididymis and the seminal vesicles, epididymal sperm count and GATA1 positive cell count. No dose-effect relationship could be established for relative weight of testes, serum testosterone levels and ZBTB16 positive cell count. In 8-month old mice no statistically significant change could be observed for relative weight of epididymis and seminal vesicles and serum testosterone levels. No dose-effect relationship could be established for the decrease in relative testes weight. From these data it can be concluded that there is no evidence that glyphosate dosed orally to mice up to 50 mg/kg bw/day during the perinatal period is an endocrine disruptor and has an adverse effect on testicular function and development. This has been corroborated by reproduction toxicity studies with rats at much higher dose levels.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the test conditions were not clearly described and the number of animals tested per

dose level is too limited.

Reliability criteria for *in vivo* toxicology studies

Publication: Pham *et al*., 2019	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	N	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity ≥ 99.2 %. Source: Sigma-Aldrich.
Only glyphosate acid or one of its salts is the tested substance	N	Also formulation was tested: Roundup 3 plus.
AMPA is the tested substance		
Study		
Test species clearly and completely described	Y?	
Test conditions clearly and completely described	Y?	Not completely described. Control and treated young prepubertal or adult mice were euthanised, and reproductive organs were dissected in 5, 20, 35 days old (d.o.), and in 8 months old (m.o.) mice. Only male mice were analysed.
Route and mode of administration described	Y	Oral via drinking water.
Dose levels reported	Y	0.5, 5, 50 mg/kg bw/day.
Number of animals used per dose level reported	Y?	5 animals derived from at least 3 to 4 different litters in each group
Method of analysis described for analysis test media	N	
Validation of the analytical method	N	
Analytical verifications of test media	N	
Complete reporting of effects observed	Y?	Not always presented in tables.
Statistical methods described	Y	
Historical control data of the laboratory reported	N	
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Not reliable		

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the test conditions were not clearly described and the number of animals tested per dose level is too limited.

1. Information on the study

Data point:	**CA 5.6.1/018**
Report author	Ren, X. *et al*.
Report year	2019
Report title	Effects of chronic glyphosate exposure to pregnant mice on hepatic lipid metabolism in offspring
Document No	doi.org/10.1016/j.envpol.2019.07.074 E-ISSN: 1873-6424
Guidelines followed in study	None
Deviations from current test guideline	Not applicable.
Previous evaluation	None
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary of the study according to OECD format

The present study aims to investigate the toxic effects of prenatal exposure to pure glyphosate or Roundup on lipid metabolism in offspring. During gestational days (GDs), ICR mice (from Institute of Cancer Research) were given distilled water, 0.5 % glyphosate solution (w/v, 0.5 g/100 mL) or 0.5 % glyphosate Roundup solution orally. The livers and serum samples of the offspring were collected on gestational day 19 (GD19), postnatal day 7 (PND7) and PND21. The results showed a significant decrease in the body weight and obvious hepatic steatosis with excessive lipid droplet formation in offspring. Moreover, the concentrations of lipids such as triglycerides (TGs), total cholesterol (T-CHO), and low-density lipoprotein cholesterols (LDL-C) increased to a significant extent in both the serum and livers. Furthermore, there were significant differences in the expression levels of the genes SREBP1C, SREBP2, Fasn, Hmgcr, Hmgcs and PPARa, which are related to lipid biosynthesis or catabolism in the liver.

Materials and methods

Animals; Ten–week-old female and male ICR mice were purchased from Nanjing Qinglongshan Experimental Animal Center (Nanjing, China). After one week of adaptation, one male and two female mice were housed in each cage from 5.00 p.m. to 8.00 a m. daily to obtain pregnant mice. Pregnant mice were placed into separate cages once the pregnancy was confirmed by a vaginal smear the following morning. This day was defined as the first day of gestation. Animals were fed with water and feed ad libitum. The temperature and relative humidity in the animal house were controlled at 23 ± 2 °C and 50 ± 10 %, respectively, and the animals were kept on a 12-h light/dark cycle. The animal experiments were approved by the Animal Welfare Committee of Nanjing Agricultural University (Nanjing, China) and implemented in accordance with the National Institutes of Health Guidelines for Animal Care and the Committee of Animal Research Institute.

Chemicals and treatment; Pure glyphosate (N-(phosphonomethyl)glycine) and Roundup (as the isopropylamine salt) were provided by Shanghai Ryon Biological Technology Co., Ltd. (Shanghai, China) and Sinochem Crop Protection Products Co., Ltd. (Shanghai, China), respectively. Glyphosate and

Roundup were diluted with distilled water to obtain 0.5 % active ingredient solutions (w/v, 5 g glyphosate/1 L solution). Then, the subjects were administered the 0.5 % glyphosate or Roundup solution through the drinking water (pH was controlled at 7.2 ± 0.2).

Animal treatment and sampling; A total of 30 pregnant mice were randomly divided into three groups: CON (control, n = 10), GLP (0.5 % glyphosate treated, n = 10), and RU (0.5 % Roundup treated, n = 10). Half of the pregnant mice (five from each group) were exposed throughout the first 19 days of pregnancy and were sacrificed on GD19. The other half of the pregnant mice were exposed throughout the pregnancy period and given distilled water after giving birth. Weekly body weights of the offspring were recorded, and their anogenital distances were measured separately to identify their sexes. Seven and 21 days after birth, the prenatally exposed offspring were sacrificed (preferably two females and two males per mother) for blood and tissue analysis. The water consumption of the pregnant mice was measured, and the real exposure dose of glyphosate in both GLP and RU groups was approximately 7 mL (Table 1). The serum was extracted through centrifugation (3500 rpm, 15 min, 4 °C) and was used to assay the biochemical indexes. Parts of the livers were stored at 80 °C for lipid concentration determination and reverse transcriptionpolymerase chain reaction (RT-PCR). Liver samples were either fixed in a 4 % paraformaldehyde solution or embedded in optimal cutting temperature compound (O.C.T. compound) provided by Sakura Finetek Japan Co., Ltd. (Tokyo, Japan) prior to frozen sectioning for the histological observation of tissue sections.

Histological preparation; Some parts of the liver tissue were fixed in 4 % paraformaldehyde solution for 24 h and then dehydrated, clarified, embedded with paraffin and sectioned. Tissue sections (5 mm) were used for hematoxylineeosin (H&E) staining. The remaining liver tissue was embedded with O.C.T. compound and sectioned using a microtome cryostat manufactured by Thermo Fisher Scientific Instrument Co., Ltd. (Shanghai, China) for Oil Red O staining.

Serum biochemical and liver lipid concentration assays; To preliminarily diagnose the liver injury and lipid content of the organisms, the following serum biochemical indexes were determined: aspartate transaminase (AST), alanine transaminase (ALT), triglyceride (TG), total cholesterol (T-CHO), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C). Additionally, liver homogenate was centrifuged to obtain the supernatant (3500 rpm, 15 min, 4 °C) to measure the TG, T-CHO, LDL-C and HDL-C content. Both the serum biochemical indexes and hepatic lipid content were assayed with commercial reagent kits purchased from the Institute of Biological Engineering of Nanjing Jiancheng (Nanjing, China).

Analysis of gene expression; Total RNA was extracted from liver tissue with the ISOGEN 2 reagent kit (from NIPPON GENE CO., LTD.) (Tokyo, Japan) according to the manufacturer's instructions. The concentration of the obtained RNA was determined by a spectrophotometer, and the purity was measured using a NanoDrop® 8000. Then, PrimeScriptTM RT Master Mix (from Takara Bio Inc.) was used to reverse transcribe RNA to cDNA, which acted as a template for the SYBR® Premix Ex Taq™ PCR kit (from Takara Bio Inc.) for real-time PCR. The expression levels of the genes SREBP1C (Sterol Regulatory Element Binding Protein 1C), SREBP2 (Sterol Regulatory Element Binding Protein 2), Fasn (Fatty acid synthase, which catalyzes fatty acid synthesis), Scd (Stearoyl-CoA Desaturase 1), Acc (Acetyl-CoA Carboxylase), Hmgcr (3-hydroxy-3-methyl-glutaryl-CoA reductase), Hmgcs1 (3-hydroxy-3-methylglutaryl-CoA synthase 1), Hmgcs2 (3-hydroxy-3-methylglutaryl-CoA synthase 2) and PPARa (Peroxisome proliferator-activated receptor alpha) were determined. The relative expression levels of the above genes were normalised to b-actin expression. All primers were designed and supplied by GenScript Bio-Tech Co., Ltd. (Nanjing, China).

Data analysis; The software packages SPSS Statistics 20.0 and GraphPad Prism (GraphPad Software, San Diego, CA, USA) were utilised to analyse the data. One-way analysis of variance (ANOVA) and Tukey's multiple comparison tests were performed. Values are expressed as the mean ± standard error of the mean (SEM), and statistical significance was set as $p<0.05$.

Results

Table 1: Effects of chronic glyphosate exposure on the performance of pregnant mice.

Items	CON	GLP	RU	*P*
Water consumption (ml)	9.69 ± 0.76^{a}	7.88 ± 0.46^{b}	7.45 ± 0.34^{b}	0.023
Feed intake (g)	9.00 ± 0.16	8.32 ± 0.55	9.63 ± 0.72	0.261
Body weight gain (g)	32.60 ± 3.30	35.96 ± 2.92	29.84 ± 0.70	0.281
Number of fetuses (n)	10.80 ± 1.50	14.40 ± 1.57	12.60 ± 2.11	0.376
Average birth weight (g)	1.73 ± 0.13	1.65 ± 0.08	2.03 ± 0.72	0.220

Each value represents the mean ± SEM of the group (n = 5).
Different letters indicate statistically significant differences. a, b $p < 0.05$.

Physical and organ development; From GD19 to PND21, the body weight in both GLP and RU groups decreased and finally saw a statistically significant reduction on PND21 ($p<0.05$) (Fig. 1). When separated according to sex, offspring showed no significant differences in either body weight or weight gain among the three groups (Table 2).

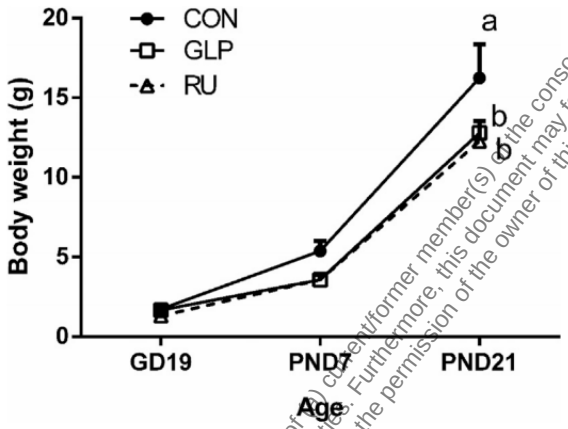


Figure 1: Effects of chronic glyphosate exposure to pregnant mice on the body weights of offspring at the ages of GD19, PND7 and PND21 (mean ± SEM). Different letters indicate statistically significant differences, p<0.05.

Table 2: Effects of chronic glyphosate exposure to pregnant mice on the physical development of the offspring (g).

Items	Female				Male			
	CON	GLP	RU	*P*	CON	GLP	RU	*P*
PND7								
Body weight	5.72 ± 0.61	4.40 ± 0.28	4.29 ± 0.59	0.429	5.24 ± 1.00	4.80 ± 0.30	4.89 ± 0.88	0.918
Body weight gain	3.19 ± 0.80	2.74 ± 0.25	3.08 ± 0.50	0.222	3.51 ± 0.88	3.15 ± 0.29	2.87 ± 0.45	0.115
PND21								
Body weight	14.69 ± 2.44	13.63 ± 0.78	13.26 ± 1.06	0.786	15.79 ± 2.21	15.10 ± 0.85	13.86 ± 1.82	0.732
Body weight gain	12.04 ± 2.01	11.98 ± 0.76	11.24 ± 0.66	0.704	14.06 ± 2.09	13.45 ± 0.82	11.84 ± 1.33	0.243

Each value represents the mean ± SEM of the group (n = 7–10).

Liver histological observation; In both GLP and RU groups, relatively elevated numbers of vacuoles exhibiting hepatic lipid droplets were observed within the hepatocytes of both female and male offspring, when compared with the CON group. Additionally, the red areas observed in the Oil Red O stained sections represent lipid substances. In females, there tended to be more lipid droplets in the GLP group than in the other two groups. In contrast, in males, both the GLP and RU groups showed excessive lipid deposits. In

addition, there were several clusters of monocytes in both the GLP and RU groups of PND7 females. It appears that glyphosate could cause inflammation in early-aged female mice.

Serum biochemical index; Compared with the CON group, TG levels showed a significant increase in the GLP group in both GD19 foetuses (p<0.01) (Table 3) and PND21 female mice (p<0.05) (Table 4). With respect to T-CHO levels, GLP mice showed a remarkable increase in both PND7 males (p<0.01) and PND21 females (p<0.05) compared with CON mice. LDL-C levels also increased in PND7 mice in both the GLP and RU groups (p<0.05) (Table 4). The increased lipid content reflects the adverse effects of glyphosate on lipid metabolism, although this disturbed effect was not detected in every individual. Furthermore, significantly elevated AST levels in PND7 females in the RU group (p<0.01) are theoretically considered to be a result of an injured liver.

Table 3: Effects of chronic glyphosate exposure to pregnant mice on the blood biochemical indexes in foetuses.

Items	CON	GLP	RU	*P*
TG (mmol/L)	0.15 ± 0.10^b	0.79 ± 0.21^a	0.30 ± 0.10^b	0.002
T-CHO(mmol/L)	2.15 ± 0.49	2.56 ± 0.52	1.22 ± 0.06	0.165
LDL-C (mmol/L)	1.03 ± 0.09	1.08 ± 0.04	0.91 ± 0.05	0.185
HDL-C (mmol/L)	0.14 ± 0.01	0.17 ± 0.02	0.17 ± 0.01	0.433
AST (IU/L)	67.88 ± 14.17	103.20 ± 10.30	76.21 ± 10.55	0.134
ALT (IU/L)	20.71 ± 1.02	47.54 ± 10.20	32.09 ± 2.35	0.091

Each value represents the mean ± SEM of the group (n = 20).
Different letters indicate statistically significant differences. a, b $p < 0.05$.

Table 4: Effects of chronic glyphosate exposure to pregnant mice on the blood biochemical indexes of PND7 and PND21 offspring.

Items	Female				Male			
	CON	GLP	RU	*P*	CON	GLP	RU	*P*
PND7								
TG (mmol/L)	1.46 ± 0.07	1.79 ± 0.26	1.60 ± 0.08	0.324	1.11 ± 0.14	1.45 ± 0.14	1.45 ± 0.20	0.240
T-CHO (mmol/L)	1.46 ± 0.07	1.60 ± 0.27	1.42 ± 0.19	0.792	2.01 ± 0.23^b	2.92 ± 0.19^a	1.97 ± 0.11^b	0.007
LDL-C (mmol/L)	1.30 ± 0.18^b	1.73 ± 0.07^a	1.42 ± 0.07^{ab}	0.047	0.58 ± 0.07^c	0.99 ± 0.12^b	1.75 ± 0.11^a	0.000
HDL-C (mmol/L)	1.12 ± 0.11	1.18 ± 0.09	0.86 ± 0.04	0.074	0.86 ± 0.12	0.88 ± 0.04	1.13 ± 0.11	0.189
AST (IU/L)	28.03 ± 6.14^b	29.09 ± 6.22^b	63.84 ± 8.03^a	0.008	65.17 ± 7.65	72.54 ± 10.41	81.71 ± 10.52	0.503
ALT (IU/L)	30.93 ± 3.77	40.51 ± 4.86	39.89 ± 4.08	0.245	29.22 ± 2.39	32.01 ± 2.89	39.27 ± 2.29	0.058
PND21								
TG (mmol/L)	1.30 ± 0.18^b	2.41 ± 0.37^a	1.36 ± 0.15^{ab}	0.022	1.01 ± 0.11	1.10 ± 0.37	1.19 ± 0.38	0.741
T-CHO (mmol/L)	1.30 ± 0.18^b	2.06 ± 0.16^a	1.37 ± 0.15^b	0.028	0.92 ± 0.07	1.01 ± 0.06	1.05 ± 0.21	0.778
LDL-C (mmol/L)	2.26 ± 0.20	2.23 ± 0.12	2.65 ± 0.18	0.293	1.24 ± 0.19	1.08 ± 0.27	0.64 ± 0.13	0.115
HDL-C (mmol/L)	1.41 ± 0.28	1.65 ± 0.31	1.29 ± 0.10	0.570	1.56 ± 0.31	1.49 ± 0.17	1.22 ± 0.29	0.713
AST (IU/L)	41.14 ± 12.38	70.64 ± 9.87	50.04 ± 12.01	0.286	39.37 ± 9.28	54.06 ± 8.10	22.56 ± 8.90	0.091
ALT (IU/L)	19.02 ± 0.43	22.50 ± 4.61	19.48 ± 3.20	0.722	14.27 ± 4.06	16.01 ± 2.34	16.34 ± 3.04	0.890

Each value represents the mean ± SEM of the group (n = 7–10).
Different letters indicate statistically significant differences. a, b $p < 0.05$.

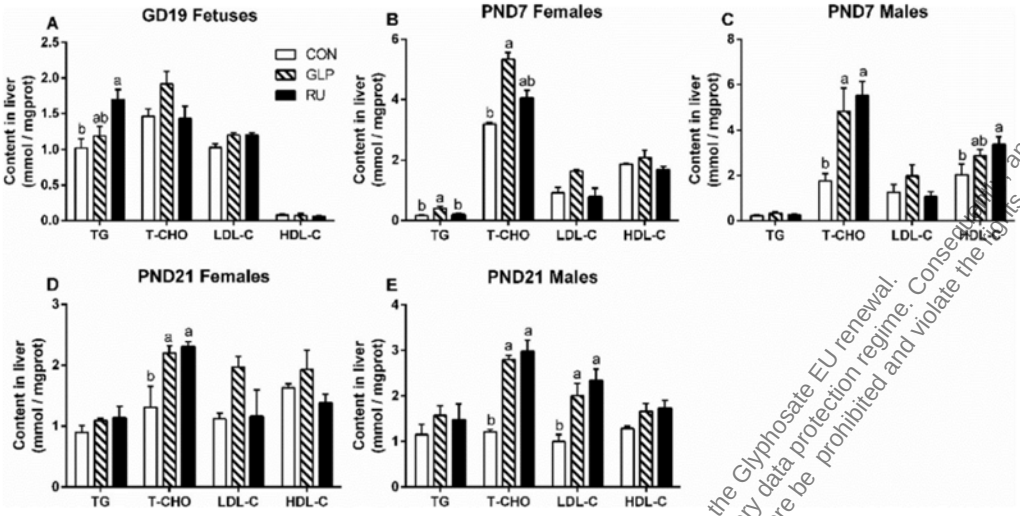


Figure 2: Effects of chronic prenatal glyphosate exposure on the lipid content in the livers of the offspring (mean ± SEM). (A) shows the TG, T-CHO, LDL-C and HDL-C content in livers of GD19 foetuses, and (B), (C), (D) and (E) show these parameters in PND7 and PND21 females and males, respectively. Different letters indicate statistically significant differences. a, b $p<0.05$.

Lipid concentration in the liver; Compared with that in CON mice, TG levels in the RU group significantly increased in GD19 foetuses and PND7 female offspring ($p<0.05$) (Fig. 2A and B). Moreover, T-CHO levels of both PND7 and PND21 offspring increased in the GLP or RU groups ($p<0.05$) (Fig. 2B-E). Elevated TG and T-CHO levels in the liver can probably cause lipid deposits. The LDL-C levels of PND21 males showed a noticeable increase in both the GLP and RU groups ($p<0.05$) (Fig. 2E), and the HDL-C levels in PND7 males were elevated in the RU group ($p<0.05$) (Fig. 2C). Both low-density and high-density lipoproteins can transport cholesterol in the extracellular environment. The elevated level of these proteins in serum is considered to be the result of increased cholesterol levels.

Expression levels of genes related to lipid metabolism in the liver; The relative expression levels of the genes SREBP1C, SREBP2, Fasn, Acc, Scd, Hmgcr, Hmgcs1 and Hmgcs2 in the GLP and RU groups showed a significant increase in GD19 foetuses and PND7 and PND21 offspring ($p<0.05$) (Fig. 3). These genes are closely related to hepatic lipid production, so their elevation contributes to increased fat storage. However, this kind of increase does not match well to the trend in serum lipid content alteration. The levels of PPARa in PND7 males and PND21 females increased remarkably in both the GLP and RU groups, which is likely due to the growing demand for lipid catabolism caused by the increased lipid content.

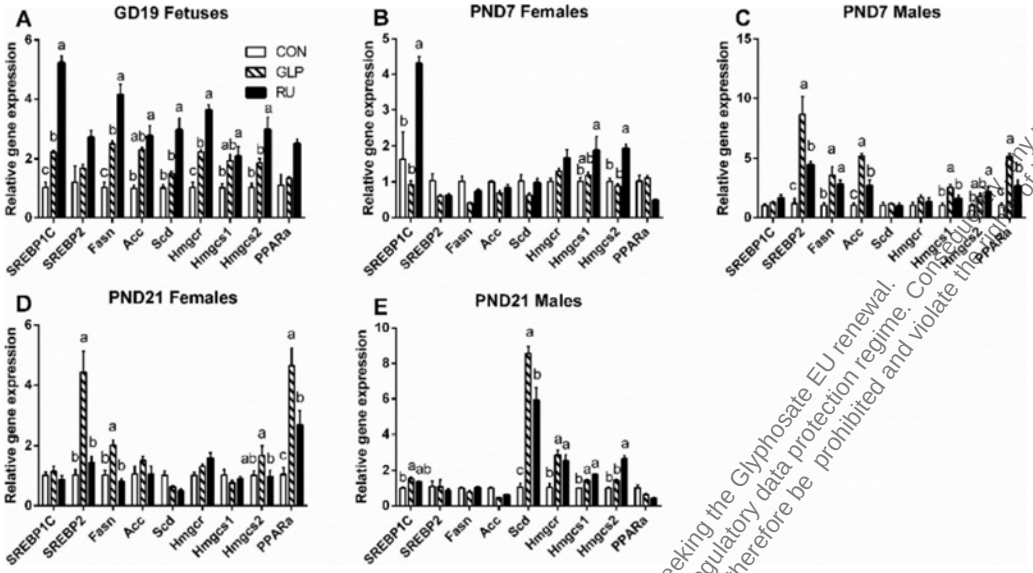


Figure 3: Effects of chronic prenatal glyphosate exposure on relative mRNA expression levels in the livers of the offspring (mean ± SEM). The relative expression levels of the genes SREBP1C, SREBP2, Fasn, Acc, Scd, Hmgcr, Hmgcs1, Hmgcs2 and PPARα genes in the livers of (A) foetuses, (B) PND7 female offspring, (C) PND7 male offspring, (D) PND21 female offspring, and (E) PND21 male offspring are shown. Different letters indicate statistically significant differences. a, b $p<0.05$.

Discussion

Previous studies have found that glyphosate could cause fatty liver disease at the level of transcriptome and proteome. The transgenerational potential of glyphosate between dams and their litter successfully raises concerns about the hepatotoxicity of glyphosate in the progeny. The present study was designed to study the toxic effects of chronic prenatal glyphosate exposure on lipid metabolism in the livers of offspring. The results suggested that chronic maternal exposure to glyphosate can lead to the disruption of lipid metabolism in the next generation. In the present study, 19 or 21 gestational days was typically chosen as the exposure period, and offspring were collected on gestational day 19 and after birth on PND7 and PND21. The average water consumption in the GLP and RU groups was approximately 7 mL, which means that the pregnant mice took an average of 35 mg glyphosate per day. Thus, the real glyphosate exposure dose in foetuses was probably less than 35 mg per day. To our knowledge, the median lethal dose (LD50) of glyphosate in mice is 5000 mg/kg body weight (bw), and the non-observed adverse effect level (NOAEL) is 500 mg/kg bw/day. Although the true administered dose of glyphosate that the offspring received in the present study is far lower than the NOAEL, several phenomena still revealed excessive lipid production. Additionally, according to the classification criteria of the EU and the OECD Globally Harmonised System, glyphosate is not listed as an acute oral toxin based on 145 studies. In chronic exposure research, the "overall NOAEL" was assessed to be 100 mg/kg bw per day based on human beings, which is also higher than the present study's dose. Taken together, a dose lower than overall NOAEL can still cause hepatic lipid metabolism disruption, although the consistency is not perfect among the tested parameters and groups in this study. Physical development is used as an important indicator of the health state of organisms. The body weights of the prenatal glyphosate-treated offspring showed a reduction compared to that of the CON group, which is in accordance with the previous results. The adverse effects on the physical development of offspring could be explained by the decreased body weight of the pregnant mice, while the feed intake of the pregnant mice showed no statistically significant changes. Therefore, the reduced body weight might be the result of energy consumption for the purpose of detoxification instead of for physical development. The liver is the most important detoxification organ that works to metabolize xenobiotics and can reflect the risk of the xenobiotics to the body system to some extent. To observe the liver state of the prenatally exposed offspring at the level of histology, H&E and Oil Red O staining were implemented in the present study. A relative increase in the number of fat vacuoles can be seen in several photomicrographs in the GLP

or RU groups compared with the CON group, which is probably due to the elevated TG and T-CHO levels determined in both the serum and liver. Lipid production and storage in organisms are mainly controlled by liver lipogenesis and catabolism. We learned from the related gene expression level data that the genes related to these two biological processes showed increased expression levels in the GLP and RU groups compared with the CON group. SREBPs are essential activators for the synthesis of fatty acids and cholesterol once combined with a lipid synthesis promoter. SREBP1c, one of the isoforms of SREBP1, regulates the process of lipogenesis that increases from the early age of GD19, while SREBP2 is responsible for cholesterol biosynthesis. Furthermore, the increasing expression levels of downstream genes, such as Fasn, Scd, Acc, Hmgcr and Hmgcrs, contribute to higher fatty acid and cholesterol biosynthesis, which could result in inevitable hepatic fat storage. For lipid catabolism, PPARa plays a crucial role in mitochondrial b-oxidation and peroxisomal fatty acid oxidation, both of which act as important biological reactions in the degradation of liver lipids. Increased PPARa levels in the prenatally exposed offspring might be due to the growing demand for lipid catabolism caused by the rising TG or T-CHO levels. However, the alterations in gene expression are not completely consistent with TG or T-CHO synthesis proteins level or fat storage in the liver sections. This finding might be due to the limited number of subjects, and we will try to explore more detailed reasons and explain these reasons in our future studies. Taken together, we believe that glyphosate could affect lipid production by disturbing lipid metabolism-related gene expression. Previous research had detected a time-dependent enhancement in triglyceride and cholesterol levels as glyphosate-treated subjects aged, whereas there were contradictory results shown in bullfrog tadpoles. To explore more mechanistic insights, a lipidomic profiling experiment was conducted, and 62 distinct lipid species were identified as altered, including triglycerides and cholesteryl esters. Additionally, glyphosate is found to be metabolised into glyoxylate, which inhibits fatty acid oxidation enzymes, all of which are considered to be tightly associated with hepatic lipid dysregulation. Together with the reported impairment of mitochondrial oxidative phosphorylation and enzymatic activity after glyphosate treatment, the efficiency of the respiratory rate and tricarboxylic acid cycle will inevitably decline, which could further induce the diversion of redundant fatty acids into other lipid metabolism pathways. There have been many studies on glyphosate-induced chromosome and DNA aberrations spanning more than 20 years. A great variety of species as well as different doses of glyphosate were used in these studies. The reactive oxygen species and oxidative injury caused by glyphosate or its metabolites are commonly thought to be the reason for the genotoxicity. In the present study, glyphosate's genotoxicity was highly likely reflected in the disruption of lipogenesis. Glyphosate also caused obvious liver damage that appeared in the hematological parameters and histopathological alterations. The increased serum ALT and AST levels in prenatally exposed offspring demonstrated severe liver damage, similar to a reported study. The leakage of liver enzymes is a remarkable indicator of hepatic injury owing to xenobiotics. Additionally, the discovery of clusters of monocytes suggested the presence of inflammatory infiltration and immune responses, but it was not widely observed in all treatment groups. Apart from the liver lesions presented in our study, leukocyte infiltration, necrosis, blood congestion, hydropic degeneration and sinusoid dilation, which could enhance the risk of glyphosate-induced steatosis progression into fatty liver disease, has been observed in hepatocytes in other experiments. Therefore, it is probable that glyphosate-induced lipid metabolism disruption could progress into steatosis if no recovery or interference therapy was performed. Although significant differences were observed between some groups, the changes in the histopathological alterations, blood biochemical indexes and expression levels of lipid metabolism related genes among the groups showed imperfect consistency. This result is highly likely caused by the limited quantity and individual variation of the subjects. Additionally, there are other factors that could have affected the results, such as sex, age and glyphosate source. Considering that the main aim of the present study was to explore the hepatotoxicity of glyphosate exposure, especially during pregnancy, on the lipid metabolism of offspring, these other factors will be future projects and will be studied in future research.

Conclusion

Chronic prenatal glyphosate exposure can probably cause lipid metabolism disruption in offspring, accompanied by an elevated lipid content in both serum and liver tissue. These alterations in hepatic lipid metabolism might result from rising lipogenesis in hepatocytes through increasing related gene expression.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The current study aimed to examine any effects on lipid metabolism in foetuses and pups following prenatal exposure to glyphosate or the glyphosate formulation, Roundup™. Ten pregnant female rats per group were exposed from gestation day 1 through 19 to drinking water containing either 0.5 % glyphosate, prepared using "pure" glyphosate (N-(phosphonomethyl) glycine), or 0.5 % glyphosate using an appropriate dilution of Roundup™. A similar group of animals were given distilled water and served as the control group. Five females per group were terminated on gestation day 19 for examination of foetuses, while the remaining dams were allowed to litter and maintain their litters to postnatal day 21. Offspring (2/sex/litter where possible) were selected on postnatal days 7 and 21 for evaluation. Foetal and offspring evaluations included liver histology, serum biochemistry, liver lipid concentration and gene expression analysis of genes related to lipid metabolism in the liver.

The study is non-GLP and does not report the following information:

- Purity of test items
- Body weight and clinical signs for pregnant animals
- Clinical observations of offspring
- Achieved dose of glyphosate in treated animals in mg/kg bw/day.
- Measures to control inter animal and intergroup variability such as
    - Time of necropsy distributed equally across groups
    - Standardisation of litter size on day 4 of lactation to mitigate variability caused by differences in liter size.
- More than a single dose level of glyphosate (0.5 % solution); thus, preventing dose-response characterisation
- Liver weight of foetuses or offspring
- Normal physiological ranges for serum and liver biochemistry in this strain of rat at this laboratory
- Clear reporting of statistical evaluation and differences
- Thorough histological evaluations of the liver with incidence and severity of any recorded findings.

Although the authors concluded that there were treatment-related effects on foetal and offspring body weight, there is no evidence from this study to suggest that glyphosate exposure has had any impact on foetal development or pup development postnatally. There was no effect on average birth weight of pups, the slight difference observed in the glyphosate-treated group should be attributed to the slightly larger mean litter size observed (14.4 pups compared to 10.8 in the control group). Although figure 1 shows a reduction in mean pup weight in the glyphosate and Roundup™ treated groups (male and females combined). Group mean body weight of pups by sex showed no statistical differences from control.

Given the small group size, the large inter animal variability observed, the lack of consistency between the same parameter across the sexes, timepoints or sampling matrices, and the deficiencies listed above, it not possible to clearly attribute any of the observed differences to glyphosate exposure. Therefore, the current study provides no evidence that glyphosate exposure causes lipid metabolism disruption in offspring following prenatal (in utero) exposure.
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate used is not sufficiently characterised, only one dose level was tested, there was large inter animal variability observed and too few animals per dose level were analysed.

Reliability criteria for *in vivo* toxicology studies

Publication: Ren *et al*., 2019	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	N	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	N	Purity not reported. Source: Shanghai Ryon Biological Technology Co, Shanghai, China.
Only glyphosate acid or one of its salts is the tested substance	N	Also formulation was tested: Roundup from Sinochem Crop Protection Products, Shanghai, China.
AMPA is the tested substance	N	
Study		
Test species clearly and completely described	?	Mice.
Test conditions clearly and completely described	?	
Route and mode of administration described	Y	Oral via drinking water.
Dose levels reported	Y	One dose level: 0.5 % in drinking water.
Number of animals used per dose level reported	Y	10/dose.
Method of analysis described for analysis test media	N	
Validation of the analytical method	N	
Analytical verifications of test media	N	
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical control data of the laboratory reported	N	
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate used is not sufficiently characterised, only one dose level was tested, there was large inter animal variability observed and too few animals per dose level were analysed.		

1. Information on the study

Data point:	**CA 5.6.1/019**
Report author	Zhang, J.W. *et al.*
Report year	2019
Report title	The toxic effects and possible mechanisms of glyphosate on

	mouse oocytes
Document No	doi.org/10.1016/j.chemosphere.2019.124435 E-ISSN: 1879-1298
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	None
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary of the study according to OECD format

The effects of glyphosate on oocyte maturation, as well as its possible mechanisms remain unclear. The present study revealed that mouse oocytes had reduced rates of germinal vesicle breakdown (GVBD) and first polar body extrusion (PBE) after treatment with 500 µM glyphosate. Reactive oxygen species (ROS) were found in mouse oocytes exposed to glyphosate, as shown by changes in the mRNA expression of related antioxidant enzyme genes (*cat, sod2, gpx*). After 14 h of exposure to glyphosate, metaphase II (MII) mouse oocytes displayed an abnormal spindle morphology and DNA double-strand breaks (DNA-DSBs). Simultaneously, mitochondria showed an aggregated distribution and decreased membrane potential in mouse oocytes exposed to glyphosate. The protein expression levels of apoptosis factors (Bax, Bcl-2) and the mRNA expression levels of apoptosis-related genes (*bax, bcl-2, caspase3*) were measured by Western blot and qRT-PCR, respectively. Meanwhile, the expression levels of autophagy-related genes (*lc3, atg14, mtor*) and proteins (LC3, Atg12) were significantly decreased in the glyphosate treatment group compared with the control group.

Materials and methods

Antibodies and chemicals: Rabbit polyclonal anti-LC3A/B (light chain 3, LC3), anti-p-MAPK, anti-g-H2AX and anti-b-actin antibodies were purchased from Cell Signaling Technology. Mouse monoclonal anti-Bax, anti-Bcl-2, antiAtg12, anti-Annexin V and anti-α-tubulin antibodies were purchased from Santa Cruz Biotechnology. The Reactive Oxygen Species Assay Kit was purchased from Beyotime Biotechnology (S0033). The Mitochondrial Membrane Potential Assay Kit with JC-1 was obtained from Beyotime (C2005). Mito Tracker Red CMXRos was purchased from Cell Signaling Technology (#9082).
Mice: Female Kunming mice (25-30 g) were purchased from the Institute of Zoology, Chinese Academy of Sciences. They were housed in a temperature-controlled room with 12D:12L (dark vs. light) and had unrestricted access to food and water under conditions of constant temperature (23 ± 2° C).

Oocyte collection and treatment: To collect fully grown GV oocytes, the mice were superovulated by injecting them intraperitoneally with 10 IU Pregnant Mares Serum Gonadotropin (PMSG) 48 h earlier. The mice were sacrificed by cervical dislocation and the ovaries were placed in M2 medium. Oocytes were released from the ovaries by puncturing the follicles with a fine needle, and denuded oocytes were collected by gentle pipetting. GV oocytes were cultured in an incubator in 50 µL droplets of culture medium under liquid paraffin oil at 37 °C in 5 % CO_2. Glyphosate was dissolved in M16 medium and diluted to a final concentration of 50, 100, 200 or 500 µM. After culturing for 2 h or 14 h, GVBD and MII oocytes were used for the subsequent experiments.

Immunofluorescent staining: To determine the levels of intracellular ROS production, denuded MII oocytes from 6 mice were incubated with M16 medium that contained 10 µM dichlorofluorescein diacetate (DCFH-DA) for 30 min at 37 °C in the dark. After washing three times with 1 % Bovine Serum Albumin/Phosphate-buffered saline (BSA/PBS), the oocytes were placed in 50 µL of M16 medium droplets and the fluorescence was observed with a confocal laser-scanning microscope (Zeiss LSM 710 META, Germany) with the same scanning parameters. The fluorescence intensity of each oocyte was analysed using ImageJ software. For mitochondrial staining, MII oocytes from 6 mice were fixed in 4 % paraformaldehyde (PFA) for 1 h and then placed in membrane permeabilisation solution containing

0.5 % Triton X-100 for 20 min at room temperature. After washing three times with 1 % BSA/PBS, the oocytes were incubated in 200 nM Mito Tracker Red CMXRos in M16 medium for 30 min in the dark. After several washes, the oocytes were costained with DAPI (4', 6-diamidino-2-phenylindole) for 5 min. Images were captured by the confocal laser-scanning microscope. To measure mitochondrial membrane potential (MMP), MII oocytes from 6 mice were incubated at 37 °C for 20 min with the 1 x JC-1 probe, then washed twice with JC-1 buffer (5 min each) to remove surface fluorescence. Images of fluorescence were captured using confocal microscopy as above. MMP was quantified as the ratio of red to green fluorescence using ImageJ software. To measure the spindle, apoptosis, autophagy and DNA damage, MII oocytes from 24 mice were stained with anti-α-tubulin, antiAnnexin-V, anti-LC3and anti-$^{\gamma}$-H2AX antibodies, respectively. The denuded MII oocytes were fixed with 4 % PFA for 30 min, permeabilised in 0.5 % Triton-X 100 for 20 min at room temperature, and blocked with 1 % BSA/PBS for 1 h at 4 °C. The oocytes were incubated with anti-α-tubulin (1:200), anti-LC3 A/B (1:200), antiAnnexin-V (1:200) and anti-$^{\gamma}$-H2AX (1:200) at room temperature for 2 h followed by three washes with 1 % BSA/PBS. The oocytes were then incubated with an appropriate secondary antibody for 2 h at room temperature and then costained with DAPI for 5 min in the dark. After three washes, oocytes were mounted on a glass slide and representative images were captured by the confocal laser-scanning microscope. ImageJ software was used to quantify the fluorescence intensity in the images.

Quantitative real-time PCR: Total RNA was extracted from 100 MII oocytes from 12 mice using the TRNpure Total RNA Kit (Nobelab, Beijing, China) according to the manufacturer's instructions. The first strand cDNA was generated with the PrimeScript™ RT Reagent Kit with gDNA Eraser (Perfect Real Time) (Takara, China). All gene expression was determined using the FS Universal SYBR green PCR master mix (Roche, Canada) under the following conditions: 50 °C for 2 min, 95 °C for 10 min, 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Each sample was tested in triplicate and *gapdh* was used as an internal control gene. Relative expression levels were analysed by the $2^{-\Delta\Delta Ct}$ method.

Western blot: A total of 200 MII oocytes from 24 mice were lysed in 1 x SDS sample buffer containing 1 x protease inhibitor Cocktail (cwbiotech) on ice for 20 min. They were boiled for 5 min at 95 °C, then subjected to 12 % SDS-PAGE and the separated proteins were transferred to polyvinylidene fluoride (PVDF) membrane. The membrane was blocked for 2 h with 5 % nonfat milk in TBST and then probed with primary antibodies for 2 h at room temperature (anti-p-MAPK antibody, 1:500; anti-Bcl-2 antibody, 1:1000; anti-Bax antibody, 1:1000; anti-LC3 antibody, 1:1000; anti-Atg12 antibody, 1:500; anti-α-tubulin antibody, 1:1000; and anti-β-actin antibody, 1:1000). After washing three times with TBST (10 min each), membranes were incubated for 1 h with the appropriate HRP-conjugated secondary antibodies (1:1000). Three washes later, the protein bands were visualised using BeyoECL Plus (Thermo Fisher Scientific), and the signals were acquired by Tanon 5500. The images were quantified using ImageJ software.

Statistical analysis: At least 50 oocytes were analysed for each experiment. For each treatment, at least three biological replicates were performed and data are presented as the mean ± standard error of the mean (SEM). Statistical comparisons were performed using the GraphPad Prism software followed by Student's unpaired two-tailed t-test and two-way ANOVA. Statistical significance was set at P value: *<0.05, **<0.001, ***<0.0005, ****<0.0001.

Results

Glyphosate composition analysis: The chemical composition of glyphosate was analysed before the start of the experiment by Ultra Performance Liquid Chromatography/Quadrupole-Time-of-Flight-Mass Spectrometry (UPLC/Q-TOF-MS). Specific results from this analysis were not reported.

Glyphosate treatment reduced the GVBD and PBE in mouse oocytes: To test the effect of glyphosate on the mouse oocytes maturation, the oocytes were cultured in culture medium supplemented with increasing concentrations of glyphosate (50, 100, 200 or 500 μM). Typical images of exposure to glyphosate on GVBD and PBE are shown in Fig. 2. After treatment with glyphosate, GVBD (Fig. 2A and B) and PBE (Fig. 2C and D) were significantly decreased in the 200 mM and 500 mM groups, while there was no significant variation in the 50 μM and 100 μM treatment groups. Hence, the 500 μM glyphosate treatment was adopted for subsequent experiments. These observations were considered to show that glyphosate exposure decreased mouse oocyte developmental competence.

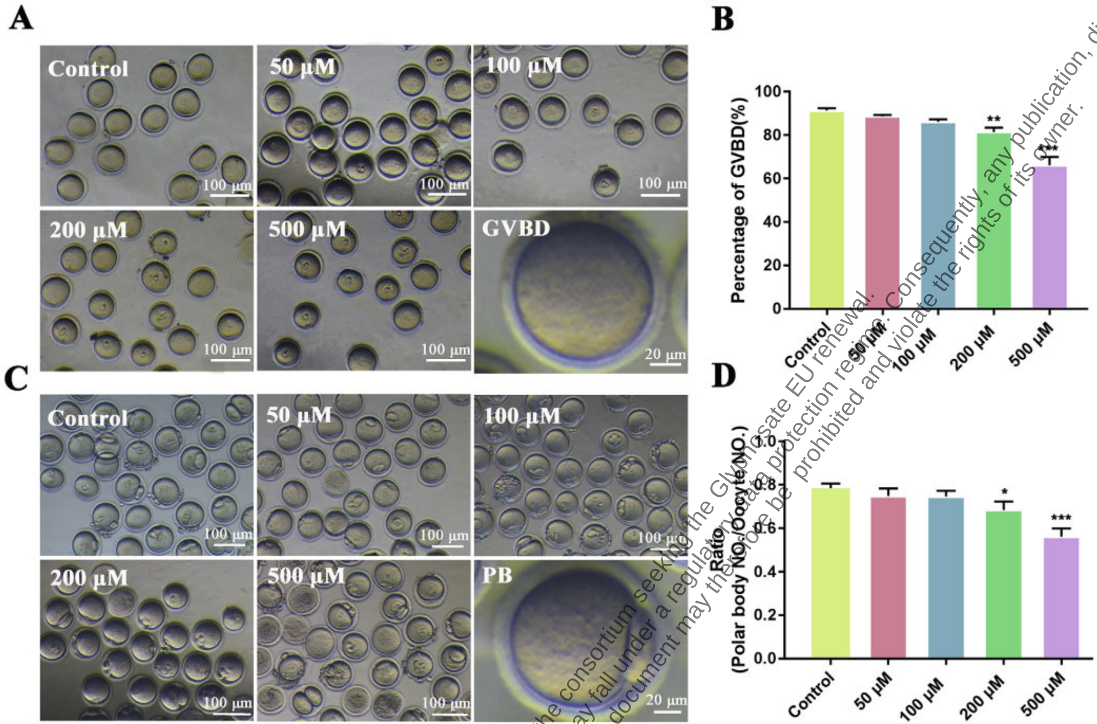


Fig. 2. Effects of glyphosate on GVBD and PBE in mouse oocytes. (A) Representative images of mouse oocytes after exposure to different concentrations of glyphosate for 2 h. (B) GVBD rates of different groups after 2 h of treatment. The data are expressed as the mean ± SEM. (C) Representative images of mouse oocytes after exposure to different concentrations of glyphosate for 14 h. (D) Quantification of the presence of PBE in control and treatment oocytes. The data are expressed as the mean ± SEM.

Glyphosate treatment increased ROS generation and DNA damage in mouse oocytes: It was tested whether defects observed in glyphosate-exposed mouse oocytes were mediated by oxidative stress. As shown in Fig. 3A and B, the fluorescence intensity of DCFH-DA was significantly higher in the 500 µM glyphosate treatment group than in the control oocytes. The levels of sod2 and gpx mRNA expression were significantly increased in glyphosate-exposed oocytes when compared with the control group (Control: sod2: 1.005 ± 0.066, gpx: 1.003 ± 0.052; 500 µM: sod2: 1.702 ± 0.148, gpx: 1.545 ± 0.181); the expression of *cat* mRNA was also increased (Control: 1.001 ± 0.036; 500 µM: 1.241 ± 0.111). These results suggested that glyphosate administration enhanced ROS production. As described above, glyphosate is cytotoxic to oocytes. As shown in Fig. 3D, there were no significant DSB foci in the control oocytes; however, there was a dense mass of γ-H2AX foci associated with chromatin in glyphosate-exposed oocytes. As shown in Fig. 3E, the fluorescence intensity of γ-H2AX also significantly increased after glyphosate treatment compared to that in the control group. These results were considered to indicate that glyphosate treatment results in DNA damage in mouse oocytes.

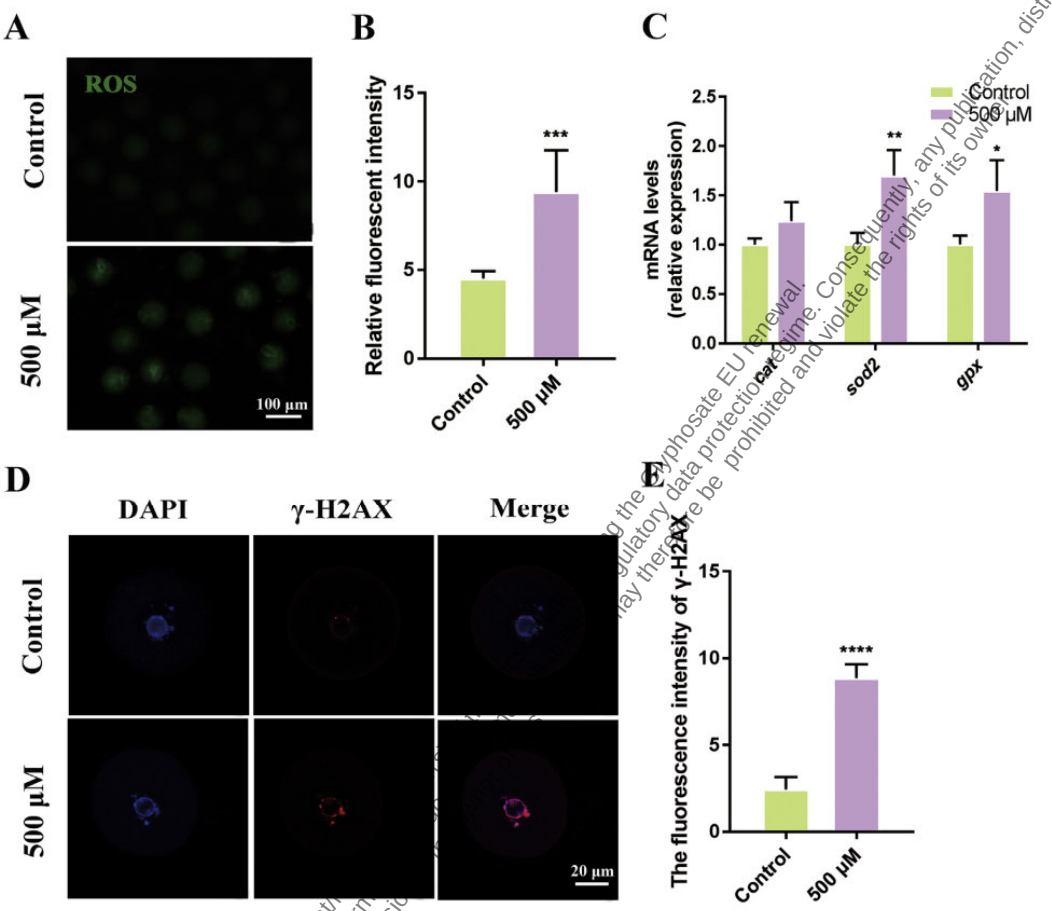


Fig. 3. Effects of glyphosate on ROS generation and DNA damage in mouse oocytes. (A) Representative images of DCFH-DA fluorescence in control and glyphosate-exposed mouse oocytes. (B) Quantitative analysis of ROS fluorescence intensity in control and glyphosate-exposed groups. The data are expressed as the mean ± SEM. (C) Relative expression levels of oxidative stress-related genes were examined by qRT-PCR. The data are expressed as the mean ± SEM. (D) Representative images of control and glyphosate-treated oocytes exhibiting γ-H2AX immunostaining. (E) The γ-H2AX fluorescence intensity obviously increased after glyphosate treatment. The data are presented as the mean ± SEM.

Glyphosate treatment disturbs spindle morphology in mouse oocytes: o investigate whether the oocytes with glyphosate treatment showed defects in spindle positioning and chromosome scattering, MII oocytes were assessed by immunocytochemical staining with anti-α-tubulin antibody and DAPI. In contrast to oocytes from the control group, which presented a normal spindle appearance and well-aligned chromosomes at the equatorial plate, the glyphosate-exposed group showed misaligned chromosomes and abnormal spindle morphology(Fig. 4A). The rate of abnormal spindles in the glyphosate treatment group was also increased (Control: 9.333 ± 0.491%; 500 µM: 15.767 ± 1.369%). as shown in Fig. 4B. The effect of glyphosate on the expression levels of spindle assembly regulatory protein was also assessed. In Fig. 4C and D, Western blots revealed that the level of p-MAPK protein was significantly reduced after 500 µM glyphosate treatment (Control: 0.856 ± 0.053; 500 µM: 0.517 ± 0.070). These results were considered to suggest that glyphosate treatment disturbs spindle morphology in mouse oocytes.

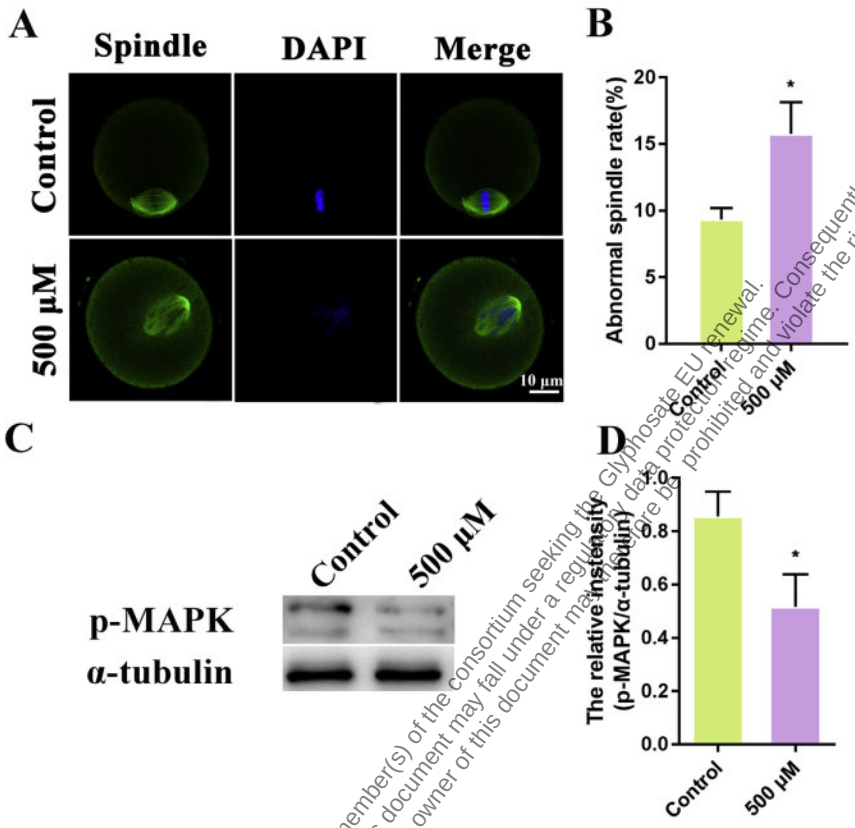


Fig. 4. Effects of glyphosate on spindle morphology and chromosome alignment in mouse oocytes. (A) Representative confocal images of spindle morphology in control and glyphosate exposed groups. (B) Percentage of cells exhibiting abnormal spindle/chromosome morphology. The data are expressed as the mean ± SEM. (C) The expression level of p-MAPK protein in oocytes from different groups was analysed by Western blot analysis. (D) The relative intensity of p-MAPK protein expression (p-MAPK/α-tubulin) was significantly reduced after treatment with 500 μM glyphosate. The data are expressed as the mean ± SEM.

Glyphosate treatment resulted in mitochondrial injury in mouse oocytes: A homogeneous distribution of mitochondria was present in the control group,but an aggregated distribution was seen in the glyphosate-exposed group by staining with Mito Tracker Red (Fig. 5A). Alterations in MMP of mouse oocytes from different groups were evaluated by staining with JC-1 (Fig. 5B). As shown in Fig. 5C, the result illustrated that the MMP was significantly lower in the glyphosate-exposed group compared with the control group (Control: 1.871 ± 0.082; 500 μM: 4.517 ± 0.155). Based on these data, glyphosate treatment was considered to interfere with mitochondrial function in mouse oocytes.

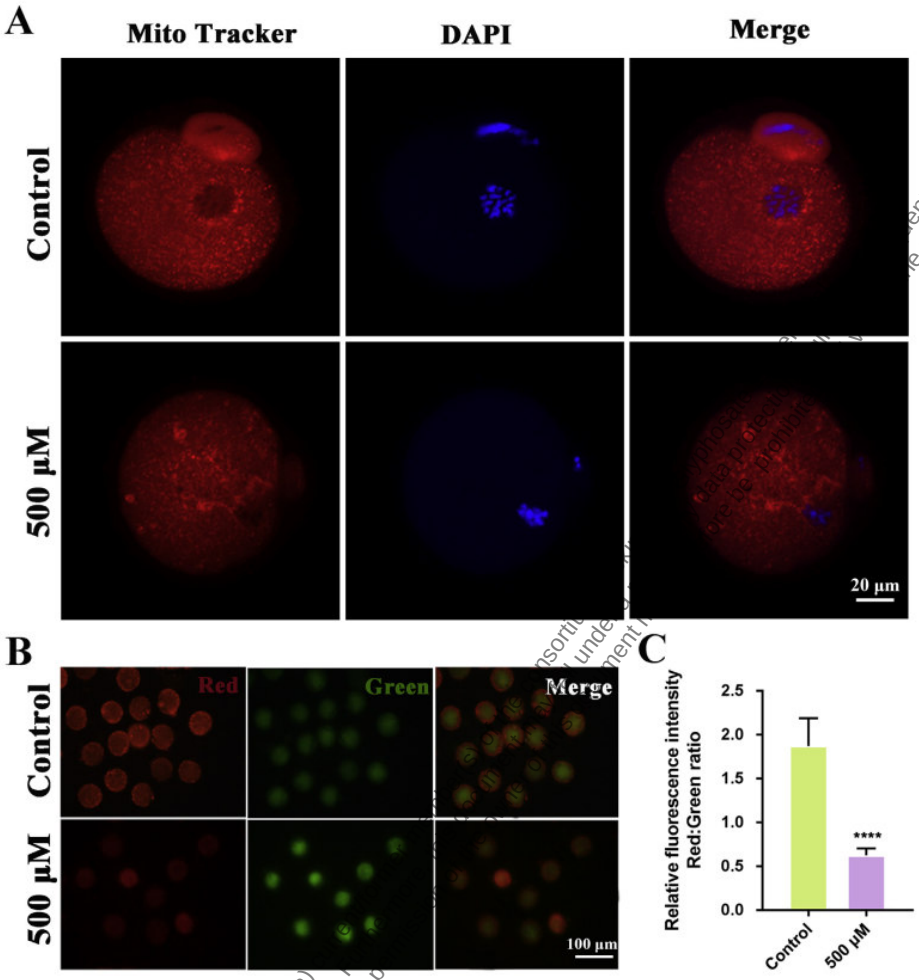


Fig. 5. Glyphosate treatment results in mitochondrial injury in mouse oocytes. (A) Representative images of mitochondrial distribution in control and glyphosate-treated oocytes by staining with MitoTracker Red. (B) Representative images of MMP in mouse oocytes from different groups stained with JC-1. (C) MMP levels (red/green fluorescence intensity) in glyphosate-exposed oocytes was significantly lower than that in the control group. The data are presented as the mean ± SEM. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Glyphosate treatment induced early apoptosis and autophagy in mouse oocytes: Annexin-V staining was conducted to identify whether early apoptosis occurred in glyphosate-treated oocytes. In the control group, Annexin-V signals were detected only in the zona pellucida, whereas the treatment group had a clear green signal in the membrane and zona pellucida Fig. 6A). In Fig. 6B, the apoptotic fluorescence intensity of oocytes was notably higher in the treatment group than in the controls. Apoptosis-related protein expression levels were also assayed via As shown in Fig. 6G and H, Western blot analysis, which showed that the expression of Bcl-2 protein decreased and the expression of Bax protein increased after exposure to 500 μM glyphosate (Control: Bcl-2: 0.645 ± 0.009, Bax: 0.427 ± 0.020; 500 μM: Bcl-2: 0.549 ± 0.011, Bax: 0.556 ± 0.018). To confirm this result, the mRNA expression levels of apoptosis-related genes were tested by qRT-PCR. The qRT-PCR results were consistent with the Western blot results. Together, these results were considered to indicate that glyphosate induced early apoptosis in mouse oocytes. Whether autophagy had occurred in glyphosate-treated oocytes was assessed by LC3 immunofluorescent staining; As shown in Fig. 6D and E, the fluorescence intensity was significantly higher in the 500 μM glyphosate treatment group than in the oocytes from the control group. The mRNA expression levels of autophagy-related genes were also assessed by qRT-PCR and showed an increasing trend (Control: *lc3*: 0.860 ± 0.013, *atg14*: 1.024 ± 0.149, *mtor*: 0.847 ± 0.171; 500 μM: *lc3*: 1.591 ± 0.109, *atg14*: 1.800 ± 0.139, *mtor*: 1.268 ± 0.242 (Fig. 6F). Western blot analysis showed that the expression of LC3 and Atg12 protein increased after exposure to 500 μM glyphosate (Control: LC3: 0.280 ± 0.021, Atg12: 0.435 ± 0.007; 500 μM: LC3:0.504 ± 0.030, Atg12: 0.580 ± 0.016). Together, these results were considered to indicate that glyphosate induced

autophagy in mouse oocytes.

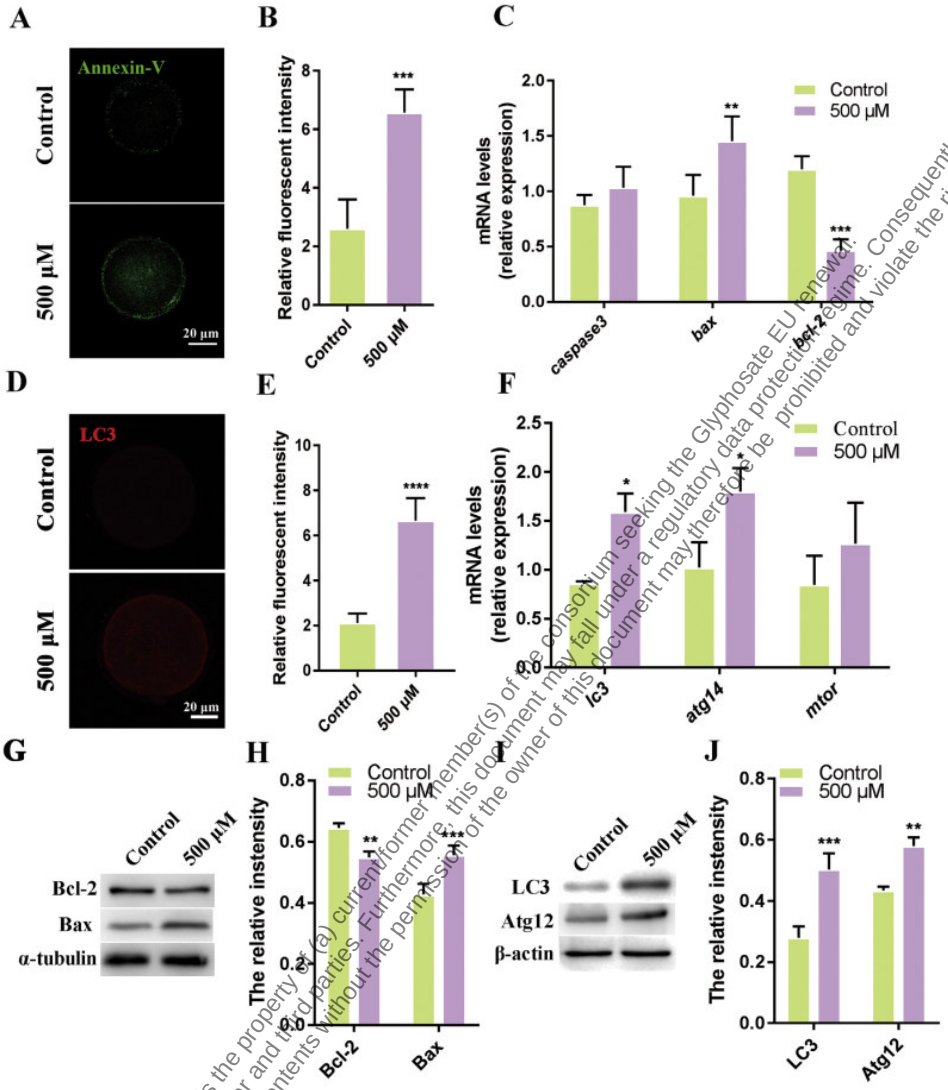


Fig. 6. Glyphosate treatment induces early apoptosis and autophagy in mouse oocytes. (A) Representative images of apoptosis (Annexin-V staining) in mouse oocytes. (B) Quantification of Annexin-V signal was recorded in control and glyphosate-exposed oocytes. (C) Relative mRNA levels of *caspase3*, *bax* and *bcl-2* in different groups of oocytes. (D) Representative images of autophagy (LC3 staining) in mouse oocytes. (E) Quantification of the fluorescence signal was recorded for the control and glyphosate-exposed oocytes. (F) The relative mRNA level of *lc3*, *atg14* and *mtor* in different groups oocytes. (G) The expression levels of apoptosis-related protein in oocytes from different groups were analysed by Western blot analysis. (H) The relative intensities of Bcl-2 and Bax protein expression were calculated. (I) The expression levels of autophagy-related proteins in oocytes from different groups were analysed by Western blot analysis. (J) The relative intensities of LC3 and Atg12 protein expression were calculated.

Discussion

In recent years, numerous studies have demonstrated the damaging effects caused by glyphosate in various organisms using different test systems. However, few studies evaluated the reproductive effects of glyphosate on female mammals. Therefore, we investigated the toxic effects of glyphosate on mouse oocyte maturation *in vitro*. Based on the experimental results, we believe that glyphosate contributed to the destruction of oocyte development . Germinal vesicle breakdown and the first polar body extrusion are markers of oocyte nuclear maturation. Our experimental results indicated that mouse oocytes exposed to glyphosate in vitro experienced a decrease in the proportion of GVBD and PBE. The decrease in the ratio of GVBD and PBE indicates that the growth and developmental ability of the oocyte is destroyed. Reactive oxygen species are excessively generated when cells are subjected to various noxious stimuli, and the oxidative system and the antioxidant system are out of balance, resulting in cell damage. Therefore, we examined the level of ROS in mouse oocytes after glyphosate exposure, and our results showed that ROS levels were increased. To survive, the expression of the antioxidant enzyme genes is increases increased.

The mRNA expression levels of sod2, cat and gpx were significantly increased in mouse oocytes exposed to glyphosate. Mouse oocytes exposed to glyphosate produced excessive ROS, which in turn affected their growth and development. Oocytes are rich in mitochondria. These mitochondria play a leading role in cell metabolism. The changes in their morphology, quantity, distribution and function are closely related to cell metabolism, proliferation and differentiation. Mitochondria are mostly uniformly distributed in the cytoplasm in oocytes with higher developmental potential, while they are aggregated in oocytes with lower developmental potential. A lack of mitochondrial rearrangement in the cytoplasm is a marker of immature cytoplasm. Our results showed that mouse oocytes exposed to glyphosate had aggregated mitochondria and a decrease in membrane potential. These findings indicated that glyphosate destroyed the function of the mitochondria in oocytes. The cytoskeleton plays an important supporting role in the oocyte maturation process. The morphology and kinetic changes of the spindle may reflect the quality of oocytes, and its integrity determines the correctness of chromosome segregation. Abnormalities in spindle morphology will lead to meiosis and fertilisation failure. MAPK is a regulated protein involved in microtubule assembly and mitotic spindle assembly. The experimental results showed that after glyphosate exposure, the expression level of p-MAPK protein in mouse oocytes was decreased. So, we proposed that glyphosate affected formation of the spindle by altering the level of p-MAPK, which in turn resulted in a reduction in the rate of mouse oocyte maturation. Excessive accumulation of ROS products has an adverse effect on lipids, proteins, and DNA. In addition, ROS damages the sugarphosphate back-bone of DNA, resulting in single- and double-strand breaks. DNA damages in cells primarily originate from oxidative stress. g-H2AX produced by phosphorylation of H2AX can be used as a biomarker to clearly reflect the extent of DNA damage and repair. Oocytes exposed to glyphosate showed an increase in g-H2AX foci, showing that glyphosate could destroy DNA integrity and produce cytotoxicity in oocytes. Oxidative stress is often associated with apoptosis and autophagy. Therefore, we first detected apoptosis in oocytes by Annexin-V staining. Our experimental results showed that the apoptotic fluorescence intensity of oocytes was increased after glyphosate exposure. The Bcl-2 gene is an inhibitor of apoptosis, and Bax is a pro-apoptotic gene that plays an important role in regulating apoptosis. In the mitochondrial mediated apoptosis pathway, abnormal intracellular signals lead to the activation of Bax, inhibit the expression of Bcl-2, induce mitochondria release of cytochrome c, form apoptotic bodies or initiate caspase cascade activation, and then lead to the occurrence of apoptosis. Western blot analysis and qRT-PCR showed that the expression of Bcl-2 was decreased and the expression of Bax was increased in glyphosate-treated oocytes. Although there was no significant difference in caspase3 mRNA expression, there was an increasing trend after exposure to glyphosate. Autophagy mainly includes phagophore, autophagy formation, autophagosome formation and degradation. The combination of PI3K complex and Atg14 is involved in the formation of phagophore. Atg gene regulates the formation of autophagosomes by forming Atg12-Atg5 and LC3-II complexes. Microtubule associated light chain protein (LC3) is present in autophagosomes; therefore, the LC3 protein is widely used as a marker for autophagosomes. Mammalian target of rapamycin, mTOR, also plays a role in the autophagy pathway. Our results showed that the fluorescence intensity of LC3 was significantly increased, and the expression levels of autophagy-related proteins and genes were increased in the glyphosate treatment group. We considered that oocytes exposed to glyphosate-induced ROS, which could further activate autophagy and early apoptosis, could lead to a decrease in oocyte quality.

Conclusion

In summary, glyphosate exposure was considered to have caused a block in mouse oocyte development, spindle assembly disruption and chromosome scattered distribution, mitochondrial aggregation and membrane potential reduction, DNA damage, and increased oxidative stress levels, which then led to cellular apoptosis and autophagy. These results were considered to provide evidence for the toxic effects of glyphosate on reproductive systems.

3. Assessment and conclusion

Assessment and conclusion by applicant:

In vitro intracellular changes in Kunming mice oocytes were evaluated after being cultured in medium

supplemented with 500 µM glyphosate. Findings included: decreased germinal vesicle breakdown, decreased first polar body extrusion, increased mRNA expression of anti-oxidant enzyme-related genes, abnormal spindle morphology, increased DNA double strand breaks, aggregated mitochondria, decreased mitochondrial membrane potential, increased protein expression of apoptosis factors, increased mRNA expression of apoptosis related genes and decreased autophagy-related genes.

No dose-response could be determined as only one concentration was tested, far in excess of that considered biologically relevant. Whilst some evaluations were conducted on oocytes harvested from a wider data set of 24 mice (protein expression levels of apoptosis factors by Western blot analysis), a number of the assessments were conducted on oocytes from just 12 mice (mRNA expression of oxidative stress-related, apoptosis-related and autophagy-related genes) or 6 mice (mitochondrial staining, measurement of mitochondrial membrane potential). This narrow source of oocytes limits the robustness of certain conclusions. Furthermore, there are insufficient details reported in the methods to establish whether mice were of the same age before oocyte harvesting or the purity of the glyphosate tested.
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because of the poor characterisation of the glyphosate tested, no cytotoxicity testing, the lack of a positive control and insufficient dose-response characterisation at biologically relevant doses.

Reliability criteria for *in vitro* toxicology studies

Publication: Zhang JW *et al*., 2019.	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	N	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	N	Purity and source not reported. Chemical analysis was performed but the results were not clear.
Only glyphosate acid or one of its salts is the tested substance	Y	
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	Y	
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	Not reported	
Test concentrations in physiologically acceptable range (< 1 mM)	Y	50, 100, 200, 500 µM. Most of the assays were carried out at 500 µM which is a concentration that cannot be reached systemically in the rat at 2000 mg/kg bw after oral intake.

Publication: Zhang JW *et al*., 2019.	**Criteria met? Y/N/?**	**Comments**
Cytotoxicity tests reported	N	
Positive and negative controls	N	No positive control used.
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because of the poor characterisation of the glyphosate tested, no cytotoxicity testing and the lack of a positive control.		

1. Information on the study

Data point:	**CA 5.6.1/020**
Report author	Johansson, H.K.L. *et al.*
Report year	2018
Report title	Exposure to a glyphosate-based herbicide formulation, but not glyphosate alone, has only minor effects on adult rat testis
Document No	doi.org/10.1016/j.reprotox.2018.09.008 E-ISSN: 1873-1708
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	No
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary of the study according to OECD format

Glyphosate has been suggested to be an endocrine disrupting chemical capable of disrupting male reproduction. There are conflicting data, however, with studies reporting effects from exposure to either glyphosate alone or to herbicide formulations, making comparisons difficult. Rat testis histopathology and androgen function following two weeks exposure to either glyphosate at 2.5 and 25 mg/kg bw/day (5x and 50x Acceptable Daily Intake, ADI, respectively), or equivalent high dose of glyphosate in a herbicide formulation; Glyfonova, were assessed.

Materials and methods

Chemicals - Glyphosate (purity ≥ 96 %) was purchased from Sigma-Aldrich, St Louis, USA.

Animals - Four–week old male Sprague-Dawley rats were randomly caged in pairs and acclimatised for 7 days. Cages were subsequently distributed into four exposure groups based on animal weight, but following exposure only one animal per cage was used for analyses. Ten animals per dose group were treated with

glyphosate orally by gavage at 2.5 and 25 mg/kg bw. Water was used as the control. After 2 weeks of exposure, the animals were decapitated under CO_2/O_2 sedation and the testes collected. From each of 10 males per dose group, one testis, selected at random, was snap frozen in liquid nitrogen and the other was fixed in 10 % formalin.

Testosterone assay - One half of each testis was used for hormone extraction. The testes were cut into two equally sized pieces. After removal of the testis cap the inner tissue (seminiferous tubules and interstitial cells) was transferred immediately to a glass vial containing 0.5 mL sterile water and reweighed to determine total tissue weight per sample. Subsequently, 2.5 mL heptane was added and the sample homogenised by manual disruption and then frozen solid. The heptane fraction was transferred and the process repeated to yield a second 2.5 mL heptane fraction that was pooled together with the first fraction. The extracts were dried under nitrogen. Before hormone analyses, the dry extracts were dissolved in EIA buffer at 4 °C overnight, then vortexed and placed in a 42 °C water bath for 10 minutes. Intra-testicular testosterone assays were performed using the Testosterone ELISA kit according to the manufacturer's instructions. Reads were obtained using 96-well plates and absorbance read at 405 nm using a microplate reader. Each sample was assayed in duplicates and result means presented as pg testosterone/g testis.

Histology - Testes were fixed in 10 % formalin, processed for paraffin embedding and sectioned at 5 µm for histological assessments. Hematoxylin & Eosin (H&E) staining was performed following standard protocols.

Immunohistochemistry - Immunohistochemistry with peroxidase was carried out on sections of formalin-fixed testes. Sections were dewaxed in petroleum and rehydrated by immersion in ethanol solutions and finally water. Antigen retrieval was carried out in 0.01 M citrate buffer (pH 6) in a microwave and then allowed to cool at room temperature. Samples were washed in PBS and blocked with 1 % bovine serum albumin (BSA) and then incubated overnight at 4 °C with primary antibody. The following day, samples were washed in PBS, blocked with 3 % H_2O_2 for 10 minutes and again washed in PBS. The samples were incubated with EnVision+System (Dako) for 30 minutes, washed, then with Liquid DAB+System (Dako) for 15 minutes and washed again. To visualize all cell nuclei, sections were counterstained with Meyers' hematoxylin. Following washing and rehydration, samples were mounted with Eukitt. The primary antibody used was against the Androgen receptor.

Immunofluorescence – Five µm sections (4/group) were dewaxed in petroleum and washed in ethanol and dehydrated. Antigen retrieval was done by heat treatment in Tris-EDTA buffer (pH 9) and then cooled at room temp before washing in PBS and blocking in 5 % bovine serum albumin (BSA). The samples were incubated with primary antibodies overnight at 4 °C. The next day, slides were brought to room temperature and incubated with secondary antibodies for 1 hour in the dark. Samples were then counterstained with 4,6-diamidino-2-phenylindole (DAPI). The primary antibodies were goat anti-HSD3β, rabbit anti-DDX4, goat anti-CYP11A1 and rabbit anti-STAR. Secondary antibodies were donkey anti-goat AlexaFluor-488 and donkey anti-rabbit AlexaFluor-568.

RNA extraction, cDNA synthesis and quantitative RT-PCR - Total RNA was extracted from a ~100 mg cross-sectional piece from each testis (n=10/group), of which 500 ng RNA was used for each cDNA synthesis. TaqMan Gene Expression Assays were Ar (Rn00560747), Ddx4 (Rn01489814), Cyp11a1, Cyp17a1, Insl3 (Rn00586632), Pcna (Rn01514538), Star and Hsd3b1 (Rn017747410). RT-qPCR assays were run on a QuantStudio 7 Flex Real-Time PCR System in a 384-well format using 3 µL diluted (1:20) cDNA as template in each 20 µL reaction. Relative transcript abundance was calculated by the comparative Ct-method with the geometric mean of the reference genes Sdha (Rn00590475) and Rpl13a (Rn00821946).

Apoptosis - One section per testis (n=5/group) was stained with terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL, Apoptag®Peroxidase in Situ Apoptosis Detection Kit) according to the manufacturer's instructions with one exception i.e. pre-dilution (1:80) of the TdT enzyme in MilliQ water before dilution with reaction buffer. Color was developed for 15 minutes using DAB+substrate. Lastly, the nuclei were stained using Mayer Hematoxylin prior to mounting using Eukitt. All positively stained cells

within the seminiferous tubules on a cross-section were counted. Cells that were clearly stained as a result of cell division rather than apoptosis were excluded, as were stained cells at the outer border of the tissue sections. The relative percentage of seminiferous epithelium to whole testis was calculated using a 15 point-grid count on three separate fields of view from each sample. Second, whole testis section areas were calculated in Adobe Photoshop CC 2017 using the measurement tool. Third, apoptotic cells were defined relative to percentage seminiferous epithelium to whole section. Data and statistical analyses are presented as number of apoptotic cells per seminiferous tubuli area.

Statistical analysis - Data were tested for normal distribution and homogeneity of variance and logarithmic transformation was applied if required. ANOVA with Dunnett's post-test was applied and $p<0.05$ was considered statistically significant. Statistical software GraphPad Prism 5 was used for analysis.

Results

Testosterone levels - No statistically significant increase in testicular testosterone levels was observed in rats treated orally at 2.5 and 25 mg/kg bw/day for 2 weeks.

Quantitative gene and protein expression analysis - In the testis from animals orally exposed to glyphosate at 2.5 or 25 mg/kg bw/day for 2 weeks no significant differences in gene expression were observed for the Leydig cell specific genes Cyp11a1, Cyp17a1, Insl3, Hsd3β1 and Star and the somatic marker gene Ar or germ cell marker gene Ddx4.

Testis histopathology - No adverse histopathology in any of the glyphosate exposed groups was observed when compared with controls. The seminiferous tubules were intact and displayed active spermatogenesis with comparable spermatogenic cycling between examined specimens (qualitative assessment). Neither were there any differences between exposed and control animals with regard to missing germ cell layers or multinucleated germ cells. The interstitial space was comparable between glyphosate treated groups and controls.

Qualitative protein expression analysis - The Leydig cell-specific steroidogenesis factors CYP11A1 and STAR were both expressed at comparative levels between the glyphosate treated animals and the controls. Also no difference in expression and distribution was noted between glyphosate treated groups and controls for the steroidogenic enzyme HSD3B1 and the germ cell-specific factor DDX4.

Apoptosis - The number of apoptotic cells was comparable between control testes and all glyphosate treated groups (5 per group).

Discussion and conclusions

Glyphosate at 2.5 mg/kg bw/day (5-fold ADI) and 25 mg/kg bw/day (50-fold ADI) did not show significant effects on intra-testicular testosterone levels, testicular gene and protein expression, testicular histopathology and apoptosis in the testes of rats treated orally for 2 weeks. This suggests that the active compound glyphosate may not contribute to previously reported endocrine disrupting effects, but rather other constituents in the commercial formulated products. This is in agreement with conclusions from the most recent expert opinion review on glyphosate commissioned by the European Food Safety Authority which conclude that there is no evidence of endocrine mode of action of glyphosate.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The effects of glyphosate on intra-testicular testosterone levels, expression of Leydig cell-specific genes Cyp11a1, Cyp17a1, Insl3, Hsd3β1 and Star and expression of somatic marker gene Ar or germ cell marker gene Ddx4, expression of Leydig cell-specific steroidogenesis factors CYP11A1 and STAR, testicular histopathology and apoptosis were investigated in male rats treated orally at 0, 2.5 and 25 mg/kg bw/day for 2 weeks. No effects were found on either of the testicular parameters tested

suggesting that glyphosate does not contribute to endocrine disrupting effects of the male reproductive system.
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because only two dose levels were used to explore the dose-effect relationship for the endpoints assessed.

Reliability criteria for *in vivo* toxicology studies

Publication: Johansson *et al*., 2018.	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity ≥ 96 %. Source: Sigma-Aldrich, St Louis, USA.
Only glyphosate acid or one of its salts is the tested substance		Also formulations were tested: Glyfonova 450 Plus, FMC corporation.
AMPA is the tested substance	N	
Study		
Test species clearly and completely described	Y	
Test conditions clearly and completely described	Y	
Route and mode of administration described	Y	Oral by gavage.
Dose levels reported	Y	Only 2 dose levels for glyphosate (2.5 and 25 mg/kg bw)
Number of animals used per dose level reported	Y	10 animals/group.
Method of analysis described for analysis test media	N	
Validation of the analytical method	N	
Analytical verifications of test media	N	
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical control data of the laboratory reported	N	
Dose-effect relationship reported	Y	Only 2 dose levels tested.
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because only two dose levels were used to explore the dose-effect relationship for the endpoints assessed.		

I. Information on the study

Data point:	**CA 5.6.1/021**

Report author	Panzacchi, S. *et al.*
Report year	2018
Report title	The Ramazzini Institute 13–week study on glyphosate-based herbicides at human equivalent dose in Sprague Dawley rats: study design and first in-life endpoints evaluation
Document No	doi.org/10.1186/s12940-018-0393-y ISSN: 1476-069X
Guidelines followed in study	Based on the National Toxicology Program's (NTP) Modified One-Generation Reproduction Study 2011.
Deviations from current test guideline	No
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary of the study according to OECD format

The current study represents the first phase of a long-term investigation of glyphosate-based herbicides (GBHs) that we are conducting over 5 years. In this paper, the study design, the first evaluation of *in vivo* parameters and the determination of glyphosate and its major metabolite aminomethylphosphonic acid (AMPA) in urine is presented.

Materials and methods

Chemicals - Glyphosate (purity of > 99.5 %), Pestanal™ analytical standard purchased from Sigma-Aldrich (Milan, Italy). The representative formulated product, Roundup Bioflow (MON 52276, containing 360 g/L of glyphosate acid in the form of 480 g/L isopropylamine salt of glyphosate (41.5 %), water (42.5 %) and surfactant (16 %)) was purchased from Consorzio Agrario dell'Emilia, Bologna, Italy.

Animals - Male and female SD rats were obtained from the colony used at the Cesare Maltoni Cancer Research Center laboratories of the Ramazzini Institute (CMCRC/RI). The animal room conditions were 22 ± 3 °C and 50 ± 20% relative humidity and a light/dark cycle of 12 hours. During the experiment the animals received standard pellet feed and tap water *ad libitum*.

Experimental design - Each of 8 virgin female SD rats (17 weeks old, 270-315 g) per dose group was placed individually in a polycarbonate cage with a single male rat of the same age and strain until evidence of copulation. Gestational day (GD) 0 was defined as the day on which sperm was found in vaginal smears. After mating, matched females were housed separately during gestation and delivery and pups were housed with the dams until weaning. The day on which parturition was completed was designated as lactating day (LD) 0 for the dam and postnatal day (PND) 0 for the offspring. On PND 28, the offspring were weaned and identified by ear punch. Sequentially, they were allocated in the same treatment group of their mother to obtain 18 animals (8 for the 6–week cohort and 10 for the 13–week cohort) per sex and for each dose group. No more than 2 males and 2 females from the same litter were included in the same cohort/treatment group. Altogether, 54 males and 54 females were enrolled in the post-weaning treatment phase. Rats were treated with glyphosate and MON 52276 at 1.75 mg glyphosate acid eq./kg bw/day in the drinking water. One group received only tap water as control. After weaning, until the end of the experiment (PND 73 ± 2 or 125 ± 2), glyphosate and MON 52276 were administered in the drinking water to F1 animals on the basis of the average body weight and average water consumption per sex and per experimental group. Males and females were considered separately because of their difference in weight gain, body weight and water consumption. Animals were checked 3 times daily on working days and 2 times daily on Sundays and non-working days. Clinical signs were checked before the start of the treatment, and at least every two days until the end of the experiment. The body weight of the dams was recorded on GD 0, 3, 6 and then daily

during gestation until parturition. During lactation, the body weight of the dams was recorded at LD 1, 4, 7, 10, 13, 16, 19, 21 and 25 and the body weight of the pups by sex and litter was determined on PND 1, 4, 7, 10, 13, 16, 19, 21 and 25. After weaning, body weight was measured twice a week until PND 73 ± 2, then weekly until PND 125 ± 2 and before terminal sacrifice. The mean individual body weights were calculated for each group and sex. Feed and water consumption of the dams were recorded twice weekly on GD 0, 3, 6, 9, 12, 15, 18, 21, and during lactation on LD 1, 4, 7, 10, 13, 16, 19, 21, 25 and 28. After weaning the daily feed and water consumption per cage were measured twice a week, until PND 73 ± 2, then weekly until PND 125 ± 2. The mean individual feed and water consumption were calculated for each group and sex. The day before terminal sacrifice, all the animals were placed individually in metabolic cages and starved for around 16 hours during which the animals had free access to water alone or to the test compound solutions. In the morning of the following day, samples of at least 5 mL of urine collected from each animal were transferred to labelled tubes for analysis of glyphosate and AMPA. Samples from 3 dams/group and from 5 rats/sex/group belonging to the 6–week and 13–week cohorts were used for analysis.

Analysis of glyphosate and AMPA in urine - Analysis of glyphosate and AMPA in drinking water, feed and urine were performed by Neotron Laboratories. The specification and results are maintained in the experimental documentation. Analysis was performed using LC-MS/MS. The limit of quantification (LOQ) for glyphosate and AMPA was 0.10 µg/L in water, 50 µg/kg in feed, and 1 µg/L in urine.

Statistical analysis - Means ± standard deviations (SD), were calculated for continuous variables. For body weight, water and feed consumption over time multilevel mixed-effect linear regression models were used, to control for within subject correlation across time also considering the litter effect during the lactation period. Analysis of variance and Dunnett's test (when applicable) were also performed to compare body weight gain in different periods and consumption of food and water as mean consumption in several periods. All tests were two tailed with results considered as statistically significant if $p<0.05$. Statistical analyses were performed by using STATA version10.

Results

Mortality, body weight, water and food consumption, clinical signs and litter data - All 24 dams and 108 rats from the 6–week and 13–week cohorts survived until sacrifice. Body weight and body weight gain of the dams during gestation and lactation were not statistically different among groups. Post-weaning body weight of female and male offspring was homogeneous and no statistically significant differences in body weight gain were observed among groups. Water and feed consumption during gestation and lactation were not different across groups. Litter sizes were fully comparable among groups, with mean number of live pups of 13.6 (range 10–16) in the control group, 13.3 (range 11–17) in the glyphosate group and 13.9 (range 11–16) in the MON 52276 group. Post weaning water and feed consumption were not affected by treatment. There was no clinical evidence of alterations in activity or behavior, reflexes, eyes or skin, respiratory, gastrointestinal, genitourinary and cardiovascular systems.

Analysis of glyphosate and AMPA in urine – The results of the analysis are presented in the Table below.

Table: Glyphosate and AMPA concentration in urine. Results are reported as mean ± standard deviations

		Dams		Offspring (6-week cohort)		Offspring (13-week cohort)	
	Treatment	Glyphosate (mg/kg)	AMPA (mg/kg)	Glyphosate (mg/kg)	AMPA (mg/kg)	Glyphosate (mg/kg)	AMPA (mg/kg)
Male	Control			0.012 ± 0.010	0.003 ± 0.003	0.011 ± 0.010	0.006 ± 0.004
	Glyphosate	–	–	0.938 ± 0.414	0.014 ± 0.007	1.684 ± 0.768	0.023 ± 0.012
	Roundup			1.174 ± 0.439	0.011 ± 0.005	2.280 ± 1.520	0.027 ± 0.016
Female	Control	0.009 ± 0.001	0.006 ± 0.002	0.013 ± 0.007	0.005 ± 0.001	0.008 ± 0.005	0.003 ± 0.005
	Glyphosate	0.480 ± 0.010	0.024 ± 0.002	0.938 ± 0.377	0.016 ± 0.010	1.354 ± 0.359	0.013 ± 0.006
	Roundup	0.700 ± 0.106	0.024 ± 0.001	0.910 ± 0.383	0.018 ± 0.007	1.524 ± 0.585	0.021 ± 0.007

The urinary concentrations of glyphosate and AMPA of rats treated with glyphosate at 1.75 mg/kg bw/day were comparable to those observed in rats treated with MON52276 at 1.75 mg glyphosate acid eq./kg bw/day, despite the limited sample size and the large standard deviations. Glyphosate and AMPA urinary levels were all below or close to the LOQ of 0.001 mg/kg in the control group. In the treated rats, the majority of glyphosate was excreted unchanged, with urinary levels about 100-fold higher than that of AMPA. For example, the mean urinary levels of glyphosate were 1.354 and 1.524 mg/kg bw in glyphosate and MON 52276 treated females in the 13–week cohort, respectively, while the corresponding AMPA levels were 0.013 and 0.021 mg/kg bw. In glyphosate and MON 52276 treated rats, a time-dependent increase in the mean urinary concentration of glyphosate was observed. In glyphosate and MON 52276 treated males, an increase of approximately 2-fold was observed of the mean urinary concentration of glyphosate in the 13–week cohort when compared to the 6–week cohort. In glyphosate treated females, the 6–week cohort showed a 2-fold higher mean urinary concentration of glyphosate than the dams after weaning, while the 13–week cohort showed a 1.5-fold increase compared to the 6–week cohort. In the MON 52276 group, the increase was less steep, but the time-dependent pattern was still evident. In glyphosate and MON 52276 treated rats, the levels of AMPA were comparable at the different time points in both males and females. Large variations were observed of the AMPA concentrations in urine, in particular those close to the LOQ as in the control groups.

Discussion

Survival, body weights, food and water consumption of rats were not affected by the treatment with glyphosate or MON 52276. No clinical changes were observed in the animals of the dosed groups. Overall, oral treatment of glyphosate and MON 52276 via the drinking water seemed to be well tolerated. Exposure to glyphosate and MON 52276 led to comparable concentrations of glyphosate and AMPA in urine, indicating that systemic exposure does occur at the selected exposure level of 1.75 mg/kg bw/day, corresponding to the US ADI. The bioavailability of glyphosate in this study is also supported by the evident increase of glyphosate concentration in urine in relation to the length of treatment. The adjuvants and the other substances present in MON 52276 did not seem to exert a major effect on the absorption and excretion of glyphosate, even though mean values of glyphosate seem to be somewhat higher in the formulation treated group. The levels in urine were also comparable between the two sexes, although a consistent inter-individual variability was observed. In rats, glyphosate in urine appears to be the most accurate biomarker of exposure to glyphosate based herbicides (GBHs). The results from this study confirm previous evidence that in rodents most of the administered dose of glyphosate (98 %) is excreted as unchanged parent compound, whereas the metabolite AMPA in urine is at around 0.2–0.3 % of the administered dose. Furthermore, with the level of exposure to glyphosate used in this pilot study, AMPA urinary values of treated animals (0.011–0.027 mg/kg) were already close to the LOQ (0.001 mg/kg) which might limit the reliability of the data. Glyphosate concentrations in urine of treated animals (0.480–2.280 mg/kg) were found to be 100-fold higher than the AMPA concentrations and at least 500-fold higher than the LOQ. Therefore, in order to assess exposure to glyphosate in rats, in particular at doses that are equal or lower than 1.75 mg/kg bw/day, glyphosate appears to be the biomarker of choice. The presence of negligible levels of glyphosate (0.003–0.013 mg/kg) in some of the urine samples of the control groups might reflect an ubiquitous environmental contamination at ultra-low doses of glyphosate, which is consistent with previous reports from other authors. As the current LOQ of glyphosate in HPLC for pelleted animal feed is 0.050 mg/kg, this represents a technical limiting factor for the testing ultra-low doses of glyphosate.

Conclusion

A pilot study was performed on the health effects of glyphosate and its formulation Roundup Bioflow (MON 52276) administered orally to rats at the US ADI of 1.75 mg/kg bw/day. Treatment with either glyphosate or MON 52276 seemed to be overall well tolerated, consistent with previous experiments performed by the US NTP. Both glyphosate and MON 52276 exposure led to comparable urinary concentrations of glyphosate and AMPA with an increasing excretion of glyphosate in urine with duration of treatment. This indicates the systemic bioavailability of glyphosate and a possible mechanism of bioaccumulaton. The adjuvants and the other substances present in the formulation did not seem to exert a major effect on the absorption and excretion of glyphosate when administered orally. The results of this

study confirm that, in rodents, glyphosate is a much more relevant biomarker in urine than AMPA, in particular at doses that are equal or lower than 1.75 mg/kg bw/day.

3. Assessment and conclusion

Assessment and conclusion by applicant:

In this study the general toxicity of glyphosate was compared against that of its reference formulation MON 52276 in pregnant rats and their progeny. Also the urinary excretion of glyphosate and AMPA was investigated. The test compounds were administered via the drinking water resulting in a daily dose of 1.75 mg glyphosate acid eq./ kg bw. The endpoints investigated were mortality, body weight, water and food consumption, and clinical signs in dams and offspring and litter data. There was no mortality and no statistically significant differences were observed among control, glyphosate, and MON 52276 groups in any of the endpoints investigated. Urinary concentrations of glyphosate and AMPA of rats treated with glyphosate at 1.75 mg/kg bw/day were comparable to those observed in rats treated with MON 52276 at 1.75 mg glyphosate acid eq. /kg bw/day. This indicates that the co-formulants in this glyphosate formulation have little influence on the oral bioavailability of glyphosate. In the treated rats, the majority of glyphosate was excreted in urine unchanged at levels of about 100-fold higher than that of AMPA and the mean urinary concentration of glyphosate increased with the duration of treatment.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because only one dose level for glyphosate and MON 52276 was considered, only 8 animals were used per dose and per sex and the method of analysis of glyphosate and AMPA in urine and its validation were not fully reported.

Reliability criteria for *in vivo* toxicology studies

Publication: Panzacchi *et al*., 2018	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	Only a part of the reproductive toxicology study was reported, one dose level of glyphosate or MON 52276 was considered and 8 females per dose group were used.
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	For the part that was reported.
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Glyphosate (purity of > 99,5 %), Pestanal™ analytical standard purchased from Sigma-Aldrich (Milan, Italy).
Only glyphosate acid or one of its salts is the tested substance	N	The representative formulated product, Roundup Bioflow (MON 52276, containing 360 g/L of

		glyphosate acid in the form of 480 g/L isopropylamine salt of glyphosate (41.5 %), water (42.5 %) and surfactant (16 %) was purchased from Consorzio Agrario dell'Emilia, Bologna, Italy.
AMPA is the tested substance	Y	Determined in urine of rats treated with glyphosate and MON 52276.
Study		
Test species clearly and completely described	Y	Male and female SD rats.
Test conditions clearly and completely described	Y	
Route and mode of administration described	Y	Oral via drinking water.
Dose levels reported	Y	1.75 mg glyphosate acid eq./kg bw/day administered as glyphosate and as MON 52276.
Number of animals used per dose level reported	Y	8 virgin female SD rats per dose group.
Method of analysis described for analysis test media	N	
Validation of the analytical method	N	
Analytical verifications of test media	N	
Method of analysis described for analysis of urine	N	No details on the conduct of the method of analysis and no complete validation data set.
Complete reporting of effects observed	Y	Limited to body weight,
Statistical methods described	Y	
Historical control data of the laboratory reported	N	
Dose-effect relationship reported	N	Not possible with one dose level of each test item.
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Reliability not assignable		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because only one dose level for glyphosate and MON 52276 was considered, only 8 animals were used per dose and per sex and the method of analysis of glyphosate and AMPA in urine and its validation were not fully reported.		

1. Information on the study

Data point:	**CA 5.6.1/022**
Report author	Perego, M.C. *et al.*
Report year	2017
Report title	Evidence for direct effects of glyphosate on ovarian function: glyphosate influences steroidogenesis and proliferation of bovine granulosa but not theca cells *in vitro*
Document No	DOI 10.1002/jat.3417 E-ISSN: 1099-1263
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	None
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Yes/Reliable with restriction

2. Full summary of the study according to OECD format

The aim of this study was to determine the potential endocrine disruptor effects of glyphosate (GLY) on ovarian function evaluating cell proliferation, steroidogenesis and gene expression using bovine granulosa cells (GC) and theca cells as *in vitro* models. GC proliferation was impaired ($P<0.05$) after exposure to GLY at 0.5, 1.7 and 5 µg/mL. GC progesterone production was not affected ($P\geq0.05$) at all doses tested, while estradiol production was inhibited ($P< 0.05$) by GLY at 5 µg/mL. At the same concentration, GLY showed no effect ($P\geq 0.05$) on theca cell proliferation and steroidogenesis. At the concentrations of 0.01 and 0.3 µg/mL), GLY had no significant effect ($P\geq0.05$) on GC proliferation and steroidogenesis. These studies, for the first time, suggest that GLY may affect the reproductive system in cattle via direct action on ovarian function; however, further studies will be required to understand better the mechanism of action and to determine the *in vivo* reproductive effects of GLY.

Materials and methods

Cell culture

Ovaries from non-pregnant beef heifers were collected from a local slaughterhouse and were treated as previously described (Lagaly *et al*., 2008; Langhout *et al*., 1991; Spicer & Aad, 2007). Thecal cells (TC) were collected from large (8–22 mm) follicles as previously described (Lagaly *et al*., 2008; Spicer & Chamberlain, 1998; Stewart *et al*., 1995). Follicular fluid was collected and final cell preparations were prepared in serum-free medium (Dulbecco's modified Eagle medium and Ham's F12) as previously described (Lagaly *et al*., 2008; Schreiber & Spicer, 2012). Trypan blue exclusion method was performed to determine viable cells (Langhout *et al*., 1991; Spicer *et al*., 1993; Tiemann *et al*., 2003a,b). Cells were then plated (2.5×10^5 in 20–80 µL of medium) on 24-well Falcon multiwell plates (Becton Dickinson, Lincoln Park, NJ, USA) in 1 mL of basal medium composed of a mixture of 1:1 Dulbecco's modified Eagle medium and Ham's F-12 containing glutamine, gentamicin and sodium bicarbonate (Sigma-Aldrich Co., St. Louis, MO, USA) as previously described (Schreiber & Spicer, 2012). Plates were maintained in a humidified 95% air and 5% CO_2 environment at 38.5 °C changing medium every 24 h. Cells were also kept in the presence of 10% foetal calf serum (FCS; Equitech-Bio, Inc., Kerrville, TX, USA) for the first 48 h of culture to procure an optimal attachment. After 48 h, cells were washed twice with serum-free medium and the different treatments were applied in serum-free medium containing 500 ng/mL of testosterone (as an estradiol [E_2] precursor; from Steraloids, Wilton, NH, USA) for 48 h. Ovine follicle-stimulating hormone (FSH; NIDDK-oFSH-20, activity: 175 × NIH-FSH-S1 U mg^{-1}, from the National Hormone and Pituitary Program, Torrance, CA, USA) was added to all treatments, because insulin-like growth factor (IGF) 1 alone does not have an effect on steroid production (Ranzenigo *et al*., 2008; Spicer *et al*., 2002).

After aspiration of follicular fluid, large follicles were bisected and GC were separated from the TC via blunt dissection and the theca interna was enzymatically digested as previously described (Aad *et al*., 2006; Spicer & Chamberlain, 1998; Stewart *et al*., 1995). The non-digested tissue was eliminated by using sterile syringe filter holders with metal screens of 149 µm mesh (Gelman, Ann Arbor, MI, USA) and filtered TC were then centrifuged at 50 g for 5 min. As described for granulosa cells (GC), TC were washed with serum-free medium and resuspended in serum-free medium containing collagenase and DNase. TC (2.0×10^5 viable cells per well) were plated and cultured as described for GC. Culture medium was also supplemented with 30 ng/mL of ovine luteinizing hormone (LH; LH activity; 2.3 × NIH-LH-S1 U mg^{-1}; from the National Hormone and Pituitary Program) because progesterone (P_4) and androstenedione (A_4) production are not induced by IGF1 in the absence of LH (Spicer & Stewart, 1996; Stewart *et al*., 1995).

Assays
Medium collected from individual wells was frozen at -20 °C for subsequent steroid analyses. Radioimmunoassays (RIA) were performed to determine concentrations of P_4, E_2 and A_4 as previously described (Lagaly *et al*., 2008; Langhout *et al*., 1991; Spicer *et al*., 1993; Stewart *et al*., 1995). The intra- and inter-assay coefficients of variation were 7 % and 13 % for the P_4 RIA, and 8 % and 17 % for the E_2 RIA, respectively.

Determination of granulosa cell and theca cell numbers
The numbers of GC and TC, in the same wells from which medium was collected, were determined via a Coulter counter (model Z2; Beckman Coulter, Inc., Hialeah, FL, USA), and used to calculate steroid production on ng or pg per 10^5 cell basis. Cells were washed twice using 0.9 % saline solution (500 µL), exposed to 500 µL of trypsin (0.25 % wt/vol= 2.5 mg/mL) for 20 min at 37 °C and then scraped from each well and enumerated as previously described (Lagaly *et al*., 2008; Ranzenigo *et al*., 2008).

RNA extraction and quantitative reverse transcription–polymerase chain reaction
At the end of the treatment period, medium was either aspirated or collected from each well depending on the experiment and cells from two replicate wells were lysed in 0.5 mL of TRI reagent solution (Life Technologies, Inc., Grand Island, NY) as previously described (Lagaly *et al*., 2008; Spicer & Aad, 2007). RNA samples were solubilised in DEPC-treated water (Life Technologies, Inc., Grand Island, NY), quantitated by spectrophotometry at 260 nm using a NanoDrop ND-1000 spectrophotometer, and stored at -80 °C. Cholesterol side chain cleavage enzyme (*CYP11A1*) and aromatase (*CYP19A1*) primers and probes for quantitative reverse transcription–polymerase chain reaction were designed using Primer Express™ software (Foster City, CA, USA) as previously reported (Lagaly *et al*., 2008; Spicer & Aad, 2007). The bovine *CYP11A1* and *CYP19A1* primer and probe sequences and information are described by Lagaly *et al.* (2008). Relative quantification of target gene mRNAs were expressed using the comparative threshold cycle method as previously described (Lagaly *et al*., 2008; Spicer & Aad, 2007).

Experimental design
Experiment 1 was performed to evaluate the effects of glyphosate (GLY) on GC proliferation and steroidogenesis. Cells were cultured for 48 h in 10% FCS, washed twice with serum-free medium as described earlier, and cells treated for 48 h in serum-free medium containing testosterone (500 ng/mL), FSH (30 ng/mL) and IGF1 (30 ng/mL; recombinant human IGF1 from R&D Systems, Minneapolis, MN, USA) with or without various doses of GLY (i.e., 0, 0.5, 5 µg/mL; Sigma-Aldrich Co.). Only the 0 and 5 µg/mL doses of GLY were tested in the absence of IGF1 to determine any possible effect of GLY on FSH-stimulated steroidogenesis. After 48 h of treatment, cells were counted and medium was collected for E_2 and P_4 determinations. In a separate set of cells, the effect of 2–day treatment with 5 µg/mL GLY on GC viability was evaluated using the trypan blue exclusion method as previously described (Adashi *et al*., 1987; Spicer & Alpizar, 1994).

Experiment 2 was designed to evaluate the effects of higher doses of GLY on GC proliferation and steroidogenesis. Cells were cultured for 48 h in 10 % FCS, washed twice with serum-free medium as described earlier, and cells treated for 48 h in serum-free medium containing testosterone (500 ng/mL),

FSH (30 ng/mL) and IGF1 (30 ng/mL) with or without the various doses of GLY (i.e., 0, 0.01, 0.3 mg mL^{-1}). After 48 h of treatment, cells were counted and medium was collected for E2 and P_4 determinations.
Experiment 3 was designed to test the effect of GLY on *CYP11A1* and *CYP19A1* mRNA abundance in GC. Cells were cultured as previously described for experiment 1 except that no testosterone was included in the medium and treatments were only applied for 24 h: no additions, FSH (30 ng/mL) plus IGF1 (30 ng/mL), and FSH plus IGF1 plus GLY (5 µ/mL). After 24 h of treatment, cells were lysed for RNA extraction as described earlier. A combined treatment of FSH and IGF1 was selected to test the GLY effect because the inhibitory effect of GLY was seen with this treatment in experiment 1.

Experiment 4 was performed to study the effect of GLY on TC proliferation and steroidogenesis. Cells were cultured for 48 h in 10 % FCS, washed twice with serum-free medium as described earlier, and cells treated for 48 h in serum-free medium containing LH (30 ng/mL) and IGF1 (0 or 30 ng/mL) with or without GLY (*i.e.*, 0 and 5 µg/mL). After 48 h of treatment, cells were counted and medium was collected for P_4 and A_4 determinations.

Experiment 5 was designed to determine the effect of GLY on serum-stimulated GC proliferation. Cells were cultured for 4 days in 10 % FCS. During the last 2 days of culture, cells were treated as follows: control (no additions) or GLY (1.7 µg/mL). At the end of treatment, cells were counted.

Statistical analysis
Experimental data are presented as the least squares means ± SEM of measurements from replicated culture wells. Each experiment was performed three times with different pools of GC collected from 10 to 20 ovaries for each pool and each treatment replicated three times within each experiment. Treatment effects and interactions were assessed using the GLM procedure of the Statistical Analysis System for Windows (version 9.2; SAS Inst. Inc., Cary, NY, USA). Main effects were treatment, experimental replicate (i.e., pool of cells) and their interaction. Steroid production was expressed as ng or pg per 10^5 cells per 24 h, and cell numbers determined at the end of the experiment were used for this calculation. Mean differences in cell growth and steroid production between treatments were determined using the Fisher's protected least significant difference procedure (Ott, 1977) $P \leq 0.05$ was considered statistically significant.

Results

Experiment 1: Dose–response of glyphosate on granulosa cell proliferation and estradiol and progesterone production in the presence of follicle-stimulating hormone with or without insulin-like growth factor 1
GLY at 0.5 and 5 µg/mL was found to decrease significantly the GC proliferation in the presence of FSH plus IGF1 (Fig. 1). Regarding steroid production, GLY at all tested concentrations (0.5 and 5 µg/mL) had no effect on GC P4 production (Fig. 2A). GLY had no effect on GC E2 production in the presence of FSH whereas GLY at 5 µg/mL decreased ($P<0.05$) E2 production in the presence of FSH plus IGF1 (Fig. 2B). Cell viability was not significantly affected by 2–day treatment with 5.0 µg/mL GLY (91.6 vs. 88.6±5.0 % for control and GLY-treated GC, respectively).

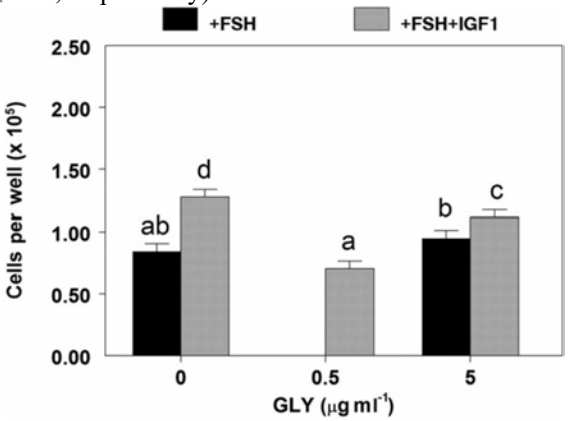


Figure 1. Effect of GLY on numbers of granulosa cells from bovine follicles (experiment 1). Cells were cultured for 48 h as described in Materials and methods, and then treated for an additional 48 h either with 30 ng/mL

FSH alone and GLY at 0 or 5 µg/ mL, or with 30ng/mL FSH and IGF1 (30 ng/mL) with GLY at 0, 0.5 or 5.0 µg/mL. All cells were treated concomitantly with 500 ng/mL of testosterone. Values are means ± SEM from three separate experiments (n = 9). Means without a common letter (a-d) differ (P<0.05). FSH, follicle-stimulating hormone; GLY, glyphosate; IGF, insulin-like growth factor.

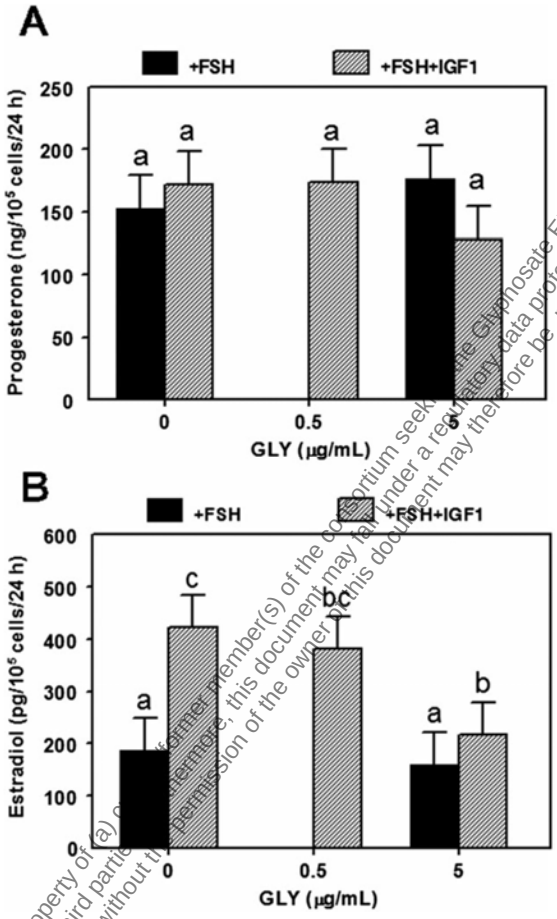


Figure 2. Effect of GLY on progesterone (A) and estradiol (B) production by granulosa cells from bovine follicles (experiment 1). Cells were cultured for 48 h as described in Materials and methods, and then treated for an additional 48 h either with 30 ng/mL FSH alone and GLY at 0 or 5 µg/mL, or with 30 ng/mL FSH and IGF1 (30 ng/mL) with GLY at 0, 0.5 or 5.0 µg/mL. All cells were treated concomitantly with 500 ng/mL testosterone. Values are means +/-SEM from three separate experiments (n = 9). Within a panel, means without a common letter (a–c) differ (P<0.05). FSH, follicle stimulating hormone; GLY, glyphosate; IGF, insulin-like growth factor.

Experiment 2: Dose–response of glyphosate on granulosa cell proliferation and estradiol and progesterone production in the presence of follicle-stimulating hormone plus insulin-like growth factor 1

GLY at all tested concentrations (0.01 and 0.3 mg/mL) had no significant effect either on GC proliferation or steroid production. Cell numbers averaged 1.25, 1.47 and 1.00 ± 0.2 (×105 cells per well) for 0, 0.01 and 0.3 mg/mL GLY, respectively. P4 production averaged 99, 154 and 91 ± 23 ng 10-5 cells per 24 h for 0, 0.01 and 0.3 mg/mL GLY, respectively. E2 production averaged 130, 157 and 187 ± 28 pg 10-5 cells per 24 h for 0, 0.01 and 0.3 mg/mL GLY, respectively.

Experiment 3: Effect of glyphosate treatment on CYP19A1 and CYP11A1 mRNA in granulosa cells

The combined IGF1 plus FSH treatment increased (P<0.05) CYP19A1 and CYP11A1 mRNA abundance

by threefold and twofold, respectively, above untreated control GC (Fig. 3A,B). GLY (5µg/mL) had no significant effect on CYP19A1 or CYP11A1 mRNA in GC co-treated with FSH and IGF1 (Fig. 3A,B).

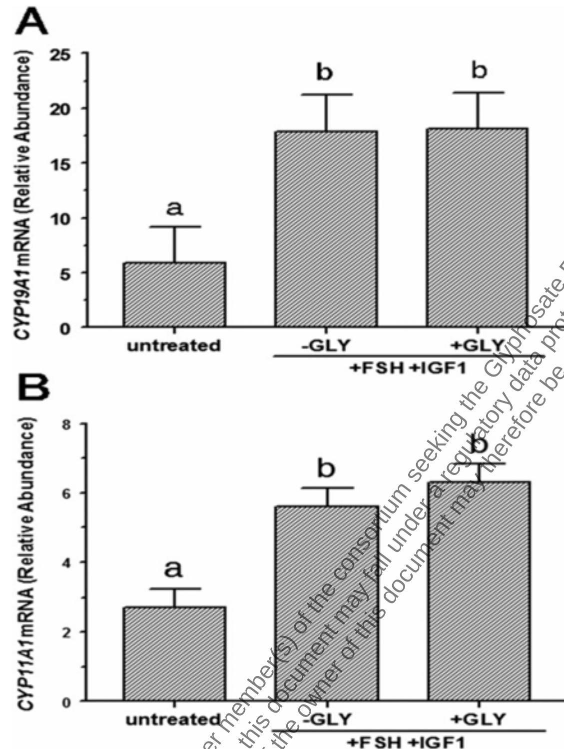


Figure 3. Effect of GLY on abundance of CYP19A1 (A) and CYP11A1 (B) mRNA in granulosa cells frombovine follicles (experiment 3). Cells were cultured for 48 h as described in Materials and methods, and then treated for an additional 24 h either with either no additions (controls), or with FSH (30 ng/mL) and IGF1 (30 ng/mL) with GLY at 0 or 5.0 µg/mL. Values are means ± SEM from three separate experiments (n = 6). Within a panel, means without a common letter (a–b) differ (P<0.05). FSH, follicle stimulating hormone; GLY, glyphosate; IGF, insulin-like growth factor.

Experiment 4: Dose–response of glyphosate on theca cell proliferation and androstenedione and progesterone production in the presence of luteinizing hormone with or without insulin-like growth factor 1

GLY at the tested concentration of 5 µg/mL had no significant effect on TC proliferation and P4 or A4 production in the presence of LH either with or without IGF1. IGF1 significantly increased cell numbers and steroid production. Cell numbers averaged 0.46, 0.53, 0.99 and 1.06 ± 0.05 for: LH alone; LH plus 5 µ/mL GLY; LH plus IGF1; and LH plus IGF1 plus 5 µ/mL GLY, respectively. P4 production averaged 40.5, 40.4, 70.3 and 78.3 ± 3.7 pg 10-5 cells per 24 h for: LH alone; LH plus 5 µg/mL GLY; LH plus IGF1 alone; and LH plus IGF1 plus 5 µg/mL GLY, respectively. A4 production averaged 1.45, 1.37, 2.75 and 2.47 ± 0.12 ng 10-5 cells per 24 h for: LH alone; LH plus 5 µg/mL GLY; LH plus IGF1 alone; and LH plus IGF1 plus 5 µg/mL GLY, respectively.

Experiment 5: Effect of glyphosate on serum-induced granulosa cell proliferation

Alone, GLY (1.7 µg/mL) increased (P<0.05) GC proliferation (Fig. 4). Cell numbers were increased by 11 % after 1 day and by 8 % after 2 days of GLY treatment (Fig. 4).

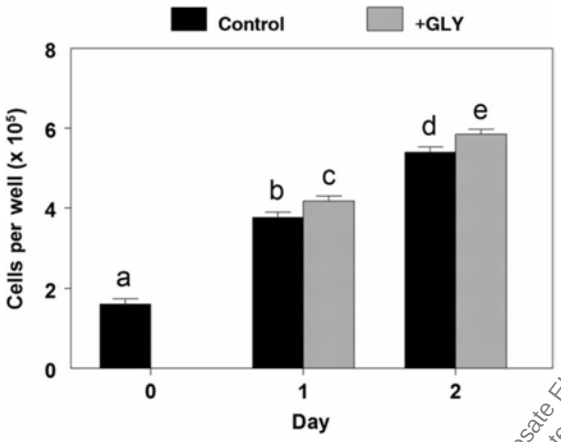


Figure 4. Effect of GLY on serum-induced proliferation of bovine granulosa cells (experiment 5). Cells were cultured for 48 h as described in Materials and methods, and then treated for an additional 0, 1 or 2 days with 10 % foetal calf serum and GLY at 0 or 1.7 µg/mL. Values are means ± SEM from three separate experiments (n = 9).Means without a common letter (a–e) differ (P<0.05). GLY, glyphosate.

Discussion

In the present study, both cell proliferation and the production of P4, A4 and E2, hormones that are connected to normal ovarian cyclicity and establishment and maintenance of pregnancy (Wood & Strauss, 2002), were evaluated to determine if GLY has effects on bovine GC and TC. GLY was tested at low doses, from 0.5 µ/mL, very far below agriculture use and authorised residues in food or feed (approximately 7000 and 800 times lower, respectively) (Gasnier et al., 2009). No effects were observed on GC P4 production in the presence of FSH either with or without IGF1, whereas GLY at 5 µg/mL had inhibitory effects on E2 production in the presence of FSH plus IGF1, showing a potential impairment on GC function that is essential for oocyte survival (Petro et al., 2012). Results of our study also showed that GLY at 5 µg/mL had an inhibitory effect on GC proliferation in the presence of FSH plus IGF1, and this inhibitory effect of 5 µg/mL GLY on cell numbers was not associated with a change in cell viability.

1.Assessment and conclusion

<u>Assessment and conclusion by applicant</u>:

In this *in vitro* study, glyphosate had minimal effects on granulosa cells (GC). In the presence of FSH only, glyphosate had no effect on GC cell viability or on progesterone or estradiol production. In the presence of FSH and IGF1, glyphosate reduced GC proliferation without a dose-response at 0.5 and 5 µg/mL but not at lower test concentrations (0.01 and 0.3 µg/mL) and did not affect progesterone production or CYP19A1 and CYP11A1 mRNA expression; estradiol production was reduced at 5 µg/mL only (not at lower test concentrations). Without FSH or IGF1, 1.7 µg/mL of glyphosate slightly increased GC proliferation in response to serum (≤11 %).

Glyphosate at 5 µg/mL had no effect on the theca cell (TC) proliferation or the production of progesterone or androstenedione.

Overall, with the exception of slight, non-dose-related alterations in GC proliferation under different test conditions, this study showed no effects of glyphosate on GC at physiologically relevant test concentrations. Glyphosate had no effect on TH.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate tested was not sufficiently characterised, no positive controls were used and the tests were conducted with only one or 2 test concentrations of glyphosate.

Reliability criteria for *in vitro* toxicology studies

Publication: Perego *et al*., 2017	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	N	Purity not reported. Source: Sigma Aldrich.
Only glyphosate acid or one of its salts is the tested substance	Y	
AMPA is the tested substance	N	
Test system clearly and completely described	Y	Bovine granulosa and theca cells.
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	Not applicable	
Test concentrations in physiologically acceptable range (< 1 mM)	Y (partly)	0, 0.5, 5, or 0, 10, 300 µg/mL (1.77 mM), or 0, 5 µg/mL.
Cytotoxicity tests reported	Y?	Viability tested at only one concentration of glyphosate.
Biochemical methods described	Y?	Some could be more detailed.
Positive and negative controls	N	No positive controls.
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	Y	Limited since max. 2 test concentrations.
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Reliability not assignable		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate tested was not sufficiently characterised, no positive controls were used and the tests were conducted with only one or 2 test concentrations of glyphosate.		

3. Information on the study

Data point:	**CA 5.6.1/023**

Report author	Dai, P. *et al.*
Report year	2016
Report title	Effect of glyphosate on reproductive organs in male rat
Document No	doi.org/10.1016/j.acthis.2016.05.009 E-ISSN: 1618-0372
Guidelines followed in study	None
Deviations from current test guideline	No
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Yes/Reliable with restrictions

1. Full summary of the study according to OECD format

Many studies have been published already on reproductive toxicity of glyphosate-based formulations, but few investigated toxicity of glyphosate alone on the male reproductive system. In this study SD rats were gavaged with glyphosate at doses of 5, 50, 500 mg/kg to detect the toxicity of glyphosate on rat testis. Glyphosate significantly decreased the average daily feed intake at dose of 50 mg/kg, the weight of seminal vesicle gland, coagulating gland as well as the total sperm count at dose of 500 mg/kg. Immunohistochemistry of androgen receptor (AR) had no difference among all groups. Testosterone, estradiol, progesterone and oxidative stress parameters showed no differences for all doses. Taken together, it had been concluded that glyphosate alone has low toxicity on male rats reproductive system.

Materials and Methods

Chemicals - Isopropylamine salt of glyphosate (90 % w/w purity) was purchased from Shanghai Ryon Biological Technology Co. Ltd., China.

Animals - 32 sexually mature 56–day old Sprague-Dawley (SD) male rats were raised in an animal house and maintained in an air-conditioned room at approx. 21 °C with a 12 h/12 h light/dark cycle. A balanced mixture of pelleted food and water were available to the rats.

Experimental design and treatment – 32 rats were randomly divided into 4 groups, 3 groups were orally given glyphosate as an aqueous solution by gavage once a day. The control group was treated in the same way with deionised water. The doses administered were 5, 50, and 500 mg/kg bw. All rats were treated for 5 weeks continuously. After the last treatment the rats were sacrificed and testis, epididymis, prostate gland and seminal vesicle removed and weighed.

Epididymal sperm parameters – Epididymal sperm was used for the measurement of total sperm count. The epididymis was minced in PBS and filtered using a nylon mesh screen. The filtrate was treated with 10 mL PBS and the number of sperm was counted using a standard hemo-cytometric method.

Hormone measurement - Serum hormones were measured by radioimmunoassay using a ^{125}I-labeled ligand double-antibody RIA Kit for total testosterone, estradiol and progesterone. The minimum sensitivity of the method was 0.02 ng/mL for testosterone, less than 0.2 ng/mL for progesterone and less than 5 pg/mL for estradiol. The intra- and inter-assay coefficients of variation (CV) were less than 10 %.

Testicular, epididymal and seminal vesicle gland histology - Following fixation of the tissues, the samples were passed through a graded series of ethanol and xylene solutions and embedded in paraffin wax. Paraffin-embedded tissues were serially sectioned at 5 µm thickness. For each rat, two non-serial sections were stained with hematoxylin/eosin (HE).

Antioxidant status analysis - The levels of catalase (CAT, U/mg protein), superoxide dismutase (SOD, U/mg protein) and malondialdehyde (MDA, µmol/g protein) were determined by the absorbance of samples

in multiskan spectrum. SOD activity was determined by an SOD assay kit with absorbance measured at 560 nm. CAT activity was determined by the H_2O_2 consumption (µmol/g protein) with absorbance measured at 405 nm. Lipid peroxidation was determined by measurement of MDA by the TBA test with absorbance at 532 nm.

Immunohistochemistry - Sections of the testes were deparaffinised with xylene and rehydrated in graded ethanol before being washed with twice-distilled water. To increase epitope exposure, the sections were heated for 15 minutes in sodium citrate buffer (0.01 M, pH 6.0) in a microwave oven. The sections were then cooled and washed with 0.01 M PBS at pH 7.2 and then blocked with 10% bovine serum albumin (BSA) in TBST (20 mM Tris-buffered saline, 0.05 % Tween 20, pH 7.5) for 1 hour at room temperature. The sections were incubated overnight at 4°C with diluted (1:400) polyclonal antibodies against androgen receptor (N-20; rabbit anti-human AR). The secondary antibody was goat anti-rabbit IgG. The binding of the antibodies were visualised using a SABC Kit Elite and 0.05 % 3,3-diaminobenzidine tetrachloride in 0.01 M PBS at pH 7.2, containing 0.01 % H_2O_2 for 2 minutes. The sections were counter stained with hematoxylin and mounted with cover slips. The specificity of the antibody was examined using 1 % BSA rather than the primary antibody.

Data analysis - All results are means ± SEM. When multiple comparisons were performed, evaluation was done using one-way ANOVA followed by Tukey's multiple comparison test. Significant differences with controls were considered when $p<0.05$.

Results

Average daily weight gain and average daily feed intake – Daily exposure to glyphosate caused a statistically significant decrease in average daily feed intake at 50 mg/kg bw/day only. Although not statistically significant there was a dose-dependent decrease in daily weight gain.

Reproductive organ weights and sperm parameters - Seminal vesicle and coagulating gland absolute weight showed a statistically significant changes amongst treatment groups whereas no such change was observed in other reproductive organs. No significant differences were observed in relative reproductive organ weights. Total sperm count was statistically significantly decreased at 500 mg/kg bw. .

Concentrations of testosterone, estradiol and progesterone in serum - Although there was a trend towards decreased serum concentrations with dose for testosterone and progesterone no statistically significant changes were noted in the serum concentrations of testosterone, estradiol and progesterone.

SOD and CAT activity, *H_2O_2 and MDA levels in testes* - There were no statistically significant changes in SOD and CAT activity and H_2O_2 and MDA levels in testes.

Testicular, epididymal and seminal vesicle gland histology - No statistically significant changes were observed in the histopathology of the testis, epididymis and seminal vesicles.

Immunohistochemical localisation of androgen receptor in the testis - No statistically significant changes were observed in androgen receptor (AR) immunoreactivity localised in the nuclei of cells, including Sertoli cells, peritubular myoid cells and Leydig cells.

Discussion and conclusions

The present study provides information on the potential effects of glyphosate on the reproductive system of the male rat. Average daily weight gain showed no substantial decrease whereas average daily feed intake was significantly decreased at 50 mg/kg bw but not at 500 mg/kg bw. It is therefore suggested that the decrease of average daily feed intake is independent of glyphosate treatment. Although there are no statistically significant differences in average weight gain, the trend is a decrease. At 500 mg/kg bw the absolute weight of seminal vesicle gland and coagulating gland and total sperm count decreased substantially. There was no significant change in oxidative stress parameters after oral administration of glyphosate at doses up to 500 mg/kg bw. Testosterone, estradiol and progesterone serum levels, as well as

AR in testis presented no significant change at all dose levels tested when compared to controls.

2. Assessment and conclusion

Assessment and conclusion by applicant:
The potential toxicity of glyphosate to the male reproductive system of the rat has been investigated after oral treatment with glyphosate for 5 weeks at dose levels up to 500 mg/kg bw. The endpoints studied were body weight, food intake, daily weight gain, absolute and relative reproductive organ weight, serum hormone levels, oxidative stress parameters, testicular histopathology and expression of AR in testis. The effects found were a significant decrease in absolute (but not relative) weight of the seminal vesicle gland and coagulating gland and a decrease in sperm count at the highest dose tested.
This publication is considered relevant but reliable with restrictions because there are deviations from regulatory guidelines for reproductive toxicicity studies and the reproductive effects seen are not corroborated by the results from guideline studies at similar dose levels.

Reliability criteria for *in vivo* toxicology studies

Publication: Dai *et al.*, 2016	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y?	Incomplete study
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity of 90 % as isopropylamine salt. Source Shanghai Ryon Biological Technology Co. Ltd., China.
Only glyphosate acid or one of its salts is the tested substance	Y	Isopropylamine salt
AMPA is the tested substance	N	
Study		
Test species clearly and completely described	Y	
Test conditions clearly and completely described	Y?	
Route and mode of administration described	Y	
Dose levels reported	Y	
Number of animals used per dose level reported	Y	8 males per group
Method of analysis described for analysis test media	N	
Validation of the analytical method	N	
Analytical verifications of test media	N	
Complete reporting of effects observed	Y?	
Statistical methods described	Y	
Historical control data of the laboratory reported	N	
Dose-effect relationship reported	Y	Results are not concordant with outcome of regulatory reproduction toxicology studies

Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Not reliable		
This publication is considered relevant but reliable with restrictions because there are deviations from regulatory guidelines for reproductive toxicology studies and the reproductive effects seen are not corroborated by the results from regulatory studies at similar dose levels.		

1. Information on the study

Data point:	**CA 5.6.1/024**
Report author	Forgacs, A.L. *et al.*
Report year	2012
Report title	BLTK1 Murine Leydig Cells: A Novel Steroidogenic Model for Evaluating the Effects of Reproductive and Developmental Toxicants
Document No	doi:10.1093/toxsci/kfs121 E-ISSN: 1096-0929
Guidelines followed in study	None
Deviations from current test guideline	No
GLP/Officially recognised testing facilities	Non-GLP
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary of the study according to OECD format

The aim of this study was to evaluate the effect of several structurally diverse endocrine disrupting compounds (EDCs) on steroidogenesis in a novel BLTK1 murine Leydig cell model. It was demonstrated that BLTK1 cells possess a fully functional steroidogenic pathway that produces low basal levels of testosterone (T) and express all the necessary steroidogenic enzymes including Star, Cyp11a1, Cyp17a1, Hsd3b1, Hsd17b3, and Srd5a1. Recombinant human chorionic gonadotropin (rhCG) and forskolin (FSK) elicited concentration- and time-dependent induction of 3',5'-cyclic adenosine monophosphate, progesterone (P), and T, as well as the differential expression of Star, Hsd3b6, Hsd17b3, and Srd5a1 messenger RNA levels. The results demonstrated that BLTK1 cells can be used to screen substances that alter intracellular cAMP, steroidogenic gene expression, and sex steroid levels. When tested in this system glyphosate was not found to induce testosterone production or alter rhCG induction of testosterone.

Materials and Methods

Chemicals - Purity and origin of the glyphosate sample tested was not reported.

Cell culture and treatment - Mouse Leydig BLTK1 (BLT-1 cells, clone K1) cells were isolated from a testicular tumor that developed in a transgenic mouse expressing the mouse inhibin α promoter/simian virus 40 T-antigen fusion gene. Cells were maintained in phenol red–free DMEM/F-12 media with 10% foetal bovine serum, 100 U/mL penicillin and 100 µg/mL streptomycin and incubated in 5 % CO_2 at 37 °C). For the evaluation of steroidogenic enzyme and receptor expression, cells were grown to 80% confluency and harvested without any treatment. For the determination of 3',5'-cyclic adenosine monophosphate (cAMP), progesterone (P), testosterone (T), and estradiol (E2), cells were grown to 80% confluency, transferred into

24-well tissue culture plates and incubated overnight. Cells were treated with DMSO, or with 0.1, 0.3, 1, 3, 10, 30, or 100 ng/mL recombinant human chorionic gonadotropin (rhCG) or with 0.1, 0.3, 1, 3, 10, 30, or 100 µM Forskolin (FSK) and media were collected at indicated times. Time course studies were conducted with DMSO, 3 ng/mL rhCG or 10 µM FSK, and media were collected after 1, 2, 4, 8, 12, 24, or 48 hours of incubation. Gene expression studies used the same study design, concentrations and time points with cells seeded into T-25 flasks.

MTT assay - BLTK1 cells placed in 96-well plates were treated with 1, 3, 10, 30, 100, 300, or 600 ng/mL rhCG, 1, 3, 10, 30, 100, 300, or 600 µM FSK or 1, 3, 10, 30, 100, 300, or 600 µM of test compound in triplicate. Media were aspirated after 24 hours and replaced with 50 µL of fresh MTT reagent (5 mg/mL thiazolyl blue tetrazolium bromide in PBS). Following 3 hours of incubation, MTT reagent was removed and replaced with 150 µL DMSO. Cells were incubated for 2 hours followed by absorbance measurements at 595 and 650 nm using an Emax precision microplate reader. Results are reported as percentage of control calculated from the relative absorbance of treated versus DMSO controls where 100 % indicates no cytotoxicity.

RNA isolation and gene expression - Total RNA was extracted from cell pellets using RNeasy Mini Kits with an additional RNase-free DNase digestion. RNA was quantified at 260 nm and purity assessed using the A260/A280 ratio, as well as by denaturing gel electrophoresis. First-strand complementary DNA (cDNA) was synthesized from RNA (1 µg) using SuperScript II reverse transcriptase and anchored oligo-dT primer. For real-time PCR (RT-PCR) evaluation of steroidogenic enzyme and receptor expression, cDNA was used as a template for PCR amplification with gene-specific primers. Quantitative RT-PCR (QRT-PCR) was used to quantify concentration- and time-dependent expression of specific genes. Reactions in 96-well plates consisted of 30 µL, including 1 µL of cDNA template, 0.1 µM forward and reverse gene-specific primers using an Applied Biosystems PRISM 7500 Sequence Detection System. Dissociation curve analysis assured single product amplification. To control for differences in RNA loading, quality and cDNA synthesis, samples were standardised to the geometric mean of three housekeeping genes: *ActB*, *Gapdh*, and *Hprt*. Results were quantified using a standard curve generated on the same 96-well plate and amplified by using purified cDNA product as template specific for each gene (serial 10-fold dilutions from 10^8 to 10^1 copies). The slope of the standard curve was used to assess amplification efficiency with all amplification efficiencies > 90 %. Fold changes were calculated relative to time-matched vehicle. Relative expression was scaled such that time-matched vehicle control expression equaled one for graphing purposes.

Dose-response modeling and statistical analyses - The ToxResponse modeler uses particle swarm optimisation to identify the best fit across five model classes: sigmoidal, exponential, linear, quadratic, and Gaussian. The best fitting model was then used to calculate half maximal effective concentration (EC_{50}) values. All statistical analyses were carried out using SAS v9.1 by ANOVA, with Dunnett's or Tukey's *post hoc* tests for concentration-response and time course data, respectively. Differences between treatment groups were considered significant when $p<0.05$ relative to time-matched DMSO control.

Results

Steroidogenic Enzyme Expression in BLTK1 Cells - Steroidogenic enzyme messenger RNA (mRNA) and protein were detected in BLTK1 cells by RT-PCR and/or Western blotting, confirming the expression of all required steroidogenic enzymes. In addition, mRNA for several potential regulatory factors including LHCGR, estrogen receptor (ER), androgen receptor (AR), and steroidogenic factor 1 (SF-1), peroxisome proliferator-activated receptors (PPARa and PPARc), the pregnane X receptor (PXR), and the aryl hydrocarbon receptor (AhR) were also detected. However, mRNA for progesterone receptor, glucocorticoid receptor, or the liver receptor homolog 1 was not detected in BLTK1 cells despite verification of RT-PCR primer specificity and functionality in mouse Hepa1c1c7 cells.

Induction of Steroidogenesis by FSK and rhCG - Temporal profiles of intracellular cAMP as well as P and T levels in media were evaluated in response to 3 ng/mL rhCG or 10 µM FSK by enzyme immunoassay (EIA). Intracellular cAMP was induced by FSK after 30 minutes (~120 pmol/mL, ~10-fold) and after 1 hour in response to rhCG (635 pmol/mL, 60-fold). However, levels quickly diminished such that no intracellular

cAMP was detected by 8 hours. Maximum P levels (200 ng/mL, 8-fold) were observed after 2 hours in response to rhCG and FSK, followed by a steady decline due to metabolism to androgens and estrogens. In contrast, T levels gradually increased reaching a maximum of ~200 pg/mL (7-fold) after 48 hours, with significant increases as early as 1 hour post treatment. Concentration-dependent induction of intracellular cAMP and secreted P and T was evaluated after 4 hours when cAMP could still be detected. 17β-Estradiol (E2) was evaluated after 48 hours as it was not consistently detected after 4 hours. cAMP, P, and T were induced 25-, 10-, and 4-fold, respectively, after 4 hours, whereas E2 was induced ~4-fold by 48 hours. The EC_{50} for cAMP induction was greater than 24 ng/mL for rhCG and greater than 29 µM for FSK. Meanwhile, EC_{50} values of 1 ng/mL rhCG and 9 µM FSK were conserved for both P and T induction, whereas E2 EC_{50} values were 10 ng/mL for rhCG and 9 µM for FSK. Intracellular cAMP levels are not only regulated by synthesis but also by degradation, which is regulated by cyclic nucleotide phosphodiesterase enzymes. The phosphodiesterase inhibitor IBMX maximizes cAMP levels in order to further induce steroidogenesis. However, IBMX co-treatment with rhCG or FSK did not increase T levels further, albeit rhCG and FSK potencies were greater with and without IBMX by FSK: 0.1 µM vs. 9.4 µM vs. 0.9 ng/mL, respectively. When tested in this system glyphosate at 300 µM did not induce T production nor alter rhCG induction of T.

Conclusion
Current test protocols and models are inadequate to screen the universe of chemicals, metabolites, and mixtures that may alter steroidogenesis. BLTK1 cells are a novel complementary rhCG-inducible Leydig-based model that can be used to assess effects on steroidogenic gene expression, intracellular cAMP, and P, T, and E2 levels in media. Their consistent response characteristics and inducibility over 30 passages also make this cell line attractive for high-throughput screening. Comprehensive characterisation of effects on intermediate steroid biosynthesis, including pregnenolone, 17-hydroxyprogesterone, DHEA, androstenedione, estrone, and DHT, as well as the differential expression of steroidogenic enzymes will also facilitate the elucidation of modes of action relevant to adverse outcome pathways in humans and other relevant species. When tested in this system glyphosate at 300 µM did not induce testosterone production or alter rhCG induction of testosterone.

1. Assessment and conclusion

Assessment and conclusion by applicant:

In this study, recombinant human chorionic gonadotropin (rhCG) and forskolin (FSK) were used as positive controls for the induction of steroidogenesis, as measured by increases in progesterone, testosterone and 17β-estradiol levels in culture media. Murine BLTK1 Leydig cells were investigated as a novel model for evaluating the effects of chemicals on steroidogenesis. The results demonstrated that BLTK1 cells can be used to screen substances that alter intracellular cAMP, steroidogenic gene expression, and sex steroid levels. When tested in this system glyphosate was not found to induce testosterone production or alter rhCG induction of testosterone.

This publication is considered relevant for glyphosate risk assessment but reliable with restrictions because the test substance was not characterised and the results of only one concentration level were reported.

Reliability criteria for *in vitro* toxicology studies

Publication: Forgacs *et al*., 2012	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	

Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y?	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	N	Purity and source not reported.
Only glyphosate acid or one of its salts is the tested substance	Y	
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	Y	
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	N	
Test concentrations in physiologically acceptable range (< 1 mM)		
Cytotoxicity tests reported	Y	
Positive and negative controls	Y	
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	N	Only one concentration was tested (300 µM) Glyphosate, did not induce or alter rhCG induction of T. Glyphosate also had no effect on T levels in BLTK1 cells
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Not reliable		
This publication is considered relevant for glyphosate risk assessment but reliable with restrictions because the test substance was not characterised and only one concentration level was tested.		

CA 5.6.2 Developmental toxicity studies

Developmental toxicity of glyphosate was tested in a variety of studies in rats and rabbits.

Rat studies

Seven toxicity studies in rats are available for the assessment of developmental toxicity of glyphosate, 5 amongst them are considered as valid and conclusive for hazard assessment. Those studies have been taken into account for the previous and current evaluation of developmental toxicity of glyphosate in the rat. Two studies are considered invalid for hazard assessment (please refer to study summaries CA 5.6.2/006 and CA5.6.2/007) but are included for the sake of completeness. No new data on developmental toxicity obtained in rats, including publically available sources, are available for the current dossier. A brief summary of the available studies conducted in the rat is provided below.

Table 5.6.2-1: Summary of developmental toxicity in rats

Annex Point	Study	Study type	Substance(s)	Reference list- related category§	Result	
					NO(A)EL [mg/kg bw/day] {previous AIR value, where different}	LOAEL Targets / Main effects {previous AIR noted effects}
CA 5.6.2/001	[redacted], 2002	Alpk: AP$_f$SD: rat (gavage, 0, 250, 500, 1000 mg/kg bw/day)	Glyphosate acid (Batch: P24, Purity: 95.6 %)	Valid, Category 2a	**Maternal:** 1000; **Develomental:** 1000	**Maternal**: no effects **Developmental**: no effects
CA 5.6.2/002	[redacted], 1995	Crj:CD (SD): rat (gavage, 0, 30, 300, 1000 mg/kg bw/day)	Glyphosate technical; HR-001 (Batch: 940908-1), Purity: 95.68 %)	Valid, Category 2a	**Maternal:** 300; **Developmental:** 1000	**Maternal:** slightly loose stool **Developmental:** no effects
CA 5.6.2/003	[redacted], 1991	Crl:CD (SD) BR VAF/Plus strain: rat (gavage, 0, 300, 1000, 3500 mg/kg bw/day)	Glyphosate technical; HR-001 (Batch: 206-Jak-25-1, Purity: 98.6 %)	Valid, Category 2a	**Maternal:** 300; **Developmental:** 300	**Maternal:** 3500 mg/kg bw/day: mortality, salivation, loose stool, noisy respiration, reduced body weight, slightly reduced food intake, increased water intake 1000 & 3500 mg/kg bw/day: noisy respiration, reduced body weight gain **Developmental:** 3500 mg/kg bw/day: reduced mean foetal weight 1000 & 3500 mg/kg bw/day: reduced / delayed ossification, increased incidence of skeletal variations
CA 5.6.2/004 and CA 5.6.2/005	[redacted] 1991	Wistar: rat (gavage, 0, 1000 mg/kg bw/day)	Glyphosate technical (Batch: 60, Purity: 98.6 %)	Valid, Category 2a	**Maternal:** 1000; **Developmental:** 1000	**Maternal:** no effects **Developmental:** no treatment-related effects
CA 5.6.2/006	[redacted], 1986	Wistar: rat (gavage, 0, 100, 500 mg/kg bw/day)	Glyphosate (Batch: not provided; Purity: not provided)	Invalid, Category 4b	**Maternal:** 500; **Developmental:** 500	**Maternal:** no effects **Developmental:** no effects
CA 5.6.2/007	[redacted], 1981	CFY: rat (diet, 0, 22, 103,	Glyphosate /active principle (Batch: not provided;	Invalid, Category 3b	**Maternal:** 544; **Developmental:**	**Maternal:** no effects **Developmental:**

Table 5.6.2-1: Summary of developmental toxicity in rats

Annex Point	Study	Study type	Substance(s)	Reference list- related category§	Result	
					NO(A)EL [mg/kg bw/day] {previous AIR value, where different}	LOAEL Targets / Main effects {previous AIR noted effects}
		544 mg/kg bw/day)	Purity: 96.8 %)		544	no effects
CA 5.6.2/008	[redacted], 1980	CD: rat (gavage, 0, 300, 1000, 3500 mg/kg bw/day)	Glyphosate technical (Batch: XHJ - 64, Purity: 98.7 %)	Valid, Category 2a	**Maternal:** 1000; **Developmental:** 1000	**Maternal:** mortality, diarrhoea, reduced body weight gain at 3500 **Developmental:** reduced number of viable foetuses, increase in early resorptions and slight increase in total post-implantation loss at 3500

§ The category describes the acceptability/reliability of the study within the AIR 5 submission. This category does not describe hazard classification.

In the developmental study performed by [redacted] (1996) (CA 5.6.2/001), no evidence of maternal and developmental toxicity due to glyphosate acid exposure during gestation was observed. Further, no teratogenic effects were apparent. Thus, the highest tested dose level of 1000 mg glyphosate acid/kg bw/day was the No-Observed-Effect-Level (NOEL) for both maternal and developmental effects.

In the second study ([redacted] (1995) (CA 5.6.2/002)), a dose of 1000 mg/kg bw/day did not lead to treatment-related toxicity except slight disturbances of the gastrointestinal tract in maternal animals indicated by slightly loose stool and decreased food consumption at the early dosing period from gestational day 6 - 9. No treatment-related changes were observed in regard to body weights and body weight gains. Gravid uterine weights, numbers of corpora lutea, implants and live foetuses, percent incidences of resorptions and foetal deaths, foetal sex ratio, foetal body weights and placenta weights were comparable among the test groups. Further, no treatment related effects including malformations or variations were noted in foetuses. Thus, the No-Observable-Adverse-Effect-Level (NOAEL) was set at 300 mg/kg bw/day for maternal rats, and 1000 mg/kg bw/day for developmental toxicity.

Materntal toxicity characterised by gastrointestinal disturbances (loose stool/diarrhoea) at 1000 mg/kg bw/day was confirmed by [redacted] (1991) (CA 5.6.2/003). Besides gastrointestinal disturbances, reduced body weight gain and/or noisy respiration were observed indicating for maternal toxicity at this dose level (CA 5.6.2/003). Further, mortality, post dose salivation and lower food consumption were observed in dams at the top dose of 3500 mg/kg bw/day. No abortions or total resorptions were detected. Implantation rate, post-implantation loss, litter size and sex ratio were similar in all groups. In foetuses, a reduction in mean foetal weight was observed at the highest dose of 3500 mg/kg bw/day. A higher incidence of rib distortion (wavy ribs) was evident in the high-dose group. In addition, reduced ossification was seen slightly more frequently at this dose level and also at 1000 mg/kg bw/day. As result, the percentage of foetuses showing skeletal anomalies (variations) was significantly increased at the mid- and high-dose levels. However, the percentage for the mid-dose level only slightly exceeded the historical background range (21.9 - 27.2 %) whereas the respective control value was atypically low in this study. The toxicological relevance of findings at 3500 mg/kg bw/day is uncertain because of the clear increase in mortality in dams at this dose level suggesting an excess of the maximum tolerated dose. Effects observed

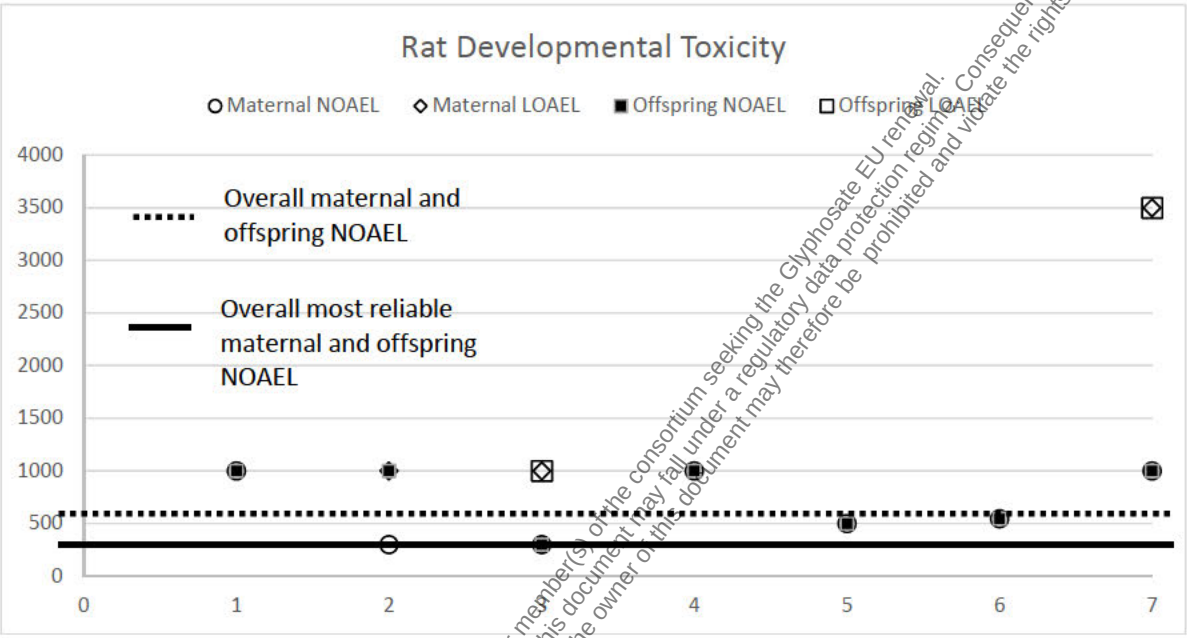
in foetuses at these high doses are attributed to secondary, non-specific consequences of maternal toxicity and not to specific developmental toxicity. No teratogenicity was observed. Treatment with glyphosate at 300 mg/kg bw/day was without adverse effect on maternal or embryofoetal parameters.

A further teratogenicity study conducted as a limit test at 1000 mg/kg bw/day is available ([redacted] (1991) (CA 5.6.2/004 and CA 5.6.2/005)). No evidence of maternal toxicity was observed. Reproduction indices and litter data were comparable to the controls and did not indicate treatement related toxicity. The incidence of foetal malformations was not increased in the treated group compared to the control. There was limited evidence of a higher incidence of delayed ossification (caudal vertebral arch, forelimb proximal and hindlimb distal phalanges) in the group receiving glyphosate. On the other hand, delayed ossification of other parts of the skeleton, in particular the skull, was more frequently seen in the control group. Thus, there was no clear and consistent impact of test compound administration on the process of ossification. The NOAEL for maternal and embryofoetal toxicity was defined as 1000 mg/kg bw/day.

Similar to [redacted], [redacted] [redacted] (1980) (CA 5.6.2/008) exposed dams to doses of glyphosate up to 3500 mg/kg bw/day. Biologically meaningful differences in appearance and behavior were noted at 3500 mg/kg bw/day when compared to the control group and included breathing rattles, reduced activity and an increase in soft stool and diarrhoea. Six animals in this dose group died of unknown reasons by gestation day 17. Reduced mean maternal body weight gain was noted over the treatment period in the 3500 mg/kg bw/day group. Further, an increase in early resorptions, a slight increase in post-implantation loss and decreases in mean foetal body weight and mean number of viable foetuses was observed at 3500 mg/kg bw/day. An increase in the number of foetuses with reduced ossification of the sternebrae was also noted in this group. No signs of maternal or developmental toxicity were noted at 300 or 1000 mg/kg bw/day. Glyphosate did not produce a teratogenic response when administered to rats up to and including 3500 mg/kg bw/day. The maternal and developmental NOAEL are considered to be 1000 mg/kg bw/day.

The available studies on developmental toxicity consistently revealed that *in utero* exposure to glyphosate did not result in teratogenicity in rats. If observed, test substance-related effects, including maternal toxicity and developmental effects occur at 1000 mg/kg bw/day. Based on the available data an "overall" NOAEL of 544 mg/kg bw/day is considered appropriate for both maternal and developmental toxicity in rats. However, given the unreliability of studies by [redacted] (1986) (CA 5.6.2/006); NOAEL 500 mg/kg bw/day) and [redacted] (1981) (CA 5.6.2/007; NOAEL 544 mg/kg bw/day), the most reliable overall NOAEL for both maternal and developmental toxicity in rats is 300 mg/kg bw/day.

Figure 5.6.2-01. Rat developmental toxicity studies, maternal and developmental NOAEL and LOAEL values, showing overall maternal and developmental NOAEL of 544 mg/kg bw/day [1. [redacted] (1996) (CA 5.6.2/001); 2. [redacted], (1995) (CA 5.6.2/002); 3. [redacted] (1991) (CA 5.6.2/003); 4. [redacted] (1991) (CA 5.6.2/004 - 005); 5. [redacted] (1986) (CA 5.6.2/006); 6. [redacted] (1981) (CA 5.6.2/007); 7. [redacted] (1980) (CA 5.6.2/008)]



1. Information on the study

Data point:	**CA 5.6.2/001**
Report author	[redacted]
Report year	2002 (study report 1996, amendment 2002)
Report title	Glyphosate Acid: Developmental Toxicity Study in the Rat (Including Amendment 001 to Glyphosate Acid: Developmental Toxicity Study in the Rat)
Report No	[redacted]/P/4819
Document No	M-301383-01-1
Guidelines followed in study	OECD 414 (2001), OPPTS 870.3700 (1998), 2004/73/EC B.31 (2004)
Deviations from current test guideline (OECD 414, 2018)	Duration of treatment was shorter than required. The following endpoints were not assessed: Weight and histopathological changes of the thyroid glands of the dams, anogenital distance (AGD) in foetuses, indication of incomplete testicular descent/cryptorchidism in male foetuses. Blood samples from dams to assess thyroid hormones (T4, T3 and TSH) were not collected. Deviations from the current version of OECD 414 (2018) are basically due to the fact that the study was aligned to an older version of the OECD test guideline 414.
Previous evaluation	Yes, accepted in RAR (2015)

GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

In a developmental study, groups of time-mated female rats of the Alpk:APfSD (Wistar-derived) strain were dosed by gavage with 0, 250, 500 or 1000 mg glyphosate acid/kg/day using deionised water as a vehicle. The day of mating was designated day 1 of gestation. The rats were dosed on days 7-16 (inclusive) of gestation which thus included the period of major organogenesis. On day 22 of gestation the rats were killed and their uteri examined for live foetuses and intra-uterine deaths. The foetuses were weighed, examined for external and visceral abnormalities, sexed, eviscerated and stained for skeletal examination.

There was no evidence of maternal toxicity attributable to glyphosate acid as assessed by the clinical condition of the animals during the study, their body weight gain and food consumption and the type and incidence of macroscopic findings *post mortem*.

There was no evidence of developmental toxicity attributable to glyphosate acid as assessed by the number, growth and survival of the foetuses. Observation of the external appearance of the foetuses, examination of the viscera and assessment of the skeletons revealed no treatment-related findings.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material:	Glyphosate acid
Description:	Technical, white solid
Lot/Batch number:	P24
Purity:	95.6% w/w a.i
CAS#:	Not reported
Stability of test compound:	Confirmed
Vehicle:	Deionised water
Test Animals:	
Species	Rat
Strain	Alpk:APfSD Wistar-derived
Age/weight on arrival	Approximately 11 weeks / 210 - 303 g
Source	[redacted]
Housing	Individually
Acclimatisation period	Not applicable
Diet	CT1 diet (Special Diet Services, Witham, Essex, UK) *ad libitum*
Water	Mains water *ad libitum*
Environmental conditions	Temperature: 21 ± 2 °C Humidity: 40 – 70 % Air changes: 25 - 30 changes / hour Photoperiod: 12 hours light / 12 hours dark

B. STUDY DESIGN

Dates of Experimental work: 1995-05-17 to 1996-03-26

In-life dates: Start: Not reported End: Not reported (QA audits conducted between May 1995 and March 1996)

Mating procedure: Virgin female rats were paired overnight (at the Breeding Unit) with males of the same strain. On the following morning, vaginal smears from these females were examined for the presence of sperm. The day when spermatozoa were detected was designated day 1 of gestation and, on this same day, successfully mated females were delivered to the experimental unit at [redacted].

Animal assignment: A total of 96 mated females was supplied over a two–week period. Twelve female rats were supplied on each of eight days. The study was divided into twenty-four replicates (randomised blocks) with each replicate containing one rat from each group. Animals were randomly assigned to test groups as shown in the following table.

Table 5.6.2-2: Glyphosate acid: Developmental toxicity study in the rat ([redacted], 1996): Animal numbers and treatment groups

Glyphosate acid (mg/kg bw/day)				
	0 (control)	**250**	**500**	**1000**
Animal Numbers	1 - 24	25 - 48	49 - 72	73 - 96

Dose selection rationale: The dose levels selected for this study were based on a dose range finding study in the pregnant rat. The highest dose level of 1000 mg/kg/day is the limit dose for this type of study.

Dose preparation and analyses: Glyphosate acid was administered in deionised water and the concentration was adjusted to give a constant volume of 1 mL/100 g body weight for each dose level. An appropriate amount of deionised water was added to a weighed amount of test substance (adjusted for purity) to provide each preparation. One preparation per concentration (i.e. 25, 50 and 100 mg/mL) was made. Each preparation was thoroughly mixed before being subdivided into aliquots. The control substance was also dispensed into aliquots. The aliquots were stored at room temperature and fresh aliquots were used for each day of the study.

A sample of each preparation was analysed prior to the start of dosing to verify the achieved concentrations of glyphosate acid in deionised water. Samples of the lowest concentration of dosing formulation was analysed to confirm the homogeneity of glyphosate acid in deionised water. The homogeneity of the 100 mg/mL formulation was not determined as part of this study and the data have been obtained from a preliminary study ([redacted] *1995*) for which the method of preparation of the dosing formulations was the same. The chemical stability of glyphosate acid in deionised water was determined by re-analysis of the lowest and highest concentrations of dosing formulation after an interval of 26 days.

Concentration analysis results: The achieved concentrations of glyphosate acid in deionised water were within 5% of nominal concentrations.
Homogeneity results: The homogeneity of glyphosate acid in deionised water at concentrations of 25 mg/mL and 100 mg/mL was within 5 % of the overall mean.
Stability results: The stability of the 25 mg/mL and the 100 mg/mL formulations was satisfactory over a period of 26 days which exceeded the period of use in this study.

Dose administration: All animals were dosed once daily from days 7 - 16 (inclusive) of gestation with 1 mL of dosing formulation per 100 g body weight using a disposable syringe and a plastic nelaton catheter. The volume given to each animal was adjusted daily according to body weight. Control animals received the appropriate volume of deionised water. Dosing was performed in group order with all animals receiving the same dose level being dosed sequentially.

Maternal Observations:
Clinical observations: All animals were observed on arrival to ensure that they were physically normal externally and were subsequently observed at least twice each day. Any changes in behaviour or clinical condition were recorded daily during the study.

Body weight: The body weight of each animal was recorded on arrival and on days 4, 7 - 16 (inclusive) and on days 19 and 22 of gestation.

Food consumption: The amount of food consumed by each animal over three day periods was measured by giving a weighed quantity of food contained in a glass jar on days 1, 4, 7, 10, 13, 16 and 19 and calculating the amount consumed from the residue on days 4, 7, 10, 13, 16, 19 and 22, respectively.

Necropsy: One rat requiring euthanasia was killed by over-exposure to halothane Ph. Eur. vapour and given a macroscopic examination *post mortem*.

On day 22 of gestation the animals were killed by over-exposure to halothane-Ph. Eur. vapour and a macroscopic examination *post mortem* was performed. The uterus from any animal without clear evidence of implantation was removed and stained with ammonium polysulphide to determine whether or not implantation had occurred.

For pregnant animals the intact gravid uterus (minus ovaries and trimmed free of connective tissue) was removed and weighed. The ovaries and uterus were then examined and the following data recorded:

Number of *corpora lutea* in each ovary
Number and position of implantations subdivided into:
- a) live foetuses
- b) early intra-uterine deaths (decidual or placental tissue only)
- c) late intra-uterine deaths (embryonic/foetal tissue plus placental tissue)

Individual foetal weights

The implantations were assigned letters of the alphabet to identify their position *in utero* starting at the ovarian end of the left horn and ending at the ovarian end of the right horn. In addition, each foetus was weighed and individually identified within the litter by means of a cardboard tag. After weighing the foetuses were killed with an intracardiac injection of approximately 0.1 mL of 200 mg/mL pentobarbitone sodium solution.

Percentage pre-implantation loss and percentage post-implantation loss were calculated.

$$\% \text{ pre-implantation loss} = \frac{\text{number of } \textit{corpora lutea} - \text{number of implantations}}{\text{number of } \textit{corpora lutea}} \times 100$$

$$\% \text{ post-implantation loss} = \frac{\text{number of implantations} - \text{number of live foetuses}}{\text{number of implantations}} \times 100$$

Foetal observations: An external examination of each foetus was made together with an examination of the oral cavity. All foetuses were then examined internally for visceral abnormalities, sexed, eviscerated and fixed in 70 % industrial methylated spirits. After approximately 24 hours the head of each foetus was

cut along the fronto-parietal suture line and the brain was examined for macroscopic abnormalities. The carcasses were then returned to 70% industrial methylated spirits for subsequent processing and staining with Alizarin Red S. The stained foetal skeletons were examined for abnormalities and the degree of ossification was assessed. The individual bones of the *manus* and *pes* were assessed and the result converted to a six point scale.

The observations were classified as major (permanent structural or functional deviations considered likely to be incompatible with survival or rarely seen) or minor defects or variants (small, generally transient deviations considered compatible with survival). The difference between the minor defect and variant classification is the frequency of occurrence in the control population of rats of this strain.

Statistical analyses: Data relating to animals which were non-pregnant, totally resorbed their litters or died intercurrently were excluded from the statistical analysis.

Maternal body weight during the dosing and post-dosing periods was considered by analysis of covariance on initial (day 7) body weight.

Maternal food consumption during the dosing and post-dosing periods, the numbers of implantations and live foetuses per female, gravid uterus weight, litter weight, mean foetal weights per litter and mean *manus* and *pes* scores per litter were considered by analysis of variance.

Maternal-performance data (excluding the animal with undetermined pregnancy status), the proportion of foetuses with each individual *manus* and *pes* score, the proportion of foetuses with each defect and the proportion of litters with each defect were considered by Fisher's Exact Test.

Pre-implantation loss, post-implantation loss, early intra-uterine deaths, late intra-uterine deaths, male foetuses, major external/visceral defects, minor external/visceral defects, external/visceral variants, major skeletal defects, minor skeletal defects and skeletal variants were analysed as follows:

- → Percentages were analysed by analysis of variance following the double arcsine transformation of *Freeman and Tukey (1950)*
- → the proportion of foetuses affected and with the exception of male foetuses the proportion of litters affected were considered by Fisher's Exact Test.

All analyses were carried out in *SAS (1989)*. For Fisher's Exact Test the proportion in each treated group was compared to the control group proportion. Analyses of variance and covariance allowed for the replicate structure of the study design. Least-squares means for each group were calculated using the LSMEAN option in SAS PROC GLM. Unbiased estimates of differences from control were provided by the difference between each treatment group least-squares mean and the control group least-squares mean. Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean using a Student's t-test, based on the error mean square in the analysis.

All statistical tests were two-sided.

II. RESULTS AND DISCUSSION

A. MORTALITY

One control animal was killed on day 7 as a result of being misdosed. There were no other mortalities.

B. CLINICAL OBSERVATIONS

Excess watery fluid in the thoracic cavity and dark red areas on the surface of the lung lobes were observed

at examination *post mortem* of the one control dam that was euthanised. The pregnancy status of the animal was not determined. There were no changes in the clinical condition of the other dams given glyphosate acid which were considered to be treatment-related.

C. BODY WEIGHT

There was no effect of glyphosate acid on maternal body weight.

Table 5.6.1-88: Glyphosate acid: Developmental toxicity study in the rat ([redacted], 1996): Intergroup comparison of maternal body weight (g) (selected timepoints, adjusted means for days 8 and 22)

	Glyphosate acid (mg/kg bw/day)			
Day	**0 (control)**	**250**	**500**	**1000**
1	255.6 ± 12.4 N=22	255.5 ± 16.4 N=24	253.5 ± 17.2 N=23	252.8 ± 24.0 N=23
8	288.2	288.1	288.0	287.5
22	406.4	410.1	411.1	408.6

D. FOOD CONSUMPTION

There was no adverse effect of glyphosate acid on maternal food consumption. The amount of food consumed by the animals given 1000 mg glyphosate acid/kg bw/day was marginally lower during the dosing period but differences from the controls were not statistically significant.

Table 5.6.1-89: Glyphosate acid: Developmental toxicity study in the rat ([redacted], 1996): Intergroup comparison of food consumption (g/day) (selected timepoints)

	Glyphosate acid (mg/kg bw/day)			
Day	**0 (control)**	**250**	**500**	**1000**
1-4	23.9 ±3.8	24.6±2.3	24.6±2.4	23.2±2.4
13-16	33.2±5.3	33.4±3.2	33.7±2.7	31.9±3.4
19-26	29.5±4.1	31.6±3.7*	30.5±3.1	30.5±3.6

* Statistically significant difference from control group mean, $p<0.05$ (Student's t-test, 2-sided)

E. NECROPSY

There were no macroscopic findings in the dams which were considered to be related to the administration of glyphosate acid.

Table 5.6.10: Glyphosate acid: Developmental toxicity study in the rat ([redacted], 1996): Intergroup comparison of macroscopic findings *post mortem*

Observation	**Glyphosate acid (mg/kg bw/day)**			
	0 (control)	**250**	**500**	**1000**
Number of females on study/ Number of females with terminal *post mortem* examination	**24/23**	**24/24**	**24/24**	**24/24**

KIDNEY				
Pelvic dilatation	1	1	0	1
Enlarged	1	0	0	0
LUNG				
Red area/s	1	0	0	0
Pale	1	0	0	0
THYMUS				
Speckled	0	1	0	0
URETER				
Distended	1	0	0	0
UTERUS				
Distended	0	0	0	1

F. FOETUSES

There was-no effect of glyphosate acid on the number, growth or survival of the foetuses *in utero*.

Table 5.6.2.1-05: Glyphosate acid: Developmental toxicity study in the rat ([redacted], 1996): Intergroup comparison of maternal performance

Observation	Glyphosate acid (mg/kg bw/day)			
	0 (control)	250	500	1000
# Animals Assigned (Mated)	24	24	24	24
# Animals Pregnant	22	24	23	24
#Pregnancy status not determined (intercurrent death)	1	0	0	0
Gravid uterus weight (g) (mean ± S D.)	89.7±14.6	87.2±19.3	91.3±15.9	89.9±18.3
Corpora Lutea/Dam (mean ± S D.)	15.8±1.2	15.7±1.4	15.5±1.4	15.5±1.3
Implantations/Dam (mean ± S D.)	14.4±1.2	12.9±3.6*	14.1±2.1	13.6±2.7
Totally resorbed at termination	0	0	0	1
Total # Litters (viable)	22	24	23	23
Live Foetuses/Dam (mean ± S D.)	12.9±2.4	12.4±3.4	13.1±2.7	12.9±2.9
Early Intra-uterine deaths (Proportion of implants affected) (mean ± S D.)	8.7±14.5	3.4±4.8**	6.2±9.8	5.5±8.4
Late Intra-uterine deaths (Proportion of implants affected) (mean ± S D.)	1.3±4.7	0.5±1.8	1.6±4.8	0.3±1.3
Litter Weight (g) (mean ± S D.)	62.4±10.4	61.2±14.5	64.3±11.5	63.6±13.7
Mean Foetal Weight (g) (mean ± S D.)	4.86±0.29	5.02±0.33	4.95±0.29	4.96±0.27
Proportion of male foetuses	51.9	54.1	53.3	51.0
Pre-implantation loss (%)	8.7	18.0**	8.8	12.0
Post-implantation loss (%)	9.9	4.0**	7.8	5.8*

* Statistically significant difference from control group mean, $p<0.05$ (Student's t-test, 2-sided)
** Statistically significant difference from control group mean, $p<0.01$ (Student's t-test, 2-sided)

Findings during external, visceral and skeletal examination:
Major defects: The incidence of foetuses with major defects was 1/284, 1/297, 1/301 and 2/296 in the control and 250, 500 and 1000 mg glyphosate acid/kg bw/day groups, respectively. Neither the type nor incidence of major defects provided evidence for an adverse effect of glyphosate acid. The defects were dissimilar in type and of single incidence.

Minor defect: The proportion of foetuses with minor external/visceral defects and the proportion of foetuses with minor skeletal defects were similar for all groups. Consideration of the specific defects provided no evidence for an adverse effect of glyphosate acid.

Variants: The proportion of foetuses with external/visceral variants and the proportion of foetuses with skeletal variants were lower in the glyphosate acid treated groups than in the control group. Consideration of the specific defects provided no evidence for an adverse effect of glyphosate acid.

Table 5.6.2.1-06: Glyphosate acid: Developmental toxicity study in the rat ([redacted], 1996): Intergroup comparison of foetal defects and variants

Observation	Glyphosate acid (mg/kg bw/day)			
	0 (control)	250	500	1000
Major External VISCERAL DEFECTS				
Prop. of foetuses affected (% mean ± S.D.)	1/284 (0.3±1.4)	0/297 (0.0±0.0)	1/301 (0.4±1.9)	2/296 (0.8±2.6)
Prop. of litters affected	1/22	0/24	1/23	2/23
Minor External VISCERAL DEFECTS				
Prop. of foetuses affected (% mean ± S.D.)	2/284 (0.6±2.0)	2/297 (0.6+2.0)	4/301 (1.4+3.0)	1/296 (0.3±1.6)
Prop. of litters affected	2/22	2/24	4/23	1/23
EXTERNAL VISCERAL VARIANTS				
Prop. of foetuses affected (% mean ± S.D.)	27/284 (9.8 ± 11.3)	25/297 (8.8±12.1)	24/301 (7.1±7.5)	17/296 (6.1±11.0)
Prop. of litters affected	12/22	11/24	13/23	9/23
Major SKELETAL DEFECTS				
Prop. of foetuses affected (% mean ± S.D.)	1/284 (0.3±1.4)	1/297 (0.3±1.5)	1/301 (0.4±1.9)	0/296 (0.0±0.0)
Prop. of litters affected	1/22	1/24	1/23	0/23
Minor SKELETAL DEFECTS				
Prop. of foetuses affected (% mean ± S.D.)	60/284 (21.1±14.5)	55/297 (18.7±13.6)	60/301 (20.2±11.0)	51/296 (17.1±12.5)
Prop. of litters affected	18/22	21/24	21/23	20/23
SKELETAL VARIANTS				
Prop. of foetuses affected (% mean ± S.D.)	208/284 (72.8±19.4)	199/297 (66.3±18.4)	200/301 (64.9±24.6)	202/296 (67.0±21.1)
Prop. of litters affected	22/22	24/24	23/23	23/23

* number affected/number examined

Table 5.6.2.1-07: Glyphosate acid: Developmental toxicity study in the rat ([redacted]., 1996): Incidence of major defects

Observation	Glyphosate acid (mg/kg bw/day)			
	0 (control)	250	500	1000
Encephalocele	1	0	0	0
Extra arch between 12th and 13th right thoracic vertebrae	0	1	0	0
Polydactyly (left hindpaw)	0	0	1	0
Hydroureter	0	0	0	1
Situs inversus totalis	0	0	0	1

Manus and pes assessment: There was no effect of glyphosate acid on the ossification of the *manus* or *pes*. The scale for assessing skeletal ossification had been presented in Appendix F of the study report.

Table 5.6.2.1-08: Glyphosate acid: Developmental toxicity study in the rat ([redacted], 1996): Intergroup comparison of manus/pes assessment

Observation	Glyphosate acid (mg/kg bw/day)			
	0 (control)	250	500	1000
MANUS Scores in the number of Litters	22	24	23	23
Score 2	0 (0.0%)	5 (1.7%)	0 (0.0%)	0 (0.0%)
Score 3	29 (10.2%)	63** (21.2%)	49* (16.3%)	32 (10.8%)
Score 4	235 (82.7%)	213** (71.7%)	231 (76.7%)	249 (84.1%)
Score 5	20 (7.0%)	15 (5.1%)	21 (7.0%)	15 (5.1%)
Score 6	0 (0.0%)	1 (0.3%)	0 (0.0%)	0 (0.0%)
MEAN	3.96±0.19	3.79±0.40*	3.90±0.22	3.95±0.19
PES Scores				
Score 3	30 (10.6%)	61** (20.5%)	22 (7.3%)	23 (7.8%)
Score 4	173 (60.9%)	157 (52.9%)	196 (65.1%)	180 (60.8%)
Score 5	80 (28.2%)	78 (26.3%)	83 (27.6%)	92 (31.1%)
Score 6	1 (0.4%)	1 (0.3%)	0 (0.0%)	1 (0.3%)
MEAN	4.17±0.27	4.02±0.44	4.18±0.33	4.24±0.35

* Statistically significant difference from control group mean, $p<0.05$
** Statistically significant difference from control group mean, $p<0.01$

III. CONCLUSIONS

There was no evidence of maternal or developmental toxicity attributable to glyphosate acid at any dose level. The dose level of 1000 mg glyphosate acid/kg bw/day was the NOEL in this study for both maternal and developmental effects.

3. Assessment and conclusion

Assessment and conclusion by applicant:

This study performed according to OECD 414 (2001) in rats, the NOEL for maternal and developmental effects was 1000 mg/kg bw/day based on no evidence of maternal or developmental toxicity attributable to glyphosate acid at any dose level.

Assessment and conclusion by RMS:

3. Information on the study

Data point:	**CA 5.6.2/002**
Report author	[redacted]
Report year	1995
Report title	HR-001: Teratogenicity Study in Rats
Report No	[redacted] 94-0152
Document No	M-301383-01-1
Guidelines followed in study	Japan MAFF Guidelines 59 NohSan No.4200, 1985 U.S. EPA FIFRA Guidelines Subdivision F, 83-3, 1984 OECD Guideline 414, 1981
Deviations from current test guideline (OECD 414, 2018)	Duration of treatment was shorter than required. The following endpoints were not assessed: Weight and histopathological changes of the thyroid glands of the dams, anogenital distance (AGD) in foetuses, indication of incomplete testicular descent/cryptorchidism in male foetuses. Blood samples from dams to assess thyroid hormones (T4, T3 and TSH) were not collected. Deviations from the current version of OECD 414 (2018) are basically due to the fact that the study was aligned to an older version of the OECD test guideline 414.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

4. Full summary

A teratogenicity study was conducted to evaluate the potential maternal and developmental toxicity of glyphosate in rats. The test substance was suspended in 0.5 % aqueous solution of sodium carboxymethylcellulose and was administered orally with stomach tube to 24 copulated Crj:CD (SD) female rats per group at dose levels of 0, 30, 300 or 1000 mg/kg bw/day from days 6 to 15 of gestation.

No adverse effects related to test substance treatment were observed for maternal rats in the 30 and 300 mg/kg bw/day groups. In the 1000 mg/kg bw/day group, 20 of 22 pregnant females showed slightly loose stool during the dosing period and/or on the following day of last dosing, and the incidence was statistically significantly high. No treatment related changes were observed in the body weights and body weight gains. Food consumption in this group was significantly decreased at an interval of days 6-9 of gestation (early dosing period).

Observation at cesarian section revealed no treatment related adverse effects in any of the parameters tested, i.e. gross pathology findings, gravid uterine weights, numbers of corpora lutea, implants and live foetuses, percent incidences of resorptions and foetal deaths, foetal sex ratio, foetal body weights and placenta weights.

In the teratological examination foetuses, no treatment related malformations or variations were noted in any of the treated groups.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material:	Glyphosate technical
Identification:	HR-001
Description:	Solid white crystals
Lot/Batch #:	940908-1
Purity:	95.68 %
Stability of test compound:	Not mentioned in the report
Vehicle:	Purified water with 0.5 % sodium carboxymethylcellulose (CMC)
Test Animals:	
Species:	Rat
Strain:	SPF Crj:CD (SD)
Source:	[redacted]
Age:	13 weeks
Sex:	Females (males used for mating only)
Females weight at dosing:	267 - 322 g
Acclimation period:	11 days
Diet/Food:	Certified diet MF Mash (Oriental Yeast Co., Ltd.), *ad libitum*
Water:	Filtered and sterilised water, *ad libitum*
Housing:	Individually in aluminum cages with wire-mesh floors and fronts.
Environmental conditions:	Temperature: 24 ± 2 °C Humidity: 55 ± 10 % Air changes: 12/hour 12 hours light/dark cycle

B. STUDY DESIGN

In life dates:
1995-03-23 to 1995-06-26

Mating procedure:
When animals were mated, pairs of males and females were housed, one pair per cage. Vaginal smears were taken from females for microscopic examination. Females showing proestrus or estrus vaginal smears were paired overnight with males on a 1:1 basis. The females were examined next morning for the presence of vaginal plugs and sperm in vaginal smears and considered to copulate when vaginal plugs and/or sperm

were observed. These mating procedures were repeated for 4 consecutive days.

Animal assignment
Four test groups were set; i.e. control, low-dose, middle-dose and high-dose groups. Copulated females were weighed on day 0 of gestation, the day in which evidence of copulation was detected, and distributed to 4 groups on that day in such a way to equalize group means and standard deviations of body weights. Each test group consisted of 24 copulated females which an individual animal number was given as follows: control group (1-24), low-dose group (25-48), middle-dose group (49-72) and high-dose group (73-96).

Dose selection rationale
The dose levels selected for this study were based on a dose range finding study in the pregnant rat. The highest dose level of 1000 mg/kg/day is the limit dose for this type of study.

Dose preparation and analyses
For each dose level, dosing solutions were prepared by suspending the specified amount of glyphosate acid in purified water with the aid of 0.5% sodium carboxymethylcellulose.

All dosing solutions prepared for each dose level were analysed for concentration of the test substance before use. For the first prepared dosing solutions, homogeneity of the test substance in vehicle was determined on the samples for the low- and middle-dose groups (30 and 1000 mg/kg/day, respectively) taken from the top, middle and bottom of the graduated glass cylinder immediately after preparation. Analytical data revealed that the test substance was distributed homogeneously in the vehicle.

Concentration analysis results: The achieved concentrations of glyphosate acid were detected at levels of 96-106% of the claimed concentrations in each dosing solution.
Homogeneity results: Analytical data revealed that the test substance was distributed homogeneously in the vehicle.
Stability results: The substance was stable in the 0.5% CMC solution for at least 14 days after preparation by the analysis conducted in the preliminary teratogenicity study ([redacted] 94-0139), dosing solution were therefore made twice at an interval of 7 days during this study.

Dose administration
The test substance was administered orally with a stomach tube to 24 copulated Crj:CD (SD) female rats per group at dose levels of 0, 300, 1000 mg/kg/day from day 6 to 15 of gestation. The volume of dosing solutions administered to females was 10 mL/kg body weight. Individual dosages were calculated based on the body weights on each day of dosing. During the dosing procedure, dosing solutions were constantly stirred with a magnetic stirrer to ensure homogeneity. Control animals only received the vehicle in the same manner.

Maternal Observations:
Clinical observations
Each female was observed for clinical signs and mortality at least once daily during the pre-dosing and post-dosing periods and at least twice daily during the dosing period.

Body weight
Individual body weights were recorded on days 0 (day on which evidence of copulation was recorded), 6-15 (daily during the dosing period) and 20 of gestation. Adjusted body weight gains were calculated by subtracting the gravid uterine weight from the body weight value on day 20 of gestation.

Food consumption
Food consumption of each female was determined at intervals of days 0-6, 6-9, 9-12, 12-15 and 15-20 of gestation.

Necropsy

All surviving females were euthanised by overdosage of ether inhalation and cesarian section was performed on day 20 of gestation.

Each female was necropsied. The ovaries and uterus were removed and the gravid uterine weight and numbers of copora lutea and implants were recorded. Then the uterus was opened and the numbers of live and dead foetuses were recorded with their positions in the uterine horns. When no uterine implants were grossly apparent, the uterus was stained with 10 % ammonium sulphide solution to detect very early resorptions.

Foetal observations

Resorbed embryos or dead foetuses were classified into implantation sites, placental remnants or macerated foetuses (including dead foetuses) according to developmental stage in which resorptions or deaths occurred. The weights of each live foetus and of each placenta were determined and recorded. Live foetuses were sexed and were euthanised by an intraperitoneal injection of pentobarbital sodium solution for examination of external abnormalities. The eyes were examined for alterations after removing the palpebral skin. Half of the foetuses were examined for visceral abnormalities according to the fresh visceral examination method. After examination, the foetuses were fixed and preserved in 10 % neutral-buffered formalin along with the ovaries and placentas. The other half of the foetuses were fixed in 70 % alcohol. Then they were eviscerated, stained with alizarin red S, cleared in 70 % glycerin, and examined for skeletal abnormalities. After examination, skeletal specimens were stored.

Statistics

Equality of variances was first evaluated using Bartlett's test for body weights, adjusted body weights, body weight gains and food consumption of maternal rats, numbers of corpora lutea, implants and live foetuses, and weights of gravid uteri, foetuses and placentas. When group variances were homogeneous, a parametric analysis of variance in one-way classifications was used to determine if any statistical difference exist among groups. If the analysis of variance was significant, Scheffé's multiple comparison test was performed to detect any statistically significant differences between the treated groups and their corresponding controls. When Bartlett's test indicated that the variances were not homogenous, Kruskal-Wallis test was used for detecting any statistical differences among groups and if significant, Scheffé-type mean rank test was performed to detect statistical differences between the treated groups and their corresponding controls. Fisher's exact probability test was used for the data on the incidences of clinical and gross pathological findings in maternal rats, incidences of maternal rats having foetuses with malformations and variations, incidences of foetal malformations and variations, and foetal sex ratio. Mann-Whitney's U-test was used for the data on the percent incidences of resorption and foetal deaths.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

During the pre-dosing period, clinical observation revealed no abnormalities in any groups. During the dosing period, no abnormalities were observed in maternal rats of the control group. At 30 and 300 mg/kg bw/day, one or two maternal rats had hair loss or scabs on the skin which have been usually observed in the historical control rats. At 1000 mg/kg bw/day, 20 out of 22 pregnant females showed slightly loose stool and the increase in its incidence was statistically significant.

Table 5.6.2.2-01: Glyphosate technical: Teratology study in the rat ([redacted] 1995): Observed clinical signs during the dosing period (gestation days 6 through 15)

Clinical sign	Glyphosate technical (mg/kg bw/day)			
	0 (control)	30	300	1000
No. of animals examined	23 (1)	24 (0)	24 (0)	22 (2)
No abnormalities detected	23 (1)	23 (0)	22 (0)	2 (0)
Loose stool	0 (0)	0 (0)	0 (0)	20 (2)***
Hair loss	0 (0)	1 (0)	2 (0)	0 (0)
Scabs	0 (0)	0 (0)	1 (0)	0 (0)

Figures represent the number of animals which showed clinical signs during each period of the study.
Figures in parentheses represent the number of animals non-pregnant.
*** Significantly different from control at p<0.001.

During the post-dosing period, slightly loose stool was also observed on the following day of last dosing (day 16 of gestation) in 9 out of 20 females that showed this finding during the dosing period at 1000 mg/kg/bw/day. Another finding observed during this period was hair loss in 1-2 maternal rats in each treated group.

Table 5.6.2.2-02: Glyphosate technical: Teratology study in the rat ([redacted], 1995): Observed clinical signs during the post-dosing period (gestation days 16 through 20)

Clinical sign	Glyphosate technical (mg/kg bw/day)			
	0 (control)	30	300	1000
No. of animals examined	23 (1)	24 (0)	24 (0)	22 (2)
No abnormalities detected	23 (1)	23 (0)	22 (0)	13 (2)
Loose stool	0 (0)	0 (0)	0 (0)	9 (0)***
Hair loss	0 (0)	1 (0)	2 (0)	2 (0)
Scabs	0 (0)	0 (0)	0 (0)	0 (0)

Figures represent the number of animals which showed clinical signs during each period of the study.
Figures in parentheses represent the number of animals non-pregnant.
*** Significantly different from control at p<0.001.

Loose stool at highest dose level was considered to be due the test substance treatment.
No deaths occurred during the study in any groups.

C. BODY WEIGHT

No significant differences were found in the mean body weights and the mean adjusted body weights of maternal rats between the control groups and any of the treated group.

No significant differences were found in the mean body weight gains of maternal rats between the control group and any of the treated groups.

Table 5.6.2.2-03: Glyphosate technical: Teratology study in the rat ([redacted], 1995): Mean body weights (g) of maternal rats

Dose level mg/kg bw/day		Body weight on Gestation day												Adjusted Weight
		0	6	7	8	9	10	11	12	13	14	15	20	
0	Mean	293	323	326	329	334	338	345	349	352	358	365	439	361
	S.D.	13	15	16	15	16	16	17	17	17	19	20	30	20
	No.	23	23	23	23	23	23	23	23	23	23	23	23	23

Table 5.6.2.2-03: Glyphosate technical: Teratology study in the rat ([redacted], 1995): Mean body weights (g) of maternal rats

Dose level		Body weight on Gestation day												Adjusted Weight
mg/kg bw/day		0	6	7	8	9	10	11	12	13	14	15	20	
30	Mean	293	325	327	332	336	341	347	351	356	363	370	451	366
	S.D.	12	11	12	13	13	13	13	13	13	13	14	22	16
	No.	24	24	24	24	24	24	24	24	24	24	24	24	24
300	Mean	293	324	326	331	334	340	346	350	353	357	365	444	359
	S.D.	12	12	13	13	14	15	15	14	18	20	19	25	14
	No.	24	24	24	24	24	24	24	24	24	24	24	24	24
1000	Mean	294	326	325	330	333	338	344	347	351	357	365	449	363
	S.D.	12	13	15	14	13	14	13	15	15	15	17	23	18
	No.	22	22	22	22	22	22	22	22	22	22	22	22	22

D. FOOD CONSUMPTION

No significant differences were found in the mean food consumption of maternal rats between the control group and any of the 30 and 300 mg/kg bw/day groups. In the 1000 mg/kg bw/day group, lower and higher values were observed in the mean food consumption at intervals of days 6-9 of gestation (early dosing period) and days 15-20 of gestation (post-dosing period), respectively, and the differences from the corresponding controls were statistically significant.

Table 5.6.2.2-04: Glyphosate technical: Teratology study in the rat ([redacted], 1995): Mean food consumption (g/day) of maternal rats

Dose level		Food consumption during gestation days				
mg/kg bw/day		0 - 6	6 - 9	9 - 12	12 - 15	15 - 20
0	Mean	22.0	22.6	23.3	21.4	23.4
	S.D.	2.2	2.5	2.1	2.2	2.0
	No.	23	23	23	23	23
30	Mean	22.0	23.6	24.0	22.5	24.3
	S.D.	1.4	2.0	2.4	2.3	1.9
	No.	24	24	24	24	24
300	Mean	21.5	21.9	22.7	20.6	23.6
	S.D.	1.8	1.7	2.2	2.5	1.4
	No.	24	24	24	24	24
1000	Mean	22.4	20.3*	21.8	21.0	25.4**
	S.D.	1.9	2.6	2.0	2.2	2.2
	No.	22	22	22	22	22

* Significantly different from control at p<0.05.
** Significantly different from control at p<0.01.

E. NECROPSY

Gross pathological examination of maternal rats at cesarean section revealed several findings such as hair loss and pelvic dilatation in the kidney in 1-2 animals in all groups including the control group. These findings were not considered to be treatment related.

Table 5.6.2.2-05: Glyphosate technical: Teratology study in the rat ([redacted], 1995): Gross pathological findings in maternal rats

Gross pathological finding	Glyphosate technical (mg/kg bw/day)

	0 (control)	30	300	1000
No. of animals examined	23 (1)	24	24	22 (2)
No. abnormalities detected	20 (1)	21	19	20 (2)
Brain: Hydrocephalus	1 (0)	0	0	0 (0)
Spleen:				
Enlargement	0 (0)	0	1	0 (0)
Thickening of capsule	0 (0)	0	1	0 (0)
Spot(s)	0 (0)	0	1	0 (0)
Adhesion to omentum	0 (0)	0	1	0 (0)
Kidney: Pelvic dilatation	2 (0)	2	0	0 (0)
Skin: Hair loss	0 (0)	1	2	2 (0)

Figures in parentheses represent the number of animals non-pregnant.

Out of 24 copulated females, 23, 24, 24 and 22 were proved to be pregnant in the control, 30, 300 and 1000 mg/kg groups, respectively.

No significant differences were found in the mean gravid uterine weights and the mean numbers of corpora lutea and implants between the control group and any of the treated group.

F. FOETUSES

There were no significant differences in the mean number of live foetuses and the mean percent incidence of resorptions and foetal deaths between the control group and any of the treated groups. There were no significant differences in the foetal sex ratio, the mean foetal body weights and the mean placental weights between the control group and any of the treated group.

Teratological examination revealed external, visceral or skeletal malformations in 1-2 foetuses in all groups including the control group. These abnormalities, however, were low in their incidences and were considered to be spontaneous occurrences. Various visceral and skeletal variations were also observed in all groups including the control group. These alterations were not considered to be related to test substance treatment because of no significant differences in their incidences between the control group and the treated groups.

Table 5.6.2.2-06: Glyphosate technical: Teratology study in the rat ([redacted], 1995): Intergroup comparison of maternal performance

Observation	Glyphosate technical (mg/kg bw/day)			
	0 (control)	30	300	1000
# Animals Assigned (Mated)	24	24	24	24
# Animals Pregnant	23	24	24	22
#Pregnancy status not determined (intercurrent death)	0	0	0	0
Gravid Uterine Weight (g) (mean ± S.D.)	78 ± 23	85 ± 12	85 ± 19	87 ± 12
Placental weight (mg) (mean ± S.D.)	516 ± 88	511 ± 93	497 ± 62	491 ± 42
No. of corpora lutea (mean ± S.D.)	16.4 ± 2.8	17.2 ± 1.1	17.4 ± 1.7	17.5 ± 1.5
No. of implantations (mean ± S.D.)	14.8 ± 4.4	16.1 ± 1.7	16.1 ± 2.5	16.8 ± 1.4
No. of Live Foetuses	13.7 ± 4.1	15.0 ± 2.1	14.9 ± 2.8	15.4 ± 2.1

% of Foetal resorption and death	7.0	6.8	7.4	8.4
Males Mean Foetal Weight (g) ± S.D.	3.58 ± 0.41	3.60 ± 0.23	3.51 ± 0.43	3.64 ± 0.18
Females Mean Foetal Weight (g) ± S.D.	3.35 ± 0.28	3.39 ± 0.36	3.37 ± 0.36	3.41 ±0 18
Sex Ratio	0.49	0.515	0.461	0.445
Pre-implantation Loss (%)	8.7	18.0**	8.8	12.0
Post-implantation Loss (%)	9.9	4.0**	7.8	5.8*

* Statistically significant difference from control group mean, p<0.05 (Student's t-test, 2-sided)
** Statistically significant difference from control group mean, p<0.01 (Student's t-test, 2-sided)

Findings in external, visceral and skeletal examination:
External malformations observed were short tail in a foetus of the 30 mg/kg bw/day group and microphthalmia in a foetus of the 1000 mg/kg bw/day group.

Visceral examination revealed two types of malformations: right aortic arch in a foetus of the 300 mg/kg bw/day group and ventricular septal defects in a foetus of each of the 300 and 1000 mg/kg bw/day groups.

Visceral variations were observed in all groups including the control group. The types and number in foetuses were thymic remnant in the neck, dilatation of the renal pelvis and left umbilical artery in 16-26, 1-2 and 0-3, respectively.

Skeletal examination revealed three types of malformations: splitting of the ossification centers of the thoracic vertebral bodies in 2, 1 and 2 foetuses in the control, 300 and 1000 mg/kg bw/day groups, respectively, asymmetry of the sternebrae with sterno-costal joint displacement in a foetus of the 300 mg/kg bw/day group, and fusion of the sternebrae in a foetus of the 300 mg/kg bw/day group, and fusion of the sternebrae in a foetus of the 1000 mg/kg bw/day group.

Skeletal variations were observed in all groups including the control group. The types and the number in foetuses were cervical ribs shortening of the 13th ribs, lumber ribs, sacralisation of the lumber vertebra and asymmetry and/or splitting of the sternebrae in 0-1, 0-1, 1-11, 0-1 and 3-5, respectively.

Table 5.6.2.2-07: Glyphosate technical: Teratology study in the rat ([redacted], 1995): Incidence of foetal malformation and variations

Observation	Glyphosate technical (mg/kg bw/day)			
	0 (control)	250	300	1000
EXTERNAL malformations				
No. of foetuses examined	314	361	358	339
Microphthalmia	0	0	0	1
Short tail	0	1	0	0
Total No. of foetuses malformed	0	1	0	1
VISCERAL malformation				
No. of foetuses examined	150	174	173	164
Right aortic arch	0	0	1	0
Ventricular septal defects	0	0	1	1
Total No. of foetuses malformed	0	0	2	1
VISCERAL variations				
No. of foetuses examined	150	174	173	164

Thymic remnant in the neck	18	26	24	16
Dilatation of the renal pelvis	1	2	1	2
Left umbilical artery	2	0	3	2
Total No. of foetuses with variations	21	28	28	20
SKELETAL malformations				
No. of foetuses examined	164	187	185	175
Splitting of ossification centers of the thoracic vertebral bodies	2	0	1	2
Fusion of the sternebrae	0	0	0	1
Asymmetry of the sternebrae with sterno-costal joint displacement	0	0	1	0
Total No. of foetuses malformed	2	0	2	3
SKELETAL variations				
No. of foetuses examined	164	187	185	175
Cervical ribs	0	0	1	0
Shortening of the 13th ribs	1	0	1	0
Lumbar ribs	4	1	3	11
Sacralisation of the lumbar vertebra	0	0	1	0
Asymmetry and/or splitting of sternebrae	4	5	5	3
Total No. of foetuses with variations	9	6	11	14

III. CONCLUSIONS

Based on slightly loose stool at the high dose, the NOAEL was set at 300 mg/kg bw/day for maternal rats, and 1000 mg/kg bw/day for developmental toxicity. It is also concluded that the highest dose level of 1000 mg/kg bw/day of glyphosate is not teratogenic to SD rat foetuses.

3. Assessment and conclusion

Assessment and conclusion by applicant:

This study using rats performed according to OECD 414 resulted in a NOAEL for maternal effects of 300 mg/kg bw/day based on slightly loose stool at the 1000 mg/kg bw/day dose level. The NOAEL for developmental toxicity was considered to be 1000 mg/kg bw/day. Teratogenic effects were not observed.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	**CA 5.6.2/003**
Report author	[redacted]
Report year	1991
Report title	The effect of glyphosate on pregnancy of the rat (incorporates preliminary investigation)

Report No	CHV 43 & 41/90716
Document No	Not reported
Guidelines followed in study	U.S. EPA FIFRA Guidelines 83-3, 1989; OECD Guideline 414, 1981
Deviations from current test guideline (OECD 414, 2018)	Duration of treatment was shorter than required. The following endpoints were not assessed: weight and histopathological changes of the thyroid glands of the dams, anogenital distance (AGD) in foetuses, indication of incomplete testicular descent/cryptorchidism in male foetuses. Blood samples from dams to assess thyroid hormones (T4, T3 and TSH) were not collected. Deviations from the current version of OECD 414 (2018) are basically due to the fact that the study was aligned to an older version of the OECD test guideline 414.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. **Full summary**

A teratogenicity study was conducted to evaluate the potential maternal and developmental toxicity of glyphosate technical in rats. The test substance was suspended in 1 % aqueous solution of methylcellulose and was administered orally, by gavage, to 25 mated Crl:CD (SD) female rats per group at dose levels of 0, 300, 1000 or 3500 mg/kg bw/day on Days 6 to 15 of gestation.

Mortality was confined to the highest dose and clinical signs of toxicity were observed at the two upper dose levels. Two high dose females were sacrificed on day 7 and 13, respectively, following noisy respiration and gasping. Since respiratory signs were apparent in most animals of this group and in few cases at the mid dose level, these deaths were considered treatment-related. One more high dose dam was killed just after a probable intubation error. Post-dosing salivation and loose faeces were further symptoms observed among animals receiving 3500 mg/kg bw/day. During the first two days of treatment, body weight gain was reduced at the high and, although only to a marginal extent, at the mid dose level. Food consumption was slightly decreased in the high dose group during the dosing period but was comparable with controls thereafter. Water intake was increased at 3500 mg/kg bw/day. Necropsy at termination did not reveal findings which could be attributed to treatment.

There were no abortions and no total resorptions. Implantation rate, post-implantation loss, litter size and sex ratio were similar in all groups. The only effect was a reduction in mean foetal weight at 3500 mg/kg bw/day. The occurrence of malformations was not significantly increased by treatment. However, the incidence of rib distortion (wavy ribs) was markedly higher in the high-dose group. In addition, reduced ossification was seen slightly more frequently at this dose level and also at 1000 mg/kg bw/day. As result, the percentage of foetuses showing skeletal anomalies (variations) was significantly increased at the mid- and high-dose levels. However, the percentage for the mid-dose level only slightly exceeded the historical background range (21.9 - 27.2 %) whereas the respective control value was atypically low in this study.

Treatment with glyphosate at 300 mg/kg bw/day was without adverse effect on maternal or embryofoetal parameters.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material:	Glyphosate technical
Description:	White solid
Lot/Batch #:	206-Jak-25-1
Purity:	98.6 %
Stability of test compound:	At least five years
Vehicle:	Purified water with 1% sodium methylcellulose (MC)
Test Animals:	
Species:	Rat
Strain:	SPF Crl:CD (SD) BR VAF/Plus strain
Source:	[redacted]
Age:	8-10 weeks
Sex:	Females
Weight at dosing:	251 - 258 g (mean group values)
Acclimation period:	11 days
Diet/Food:	Labsure Laboratory Animal Diet No. 1, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	In groups of five in suspended galvanised metal cages equipped with solid sides and back, wire mesh front, floor and top.
Environmental conditions:	Temperature: 21.0 – 25.0 °C Humidity: 39 – 64 % Air changes: not reported 12 hours light/dark cycle

B. STUDY DESIGN

In life dates
1989-11-16 to 1989-12-12

Mating procedure
Time-mated animals were received from the breeder in two batches (A and B). The day of mating, as judged by appearance of sperm in a vaginal smear or by presence of a vaginal plug was considered as Gestation Day 0.

Animal assignment
Animals were assigned to four groups using arrival weights by computerised stratified randomisation to give approximately equal initial group mean body weights within each batch. Each test group consisted of 25 females (10 in batch A and 15 in batch B) which an individual animal number was given as follows: control group (1-25), low-dose group (26-50), middle-dose group (51-75) and high-dose group (76-100).

Dose selection rationale
The dose levels selected for this study were based on a dose range finding study in the pregnant rat. The highest dose level of 1000 mg/kg/day is the limit dose for this type of study.

Dose preparation and analyses
The highest dose formulation was prepared by suspending an appropriate amount of test substance in 1 % aqueous methylcellulose. Dose formulation for the lower dose groups were prepared by serial dilution with

1% methylcellulose. Formulations were prepared daily and used within three hours of preparation. Samples were taken for analysis on the first day of treatment for each of the two batches of animals.

Concentration analysis results: The achieved concentrations of glyphosate acid with the exception of one result (Batch A, Group 4) which was 17.4 % above the nominal concentration, mean results were within 5 % of nominal values.
Homogeneity results: Analytical data revealed that the test substance was distributed homogeneously in the vehicle.
Stability results: The substance was stable in the 1 % aqueous methylcellulose solution for at least 72 hours after preparation.

Dose administration
The test substance was administered orally, by gavage, to 25 mated female rats per group at dose levels of 0, 300, 1000 and 3500 mg/kg bw/day from day 6 to 15 of gestation. The dose volume was 1 mL/100 g bw. Dose volumes were calculated for individual animals on day 6 and adjusted according to body weight on days 8, 10, 12 and 14.

Maternal Observations:
Clinical observations
Each female was observed daily for mortality, obvious changes and reaction to treatment.

Body weight
Individual body weights were recorded initially (day 1) and on days 3, 6, 8, 10, 12, 14, 16, 18 and 20 of gestation.

Food consumption
Food consumption of each female was determined at intervals of days 1-3, 3-6, 6-8, 8-10, 10-12, 12-14, 14-16, 16-18 and 18-20 of gestation.

Water consumption
Water consumption was determined daily from day 2 through to termination.

Necropsy
All surviving females were euthanised by CO_2 asphyxation, dissected and examined for congenital abnormalities and macroscopic pathological changes in maternal organs. Abnormal tissues were preserved at the discretion of the *post mortem* pathologist. The ovaries and uteri were examined immediately to determine the number of corpora lutea, number and distribution of live young, number and distribution of embryofoetal deaths.

Foetal observations
Embryofoetal deaths were classified as early (only placenta visible) or late (both placental and embryonic remnants visible). Uteri or individual uterine horns without visible implantations were immersed in a 10% solution of ammonium sulphide to reveal evidence of embryonic death at very early stages of implantation. Live young were examined externally, weighed and individual identified. Approximately half the foetuses in each litter were preserved in Bouin's solution for subsequent free-hand sectioning to discover visceral abnormalities (Wilson technique); the remainder were fixed in 74 OP industrial methylated spirit for subsequent macroscopic examination, evisceration, clearing and alizarin staining (modified Dawson technique) for skeletal examination. Young showing suspected abnormalities were processed by the more appropriate technique for clarification of initial observations. All foetuses were sexed by gonadal inspection following preservation.

Statistics
Group values: Group mean values, except for food and water consumption, were calculated using only animals with live young at termination. As animals were group-housed, food and water consumption values

are based on all animals regardless of pregnancy status.

Statistical analyses were routinely performed on litter data. Significance tests were normally two-tailed. The basic sample unit was the litter. Mean values of litter size, pre- and post-implantation loss, litter weight, mean pup weight and the incidence of anomalous offspring were analysed by the Kruskal-Wallis test followed, if significant, by the non-parametric equivalent of Williams' test for intergroup comparison with the control. Where 75 % of the values for a given variable consisted of one value. A Fisher's exact test was used.

II. RESULTS AND DISCUSSION

A. MORTALITY

At 3500 mg/kg bw/day, one female was sacrificed on Day 10 of pregnancy immediately after dosing following a probable intubation error (white fluid was found in the thoracic cavity). A further two females were sacrificed on day 7 and 13, respectively, following signs of respiratory distress (noisy respiration/gasping). *Post mortem* observation did not reveal the cause of distress, but the marked signs were a continuation of the signs representative for this group and these two deaths are considered to be related to treatment. There were no further deaths.

B. CLINICAL OBSERVATIONS

Signs of reaction to treatment at 3500 mg/kg bw/day amongst survivors included post-dose salivation in all animals with wet coats observed in approximately half the animals. Loose faeces on the cage traypaper were seen from day 7 or 8 of pregnancy and persisted throughout the treatment period. Noisy respiration and/or gasping were observed in 17/22 females on one or more occasions. At 1000 mg/kg bw/day, two animals showed noisy respiration on one occasion. There were no clinical signs observed at 300 mg/kg bw/day.

Table 5.6.2.3-01: Glyphosate technical: Teratology study in the rat ([redacted], 1991): Summary of maternal performance and clinical signs

Clinical sign	Glyphosate technical (mg/kg bw/day)			
	0 (Control)	300	1000	3500
No. of animals in group	25	25	25	25
Non-pregnant	2	2	0	0
Sacrificed	0	0	0	3
With live young on Day 20	23	23	25	22
Clinical signs				
Salivation, occasionally	0	0	0	22
Loose faeces	0	0	0	22
Noisy respiration	0	0	2	15
Wet coats	0	0	0	13
Gasping	0	0	0	5
Area of hair loss/scabbing	2	3	3	0

C. BODY WEIGHT

At 3500 mg/kg bw/day, the rate of body weight gain was markedly reduced during the first two days of treatment when compared to the concurrent control values. Thereafter, apart from a slight reduction in the rate of body weight gain during days 12 to 14 of pregnancy, the rate of body weight gain was comparable with the controls, although absolute parity with the control group was not attained by day 20. At 1000 mg/kg bw/day, there was a marginal reduction in the rate of body weight gain during the first two days of treatment

when compared with the concurrent control group. Thereafter, the pattern of body weight change was similar to the controls. At 300 mg/kg bw/day, the pattern of body weight gain was generally similar to controls throughout.

Table 5.6.2-3: Glyphosate technical: Teratology study in the rat ([redacted], 1991): Mean body weights (g) and body weight gain (g) relative to start at treatment on Day 6

Parameter	Glyphosate technical (mg/kg bw/day)			
	0 (control)	300	1000	3500
Mated females	25	25	25	25
No of animals included in assessment	23	23	25	22
Body weights				
Day 1	194.5	196.6	196.9	199.6
Day 3	221.8	223.1	224.2	227.8
Day 6	250.5	254.0	253.3	257.8
Day 8	266.0	270.1	266.6	260.3
Day 10	282.6	287.0	283.6	275.0
Day 12	302.0	306.4	302.9	292.9
Day 14	319.1	324.5	319.1	303.4
Day 16	344.0	347.3	343.9	321.5
Day 18	376.5	378.2	374.9	354.8
Day 20	414.4	413.6	405.4	390.5
Body weight gain relative to Day 6				
Day 1	-56.0	-57.4	-56.4	-58.2
Day 3	-28.7	-30.9	-29.2	-30.0
Day 6	0.0	0.0	0.0	0.0
Day 8	15.5	16.0	13.3	2.5
Day 10	32.1	33.0	30.3	17.2
Day 12	51.5	52.4	49.6	35.1
Day 14	68.7	70.5	65.8	45.5
Day 16	93.6	93.3	90.6	63.7
Day 18	126.0	124.1	121.6	97.0
Day 20	163.9	159.5	152.1	132.6

D. FOOD CONSUMPTION

At 3500 mg/kg bw/day, food consumption was decreased throughout the treatment period, thereafter, food intake was comparable with controls to termination. At lower dosages, food consumption was comparable with controls throughout.

Table 5.6.2-4: Glyphosate technical: Teratology study in the rat ([redacted], 1991): Summary of food consumption (group means)

Parameter	Glyphosate technical (mg/kg bw/day)			
	0 (control)	300	1000	3500
Mated females	25	25	25	25
No. of cages	5	5	5	5
No. of animals	25	25	25	25+
Food consumption (g/rat/day) during				
Days 3-5	27	27	28	28

Parameter	Glyphosate technical (mg/kg bw/day)			
	0 (control)	300	1000	3500
Days 6-7	28	28	27	21
Days 8-9	29	30	29	25
Days 10-11	31	31	31	29
Days 12-13	31	30	30	25
Days 14-15	31	31	31	27
Days 16-17	35	34	36	36
Days 18-19	34	33	34	36

+ Initial group size, group size reduced by mortalities, see Mortality

E. NECROPSY

Among animals killed at day 20, the only macroscopic finding was hair loss/scabbing in occasional animals, including controls, and was not related to the test item.

Out of 25 mated females, 23, 23, 25 and 22 had live young at day 20 in the control, and 300, 1000 and 3500 mg/kg bw/day groups, respectively. There were no instances of total resorption in any group. There were no treatment-related effects on the implantation rate, embryonic or foetal losses. There was an apparent increase in pre-implantation loss at 3500 mg/kg bw/day but since treatment commences after implantation, this is not considered to be treatment-related.

F. FOETUSES

There were no significant differences in the mean number of litter size.

At 3500 mg/kg bw/day, both litter and mean foetal weights were reduced in comparison with controls with differences from control values for mean foetal weight attaining statistical significance. At lower dosages, the slight differences observed were not considered to be attributable to treatment.

There were no significant differences in foetal sex ratios between the control group and any of the treated groups.

Table 5.6.2-5: Glyphosate technical: Teratology study in the rat ([redacted], 1991): Litter data – group mean values

Observation	Glyphosate technical (mg/kg bw/day)			
	0 (control)	300	1000	3500
# Animals assigned (mated)	25	25	25	25
# Animals with live young on Day 20	23	23	25	22
#Pregnancy status not determined (intercurrent sacrifice)	0	0	0	3
Mean no. of corpora lutea	16.3	15.5	16.4	16.9
Mean no. of implantations	14.6	13.6	14.0	13.6
Pre-implantation loss (%)	10.0	13.1	14.6	19.3**
% of foetal resorption and death (post implantation loss)	6.1	7.3	5.7	3.6
Mean no. of live foetuses/litter	13.7	12.7	13.2	13.1
Mean Foetal Weight (g)	3.96	3.90	3.89	3.71**
Sex Ratio (% male foetuses)	50.9	45.5	47.5	45.5

** Statistically significant difference from control group mean (Kruskal-Wallis H-statistic followed, if significant, by intergroup comparison with the control (distribution-free Williams' test, significant at $p<0.01$)

Findings in external, visceral and skeletal examination:
A total of 1, 2, 1 and 3 foetuses in Groups 1 to 4 respectively were malformed; this low incidence does not suggest any treatment-related aetiology. The incidence of foetuses with visceral anomalies was low and did not indicate any adverse treatment-related effects. The only skeletal change clearly influenced by treatment was rib distortion (wavy ribs) for which the incidence was markedly increased at 3500 mg/kg bw/day. The marginally higher incidence at 1000 mg/kg bw/day was of uncertain relationship to treatment. The only other skeletal change considered worthy of mention was reduced ossification for which the incidence was higher in all treated groups compared to the concurrent control. However, as comparison with the background control data showed the incidence of these skeletal changes at 3500 mg/kg bw/day to be only slightly outside the expected range, and no clear dosage-response was apparent, the effect on these skeletal changes (reduced ossification) were not considered to be attributable to treatment with the test item.

Table 5.6.2-6: Glyphosate technical: Teratology study in the rat ([redacted], 1991): Summary of foetal malformations and anomalies

Observation	Glyphosate technical (mg/kg bw/day)			
	0 (control)	300	1000	3500
Malformations				
No. of foetuses examined	314	292	329	289
Total no. of foetuses with malformations (affected litters)	1 (1))	2 (2)	1 (1)	3 (2)
Mean % malformations	0.3	0.8	0.3	1.1
Background range (% affected foetuses)	0.3 – 1.7			
Visceral anomalies				
No. of foetuses examined	158	147	162	144
Total no. of foetuses with malformations (affected litters)	9 (8)	11 (8)	5 (4)	11 (10)
Mean % affected foetuses	5.8	6.9	2.7	7.6
Background range (% affected foetuses)	4.6 – 5.8			
Skeletal anomalies				
No. of foetuses examined	155	143	166	142
Total no. of foetuses with malformations (affected litters)	19 (11)	36 (16)	46 (19)	55 (19)
Mean % affected foetuses	11.7	22.6	28.4*	35.7**
Background range (% affected foetuses)	21.9 – 27.2			

* Statistically significant difference from control group mean (Kruskal-Wallis H-statistic followed, if significant, by intergroup comparison with the control (distribution-free Williams' test, significant at $p<0.05$)
** Statistically significant difference from control group mean (Kruskal-Wallis H-statistic followed, if significant, by intergroup comparison with the control (distribution-free Williams' test, significant at $p<0.01$)

Table 5.6.2-7: Glyphosate technical: Teratology study in the rat ([redacted], 1991): Summary of foetal skeletal and visceral malformations (group incidences)

Observation	Glyphosate technical (mg/kg bw/day)			
	0 (control)	300	1000	3500
No. of foetuses examined (no. of litters examined)	314 (23)	292 (23)	329 (25)	289 (22)
No. of foetuses affected (no. of litters affected)	1 (1)	2 (2)	1 (1)	3 (2)
	Skeletal and visceral malformations			
Description	**Incidence**#			

Table 5.6.2-7: Glyphosate technical: Teratology study in the rat ([redacted], 1991): Summary of foetal skeletal and visceral malformations (group incidences)

Observation	Glyphosate technical (mg/kg bw/day)			
	0 (control)	300	1000	3500
Cranial				
Microphthalmia	0 (0)	1 (1)	0 (0)	0 (0)
Palatine irregularity	0 (0)	0 (0)	0 (0)	2 (2)[b,c]
Naso-pharangeal fistula	0 (0)	0 (0)	0 (0)	1 (1)[b]
Cervical				
Multiple vertebral irregularities	0 (0)	0 (0)	0 (0)	1 (1)
Connected 5th to 6th vertebral arches	0 (0)	0 (0)	0 (0)	1 (1)[c]
Thoracic				
Interventricular septal defect	0 (0)	0 (0)	1 (1)[a]	1 (1)[b]
Abdominal/Lumbar				
Marked distension of urinary bladder	1 (1)	0 (0)	0 (0)	0 (0)
Absent cleft of median liver lobe	0 (0)	0 (0)	1 (1)[a]	0 (0)
Other				
Termination of vertebral column (sacral) - includes anury	0 (0)	1 (1)	0 (0)	0 (0)

Individual foetuses may occur in more than one category, superscripts refer to the same foetus

Table 5.6.2-8: Glyphosate technical: Teratology study in the rat ([redacted], 1991): Summary of foetal visceral anomalies (group incidences)

Observation	Glyphosate technical (mg/kg bw/day)			
	0 (control)	300	1000	3500
No. of foetuses examined (no. of litters examined)#	158 (23)	147 (23)	162 (25)	144 (22)
No. of foetuses affected (no. of litters affected)#	9 (8)	11 (8)	5 (4)	11 (10)
	Visceral anomalies			
Description	**Incidence***			
Subcutaneous/subdural haemorrhage: trunk, tail, limbs, head	3 (3)	3 (3)	1 (1)	0 (0)
Cranial				
Small eye	0 (0)	1 (1)	0 (0)	0 (0)
Moderate haemorrhage anterior chamber of eye	0 (0)	0 (0)	0 (0)	1 (1)
Cervical				
Reduced side of thyroid	0 (0)	0 (0)	0 (0)	1 (1)
Thoracic				
Anomalous cervicothoracic arteries	0 (0)	1 (1)	0 (0)	0 (0)
Interventricular septal defect (small)				
Abdominal/Lumbar				
Intra-abdominal haemorrhage	0 (0)	0 (0)	2 (2)	1 (1)
Abdominal lobation of liver	2 (2)	2 (2)	0 (0)	4 (3)
Increased dilation of renal pelvis/ureter				
Displaced testis	0 (0)	0 (0)	0 (0)	1 (1)

Malformed foetuses are excluded
* Individual foetuses may occur in more than one category

Table 5.6.2-9: Glyphosate technical: Teratology study in the rat ([redacted], 1991): Summary of skeletal anomalies (group incidences)

Observation	Glyphosate technical (mg/kg bw/day)			
	0 (control)	300	1000	3500
No. of foetuses examined (no. of litters examined)#	155 (23)	143 (23)	166 (25)	144 (22)
No. of foetuses affected (no. of litters affected)#	19 (11)	36 (16)	46 (19)	57 (18)
Description	**Incidence##**			
Reduced ossification of:				
one or more cranial centres	3 (3)	2 (2)	12 (8)	10 (5)
cervical vertebral arches	0 (0)	0 (0)	1 (1)	0 (0)
sacro-caudal vertebral arches	3 (2)	8 (6)	17 (11)	15 (10)
one or more cantres of pelvic girdle	0 (0)	3 (3)	4 (3)	6 (4)
Cranial				
Area of ossification within suture	0 (0)	1 (1)	1 (1)	0 (0)
Cervical				
Cervical ribs	0 (0)	2 (2)	0 (0)	1 (1)
Thoracic				
Irregular ossification of vertebral centre	2 (2)	1 (1)	3 (3)	3 (3)
Distortion of ribs	1 (1)	0 (0)	3 (2)	28 (11)
Shortened 13th rib(s)	0 (0)	0 (0)	2 (1)	0 (0)
Lumbar				
Lumbar rib(s)	13 (8)	24 (13)	22 (12)	24 (12)
One extra thoracolumbar vertebra	1 (1)	2 (2)	0 (0)	6 (3)

\# Malformed foetuses are excluded
\## Individual foetuses may occur in more than one category

III. CONCLUSIONS

Within the context of this study, glyphosate shows obvious effects on maternal parameters and embryofoetal development at 3500 mg/kg/day (although does not induce malformations), and shows slight maternal effects at 1000 mg/kg/day. It can be concluded that glyphosate shows no selective embryofoetal toxicity and that the no-effect-level for embryofoetal development is 1000 mg/kg/day.

3. Assessment and conclusion

Assessment and conclusion by applicant:

In this developmental toxicity study according to OECD 414, the maternal NOAEL was set at 300 mg/kg bw/day based on mortality, clinical signs, decreased body weight gain and reduced food and water intake indicating a clear adverse effect of glyphosate administration at 3500 mg/kg bw/day and a possible impact at 1000 mg/kg bw/day with noisy respiration and decreased body weight gain. There was no evidence of teratogenicity in this study. Secondary to maternal toxitcity, reduced foetal weight, delayed ossification and a lower number of viable foetuses was observed at the extremely high top dose of 3500 mg/kg bw/day and reduced/delayed ossification and increased incidence of skeletal variations were observed at 1000 and 3500 mg/kg bw/day. The developmental NOAELwas considered to be 300 mg/kg bw/day.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.6.2/004
Report author	[redacted]
Report year	1991
Report title	Teratogenicity study in Wistar rats
Report No	ES.883.TER-R
Document No	Not reported
Guidelines followed in study	Not stated but in general accordance with OECD Guideline 414, 1981
Deviations from current test guideline (OECD 414, 2018)	Duration of treatment was shorter than required. The following endpoints were not assessed: weight and histopathological changes of the thyroid glands of the dams, anogenital distance (AGD) in foetuses, indication of incomplete testicular descent/cryptorchidism in male foetuses. Blood samples from dams to assess thyroid hormones (T4, T3 and TSH) were not collected. Deviations from the current version of OECD 414 (2018) are basically due to the fact that the study was aligned to an older version of the OECD test guideline 414.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

1. Information on the study

Data point:	CA 5.6.2/005
Report author	[redacted]
Report year	1991
Report title	Amendment to final report - Teratogenicity study in Wistar rats
Report No	ES.883.TER-R
Document No	Not reported
Guidelines followed in study	Not stated but in general accordance with OECD Guideline 414, 1981
Deviations from current test guideline (OECD 414, 2018)	Duration of treatment was shorter than required. The following endpoints were not assessed: weight and histopathological changes of the thyroid glands of the dams, anogenital distance (AGD) in foetuses, indication of incomplete testicular descent/cryptorchidism in male foetuses. Blood samples from dams to assess thyroid hormones (T4, T3 and TSH) were not collected.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid

Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

A teratogenicity study was conducted as a limit test to evaluate the potential maternal and developmental toxicity of glyphosate technical in rats. The test substance was suspended in groundnut (peanut) oil and was administered orally, by gavage, to 20 mated female Wistar rats at a dose level of 1000 mg/kg bw/day on days 6 to 15 of gestation. Thirty control rats received the vehicle, refined groundnut (peanut) oil. Both groups received a dose volume of 5 mL/kg bw. The limit dose of 1000 mg/kg bw/day had been selected on the basis of preliminary tests which were also briefly described in the main study report. Investigations were carried out according to OECD guideline 414 (1981). Appropriate statistical methods were applied.

There was no evidence of maternal toxicity. Mortality and clinical signs of toxicity were not noted. Body weight, body weight gain and food intake were not impaired. There was no impact on reproduction indices or litter data. The incidence of foetal malformations was not increased in the treated group compared to the control. There was limited evidence of a higher incidence of delayed ossification (caudal vertebral arch, forelimb proximal and hindlimb distal phalanges) in the group receiving glyphosate. On the other hand, delayed ossification of other parts of the skeleton, in particular the skull, was more frequently seen in the control group. Thus, there was no clear and consistent impact of test compound administration on the process of ossification.

Glyphosate technical, when administered at a limit dose of 1000 mg/kg bw/day from day 6 to 15 of gestation did not induce maternal or embryofoetal toxicity and no teratogenic potential was observed.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material:	Glyphosate technical
Description:	Solid white coloured crystal, odourless
Lot/Batch #:	60
Purity:	98.6 %
Stability of test compound:	Minimum 2 years under normal conditions
Vehicle:	Postman brand refined groundnut (peanut) oil
Test Animals:	
Species:	Rat
Strain:	Wistar
Source:	[redacted]
Age:	approx.18-20 weeks
Sex:	Females
Weight at dosing:	199 - 200 g (mean group values)
Acclimation period:	At least 10 days
Diet/Food:	Pelleted rat feed from M/S Lipton India Ltd., *ad libitum*
Water:	Protected, deep borewell water, passed through activated charcoal filter and exposed to UV rays in Aquagard on-line water filter cum-purifier, provided in glass bottles, *ad libitum*

Housing:	After mating: single housing in polypropylene rat cages; clean sterilised paddy husk bedding.
Environmental conditions:	Temperature: 22 ± 3 °C
	Humidity: 67 %
	Air changes: 10-15 per hour
	12 hours light/dark cycle

B. STUDY DESIGN

In life dates: November 1990 to December 1990

Mating procedure: In the final study randomly selected 1 male: 3 females were cohabited overnight. When a vaginal plug or spermatozoa were found in the vaginal smear the next morning, that day was considered day 0 of pregnancy.

Animal assignment: The day '0' pregnant rats were assigned to a single group until there were 35 rats in 0 mg/kg group and 25 rats in the 1000 mg/kg bw/day group.

Dose selection rationale: The dose levels selected for this study were based on two dose range finding studies, one in non-pregnant and one in pregnant rats. In the first study, groups of 3 non-pregnant rats were treated orally, by gavage, at 100, 400 and 1000 mg/kg bw/day for 10 consecutive days. Three female rats received the vehicle only, and served as the control group. No mortalities occurred during this study. No clinical signs were noted in the control and low dose animals. At 400 mg/kg bw/day one animal appeared emaciated; at 1000 mg/kg bw/day emaciation, urinary incontinence and red nasal discharge were noted for one animal. Body weight gain was unaffected up to and including 400 mg/kg bw/day but was slightly reduced at 1000 mg/kg bw/day (- 5.1% compared to the control).
Feed intake was not affected up to 1000 mg/kg bw/day.

In the second range finding study, 5 mated rats were treated orally, by gavage, at 1000 mg/kg bw/day from day 6 until day 15 of gestation. Historical control data from 70 animals were used as reference data. No mortalities occurred during this study. At 1000 mg/kg bw/day, urinary incontinence was noted in two animals and loose stool was noted for one animal. Body weight gain in test item-treated animals was slightly reduced (67.2 % compared to 75.0 % in the historical control), while feed intake was similar to that of the historical control (13.5 g/rat/day versus 15.0 g/rat/day in the control). At caesarean section on day 20 of gestation no effects of treatment were noted.

Dose preparation and analyses: A known amount of test compound was weighed, ground using a mortar and pestle and suspended in a known volume of the groundnut oil to get the concentrations of 0 and 1000 mg/kg in 5 mL of the vehicle. Fresh suspension of the test compound was prepared for administration each day. Analysis of dose formulations is not reported.

Dose administration: The test substance was administered orally by gavage.

Maternal Observations:
Clinical observations: Each female was observed twice daily for mortality, obvious changes and reaction to treatment.

Body weight: Individual body weights were recorded of all rats prior to mating. Mated rats were weighed on day 0 and daily from day 6 to 15 and on day 20. From these values the body weight gain was calculated for the following intervals: days 0-6, 6-15, 15-20 and 0-20. Corrected body weight was derived by subtracting gravid uterus weight from day 20 body weight.

Food consumption: Food consumption of each female was determined at intervals of days 0-6, 6-16, and

16-20 and throughout gestation day(days 0-20).

Necropsy: All females included in the study, dead moribund and terminally sacrificed were subjected to gross pathology. Tissues with gross lesions were preserved for histopathological examination if deemed necessary. On day 20 all surviving rats were sacrificed under ether anaesthesia and subjected to caesarean section. The abdomen was opened, and the uterus exposed. The uterus along with the ovaries was excised and weighed. The following observations were made: uterine weight, number of corpora lutea, number of implantations, embryonic and foetal resorptions.

Foetal observations: The following foetal data were recorded: total number of foetuses, number of dead foetuses, number of abnormal foetuses, number of live foetuses (including foetal weight), number of male foetuses, number of male foetuses, sex ratio. All foetuses were examined for external malformations, sex and weight. Afterwards foetuses were killed using excess ether anaesthesia. Approximately half the foetuses in each litter were fixed in 70 % ethanol and examined by modified Wilson's technique. Visceral malformations recorded were categorised as normal variants, minor and major malformations. The remaining half of the foetuses were fixed in 70 % ethanol, skinned, eviscerated, dehydrated in 95 % ethanol, macerated in 2 % KOH and stained with Alizerin Red-S in Mall's solution. The stained foetuses were cleared in grades of glycerol and evaluated for skeletal malformations. The malformations recorded were classified as normal variants, minor and major malformations.

Statistics: For statistical analysis the litter was used as the basic sampling unit. The following statistical methods were used:
1) Maternal body weight and body weight gain, food intake, no. of corpora lutea, no. of implantations, mean foetal weight by Bartlett's test followed by ANOVA and Dunnett's test with statistical significance at P=0.05.
2) Day 0 and absolute body weight by paired t-test.
3) Litter size by Student's t-test
Sex ration, no. of rats with resorptions, no. of rats with complete resorption, incidence of malformations by 2 x 2 contingency table.
Results of statistical analyses are designated as significantly higher (+) /lower (-) than control value at P=0.05.

II. RESULTS AND DISCUSSION

A. MORTALITY
No mortality occurred in this study.

B. CLINICAL OBSERVATIONS
Common clinical signs seen in a few animals in the study groups were: weakness and dullness, soft faeces, red coloured nasal discharge, snuffling, lacrimation and urinary incontinency. Their incidence was similar in both the control and test item group.

Table 5.6.2-10: Glyphosate technical: Teratogenicity study in Wistar rats ([redacted] 1991): Summary of clinical signs

Clinical sign	Glyphosate technical (mg/kg bw/day)	
	0 (control)	1000
No. of animals in group	30	20
Mortality	0	0
Clinical signs		
Weak, dull	2	0
Soft faeces	2	2

Nasal discharge	2	4
Red coloured nasal discharge	2	0
Lacrimation	0	1
Urinary incontinency	1	1
Snuffling	6	3

C. BODY WEIGHT

There were no statistically significant differences in body weight throughout the study. The mean body weight gain during pre-treatment, treatment, post-treatment or the overall gestation period did not show any statistically significant differences between the control and the treated group. Similarly, corrected body weight gain at the end of gestation was significantly increased (P=0.01) in both groups compared to day 0 weights.

Table 5.6.2-11: Glyphosate technical: Teratogenicity study in Wistar rats ([redacted] 1991): Mean body weights and corrected body weight gain (g) relative to start at treatment on day 6 (group means ± SD)

Parameter	Glyphosate technical (mg/kg bw/day)	
	0 (control)	1000
Mated females	35	25
No. of animals included in assessment (pregnant at term)	30	20
Body weights at		
Day 0	186 ± 18.5	188 ± 13.9
Day 6	199 ± 18.2	200 ± 16.5
Day 15	218 ± 19.7	220 ± 17.6
Day 20	258 ± 27.2	253 ± 30.2
Corrected body weight gain (Day 20 bw – uterine weight)	208 ± 23.3	210 ± 21.6

D. FOOD CONSUMPTION

There were no intergroup differences in food consumption between treated and control groups. Statistically significantly lower food consumption was noted in the treated group during the pre-treatment period.

Table 5.6.2-12: Glyphosate technical: Teratogenicity study in Wistar rats ([redacted] 1991): Summary of food consumption (group means ± SD)

Parameter	Glyphosate technical (mg/kg bw/day)	
	0 (control)	1000
Mated females	35	25
No of animals included in assessment (pregnant at term)	30	20
Food consumption (g/rat/day) during		
Days 0-6	17 ± 2.1	15 ± 2.4*
Days 6-16	15 ± 1.9	15 ± 2.0
Days 16-20	17 ± 3.9	17 ± 3.1
Days 0-20	16 ± 1.9	16 ± 2.1

* Significantly lower compared to the control (Dunnett's test, P=0.05)

E. NECROPSY

Dams necropsied during the study showed stray incidence of petechiae, emphysema, congestion, focal consolidation of lung and higher incidence of mottled liver. These incidences did not show any relationship with the test item.

The number of corpora lutea, implantations, embryonic and foetal resorptions, pre-implantation and post-implantation loss were similar in both groups. The number of dams with any resorption was lower in the treated group than in the control, which was considered an incidental finding.

Table 5.6.2-13: Glyphosate technical: Teratogenicity study in Wistar rats ([redacted] 1991): Summary of maternal performance and litter data (group means ± SD)

Observation	Glyphosate technical (mg/kg bw/day)	
	0 (control)	1000
No. animals assigned (mated)	35	25
No. animals with live young on Day 20	30	20
No. pregnancy status not determined (intercurrent sacrifice)	0	0
No. of corpora lutea	11 ± 1.7	11 ± 2.0
No. of implantations	10 ± 2.5	9 ± 3.2
Pre-implantation loss (%)	38 (13)	35 (20)
No. of embryonic resorptions (%)	24 (8)	19 (11)
No. of foetal resorptions (%)	0 (0)	0 (0)
Post implantation loss (%)	24 (8)	19 (11)
No. of dams with any resorptions (%)	15 (50)	11 (55)
No. of dams with total resorption (%)	0 (0)	0 (0)
Total no. of foetuses	261	157
Mean litter size	9	8
No. of dead foetuses/litter	0	0
No. of abnormal foetuses	0	0
Mean foetal weight (g)	3.6 ± 0.4	3.7 ± 0.3
Foetal sex ratio (male:female)	1:1	1:1.3

F. FOETUSES

There were no significant differences in litter size or the incidence of dead or abnormal foetuses.

No differences between the test item group and the control were noted for foetal body weights or foetal sex ratios.

Findings in external, visceral and skeletal examination:

At external examination, the incidence of normal variations like haemorrhagic spots was slightly higher in the treated group than in the control. However, the difference was not statistically significant. Incidences of minor malformations like small foetus was noted in both groups. There was one incidence of a foetus with short snout in the control group. No incidence of major external findings was noted in either of the study groups.

Table 5.6.2-14: Glyphosate technical: Teratogenicity study in Wistar rats ([redacted] 1991): Summary of foetal external findings (% foetuses affected)

Observation	Glyphosate technical (mg/kg bw/day)	
	0 (control)	1000
No. of foetuses examined	261	157
No. of litters examined	30	20

Variations		
Haemorrhagic spot	5.4	10.2
Minor Malformations		
Foetus small	0.8	0.6
Snout short	0.4	0.0
Major Malformations		
No. of foetuses with major malformations	0	0

Visceral examination and intergroup statistical comparison of visceral findings revealed that the incidence of variations like tortuous ureter was noted in the control and treated group. A few incidences of minor malformations seen in both the control and treated group were unascended kidney, slight renal pelvis dilation, unusual lung lobulation, retinal folding, displacement of umbilical artery and displacement of vitelline vein. None of these observations reached the level of statistical significance. No major visceral malformations were noted.

Table 5.6.2-15: Glyphosate technical: Teratogenicity study in Wistar rats ([redacted] 1991): Summary of foetal visceral findings (% foetuses affected)

Observation	Glyphosate technical (mg/kg bw/day)	
	0 (control)	1000
No. of foetuses examined	122	74
No. of litters examined	30	20
Variations		
Ureter - tortuous	35.3	31.1
Minor Malformations		
Testis - undescended	1.6	0.0
Renal pelvis – slight dilation	5.7	4.0
Uterus - short	1.6	0.0
Lung – unusual lobulation	0.0	1.4
Retinal folding	0.8	0.0
Umbilical artery - displaced	0.8	0.0
Vitelline vein - displaced	0.0	1.4
Major Malformations		
No. of foetuses with major malformations	0	0

The incidence of variations like delayed ossification was noted in many bones, both in the control and treated group. However, the incidence of delayed ossification of caudal vertebral arch, forelimb-proximal phalanx and hindlimb-distal phalanx was significantly higher in the treated group compared to the control. However, the incidence of incomplete to partial ossification of parietal and interparietal of the skull was less in the treated group. The incidence of minor and major skeletal malformations present to a small extent in both groups, did not show any statistically significant intergroup differences.

Table 5.6.2-16: Glyphosate technical: Teratogenicity study in Wistar rats ([redacted] 1991): Summary of foetal major skeletal malformation findings (% foetuses affected)

Observation		Glyphosate technical (mg/kg bw/day)	
		0 (control)	1000
No. of foetuses examined		140*	83
No. of litters examined		30	20
Major Malformations			
Malformed	Supraoccipital bone	0.7	0.0
	Parietal bone	0.7	2.4

	Interparietal bone	2.1	3.6
	Temporal bone	0.7	1.2
	Basisphenoid	1.4	1.2
	Sternebra #6	0.0	1.2
	Cervical vertebral arch # 3-7	0.7	0.0
	Scapula	0.7	0.0
Incomplete	Palatine	0.0	2.4
	Zygomatic arch	2.1	0.0
	Cervical vertebral arch #5	0.7	0.0
	Cervical vertebral arch #6	1.4	0.0
Fused	Sternebrae #1-6	0.7	0.0
No. of foetuses with major malformations		17	10
No. of dams with foetuses with major malformations		8	6

* One foetus noted with short snout during visceral examination was also evaluated for skeletal changes

III. CONCLUSIONS

Glyphosate technical, when administered at a limit dose of 1000 mg/kg bw/day from day 6 to 15 of gestation did not induce maternal or embryofoetal toxicity and no teratogenic potential was observed. Accordingly, the NOAEL for maternal and embryofoetal toxicity was 1000 mg/kg bw/day.

5. Assessment and conclusion

Assessment and conclusion by applicant:

This study (performed according to OECD 414) showed no evidence of maternal toxicity. The incidence of foetal malformations was not increased in the treated group compared to the control. There was limited evidence of a higher incidence of delayed ossification (caudal vertebral arch, forelimb proximal and hindlimb distal phalanges) in the group receiving glyphosate. On the other hand, delayed ossification of other parts of the skeleton, in particular the skull, was more frequently seen in the control group. Thus, there was no clear and consistent impact of test compound administration on the process of ossification. Thus, the NOAEL for both maternal and developmental toxicity is assumed to be 1000 mg/kg bw/day.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	**CA 5.6.2/006**
Report author	[redacted]
Report year	1986
Report title	Report on effect of glyphosate technical of Excel Industries Ltd. Bombay, India; on reproductive process. Segment II – Teratological study
Report No	Not indicated
Document No	Not indicated
Guidelines followed in study	Not mentioned in Monograph (2000)
GLP	No (not compulsory at that time)

Previous evaluation	Yes. The study was evaluated and not accepted (Monograph 2000).
Short description of study design and observations:	20 mated female Wistar rats per group were administered glyphosate (Excel Industries Ltd. Bombay, India; purity not reported) at dose levels of 0 (control, vehicle not stated), 100 and 500 mg/kg bw/day on gestational days 6 through 15 by gavage. Dams were sacrificed and foetuses delivered on day 20.
Short description of results:	Neither maternal nor reproduction and embryo-foetal effects were observed up to the highest dose tested. Thus, the NOEL for both maternal and developmental toxicity was 500 mg/kg bw/day in this study.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	The study was considered not acceptable due to several serious reporting deficiencies.
Reasons why the study report is not available for submission	The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance" (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL
Category study in AIR 5 dossier (L docs)	Category 4b

2. Information on the study

Data point:	**CA 5.6.2/007**
Report author	[redacted]
Report year	1981
Report title	Teratological investigation of glyphosate in rats and rabbits
Report No	Not stated
Document No	Not stated
Guidelines followed in study	Not stated (pre-guideline)
Deviations from current test guideline (OECD 414, 2018)	Treatment duration was slightly shorter than required; group size smaller than required; missing endpoints: no data on food consumption reported, weight and histopathological changes of the thyroid glands of the dams, anogenital distance (AGD) in foetuses, indication of incomplete testicular descent/cryptorchidism in male foetuses, blood samples from dams to assess thyroid hormones (T4, T3 and TSH) were not collected;, no data on results of foetal or visceral skeletal examination
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, not conducted under GLP (GLP was not compulsory at the time the study was performed)
Acceptability/Reliability:	Invalid
Category study in AIR 5	Category 3b

dossier (L docs)	

3. Full summary

In this developmental toxicity study, groups of 12-15 time-mated female CFY rats were administered glyphosate from Days 6 to 18 of gestation as a dietary admix. In both, the control and high dose group 10 additional females were treated and allowed to give birth and raise their litters until day 28 post partum ('postnatal group'). Concentrations of glyphosate in the diet are not reported, but from Days 6 to 18 of gestation admix of the test item was reported to result in to test item intakes of 22, 103 and 544 mg/kg bw/day, respectively. For the high dose postnatal females a test item intake of 558 mg/kg bw/day was reported. Females of the 'prenatal group' were sacrificed in a CO_2 chamber on Day 20 of gestation. After opening the uterine horns the total number of implantations, of live, dead and resorbed embryos/foetuses were counted. The mean litter and foetal weights, gestational growth, the mean body weight gain and food consumption were calculated. Live foetuses in the prenatal groups were fixed and examined for visceral and skeletal abnormalities. The following foetal organs were examined histologically: heart, lungs, thymus, liver, intestines, pancreas, spine, skeletal muscles, intervertebral discs and nervous tissue.

Females of the postnatal group and their offspring were sacrificed on day 28 post partum. Gross and histopathology was conducted on each animal. The following organs were histopathologically examined: heart, lungs, thymus, stomach, liver, kidney, spleen and reproductive organs.

Treatment with glyphosate had no effect on the maternal organism. Body weight was similar in the treated groups, body weight gain in the mid and high dose group was slightly lower than in controls throughout pregnancy, gross examination and histopathology carried out on foetuses of the 'prenatal group' indicated no changes in any group. In the postnatal group no toxic symptoms or gross pathology alterations were observed in high dose or control females; and no macroscopic or microscopic changes were noted in their offspring.

Based on these results the non-teratogenic level is larger than 544 mg/kg bw/day.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material:

Identification:	Glyphosate /active principle
Description:	White odourless adhesive powder
Lot/Batch #:	Not stated
Purity:	96.8 %
Stability of test compound:	Not stated
Vehicle:	Laboratory rodent food

Test Animals:

Species:	Rat
Strain:	CFY
Source:	Not stated
Age:	Not stated
Sex:	Female
Weight:	210-275 g (group mean values)

Acclimation period:	Not stated	
Diet/Food:	Laboratory rodent food, *ad libitum* (before and after the treatment period)	
Water:	*ad libitum*	
Housing:	Individually, in plastic cages	
Environmental conditions:	Temperature:	22 ± 2 °C
	Humidity:	55-75 %
	Air changes:	8 per hour
	Light regime	12/12 hours

B. STUDY DESIGN

In-life dates
Not stated.

Mating procedure
In this developmental toxicity study two female rats and one male rat were housed in the same cage for mating; the males were changed every second day. To detect mating the presence of sperm in native vaginal smears was checked daily; the presence of vaginal plugs was recorded.

Animal assignment
Groups of 12 - 15 mated female CFY rats were assigned to four dose groups in the prenatal part of the study. An additional 10 females were added to the control and high-dose groups and were assigned to be the postnatal part of the study.

Dose selection rationale
Not provided.

Dose preparation and analyses
Not provided.

Dose administration
Treatment was via the dietary route. Concentrations of glyphosate in the diet are not reported, but from days 6 to 18 of gestation admix of the test item had reported intakes of 22, 103 and 544 mg/kg bw/day, respectively. For the high-dose postnatal females a test item intake of 558 mg/kg bw/day was reported.

Maternal Observations:
Clinical observations: Not stated.

Body weight
Individual body weights were generally recorded daily during gestation and lactation. However, not on every day body weights were recorded for all animals.

Food consumption
Not stated.

Necropsy

Prenatal group
Females were sacrificed in a CO_2 chamber on day 20 of gestation. After opening the uterine horns the total number of implantations, of live, dead and resorbed embryos/foetuses were counted. The mean body weight

gain and food consumption were calculated.

Postnatal group
Females and their offspring were sacrificed on day 28 *post partum*. Gross and histopathology was conducted on each animal.

Foetal observations
The mean litter and foetal weights and gestational growth were recorded. Half of the live foetuses in the prenatal groups were fixed in 4 % neutral formalin and embedded in paraffin. Sagittal sections of 5-8 µm were prepared. Cerebral, abdominal, thoracal morphological abnormalities were examined by Wilson technique. In the other half of foetuses, skeletal abnormalities were examined according to Simon and Hern (1971). The following foetal organs were examined histologically in the prenatal group: heart, lungs, thymus, liver, intestines, pancreas, spine, skeletal muscles, intervertebral discs and nervous tissue. The following foetal organs were histopathologically examined in the postnatal group: heart, lungs, thymus, stomach, liver, kidney, spleen and reproductive organs.

Statistical analyses
Group means and deviations were calculated according to Finney (1952).

II. RESULTS AND DISCUSSION

A. MORTALITY
None.

B. CLINICAL OBSERVATIONS
No effect on behaviour or appearance was observed in either the prenatal or postnatal dams.

C. BODY WEIGHT
Body weight gain was similar in the treated prenatal groups compared to control; when the gestation period (day 1-20) is considered the body weight gain in the mid- and high-dose groups were slightly below the control level. In the postnatal group the body weight gain was similar in the treated high-dose group compared to control (when using the recalculated value).

Table 5.6.2-17: Glyphosate technical: Teratological investigation of glyphosate in rats and rabbits ([redacted] 1981): Summary of body weight data (group means) - Prenatal group

Parameter	Glyphosate (mg/kg bw/day)			
	0 (control)	22	103	544
Mated females	13	12	15	15
Body weights (g) at				
Day 6	255	261	280[1]	280
Day 10	275	276	294	295
Day 19	304	309	331	338
Body weight gain day 1-20 (g)	81	85	71	68

[1] No value was given for Day 6, this is the mean for Day 7

Table 5.6.2-18: Glyphosate technical: Teratological investigation of glyphosate in rats and rabbits ([redacted], 1981): Summary of body weight data (group means) – Postnatal group

Parameter	Dose Group (mg/kg bw/day)			
	0 (control)	22	103	558

Mated females	10	0	0	10
Body weights (g) at				
Day 6 post coitum	266			262
Day 11 post coitum	295			297
Day 16 post coitum	328			338
Day 19 post coitum	310			344
Body weight gain Day 1-21 post coitum (g)	123			117[1]
Day 0/1[2] post partum	278			293
Day 8/9[2] post partum	288			311
Day 14/15[2] post partum	299			313
Day 21/22[2] post partum	274			305
Day 28 post partum	275			295

[1] in the report, an obviously wrong value of 73 g is given; recalculation resulted in the presented value
[2] weighing day for the control/weighing day for the high dose

D. FOOD CONSUMPTION
Not reported.

E. NECROPSY
No macroscopic changes were noted in the control and glyphosate-treated females in either the prenatal or postnatal groups.

F. FOETUSES
A total of 13, 12, 15 and 15 pregnant females from the prenatal group survived to termination and 134, 134, 192 and 185 live foetuses were recorded for the 0 (control), 22, 103 and 544 mg/kg bw/day dose groups, respectively. There were no significant intergroup differences in the numbers of implantations (see table below). The incidence of dead foetuses was slightly higher in the mid-dose group than in the controls but without a dose response. The percentage of foetal loss was highest in the control group while for the high-dose group the lowest values were noted. No total litter loss was recorded in any group. Litter size at caesarean necropsy was comparable in all treatment groups.

Table 5.6.2-19: Glyphosate technical: Teratological investigation of glyphosate in rats and rabbits ([redacted] 1981): Summary of litter parameters – Prenatal group

Parameter	Glyphosate (mg/kg bw/day)			
	0 (control)	22	103	544
No. of mated females	13	12	15	15
Mean No. of implantations/dam	12.08	12.58	14.67	13.00
Mean No. of dead foetuses/dam	1.77	0.67	1.87	0.67
Foetal loss (%)	14.65	5.30	12.73	5.13
Mean No. of live foetuses/dam	10.31	11.92	12.80	12.33
Mean litter weight (g)	25.46	28.23	28.13	30.53
Mean foetal weight (g)	14.65[1]	5.30[1]	2.07[2]	2.50[2]
Externally abnormal foetuses	0	0	0	0

[1] values as given in the report, but unrealistic for rat foetuses at term, in looking at the table in the report it appears the values for foetal loss (%) were accidently repeated here.
[2] values as given in the report, but untypically low for rat foetuses at term

External, visceral and skeletal examination
No externally abnormal foetuses were noted in any prenatal group. No treatment related skeletal or visceral effects were observed.

Postnatal group
Pup growth during lactation was faster than in the controls. No treatment related micro- or microscopic effects were observed. The lactation index was 97% in both the treated and control group.

III. CONCLUSIONS

No treatment related effects were observed in the dams. Body weight was similar in the treated groups, the body weight gain in the mid- and high-dose groups was slightly lower than in controls throughout the pregnancy. No gross or histopathological effects were observed in the pre- and postnatal groups. No treatment related effects were observed with foetuses or neonates. Based on these results the non-teratogenic level is larger than 544 mg/kg.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The NOAEL for maternal as well as development toxicity was considered to be 544 mg/kg bw/day based on no evidence of maternal or developmental toxicity attributable to glyphosate in either the pre- or postnatal groups.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	**CA 5.6.2/008**
Report authors	[redacted]
Report year	1980
Report title	Teratology study in rats
Report No	401-054
Document No	M-644179-02-1
Guidelines followed in study	Study was performed before adoption of OECD Guideline, but in general accordance with OECD Guideline 414 (1981)
Deviations from current test guideline (OECD 414, 2018)	The following endpoints were not assessed: food consumption, weight and histopathological changes of the thyroid glands of the dams, anogenital distance (AGD) in foetuses, indication of incomplete testicular descent/cryptorchidism in male foetuses, blood samples from dams to assess thyroid hormones (T4, T3 and TSH) were not collected. Deviations from the current version of OECD 414 (2018) are basically due to the fact that the study was aligned to an older version of the OECD test guideline 414.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, not conducted under GLP (GLP was not compulsory at the time the study was performed)
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Pregnant COBS® CD@ rats were used to determine the teratogenic potential of technical glyphosate. Dosage levels of 0, 300, 1000 and 3500 mg/kg bw/day were administered orally by gavage as a single daily dose on days 6 through 19 of gestation at a constant volume of 10 mL/kg. The control group received the vehicle, 0.5 % aqueous Methocel®, on a comparable regimen. Cesarean sections were performed on all surviving females on gestation day 20. Biologically meaningful differences in appearance and behavior were noted in the 3500 mg/kg bw/day dosage group when compared to the control group and included breathing rattles, reduced activity and an increase in soft stool and diarrhoea. Six animals in this dosage group died by gestation day 17. The causes of death could not be determined at necropsy. A reduced gain in mean maternal body weight was noted over the treatment period in the 3500 mg/kg bw/day dosage group, as well as an increase in early resorptions, a slight increase in post-implantation loss and decreases in mean foetal body weight and the mean number of viable foetuses. An increase in the number of foetuses with reduced ossification of the sternebrae was also noted in this dosage group. No signs of maternal or developmental toxicity were noted at 300 or 1000 mg/kg bw/day.

Treatment with glyphosate produced signs of maternal and foetal toxicity in the 3500 mg/kg/day dosage group, as evidenced by maternal deaths, an increase in early resorptions and decreases in mean foetal body weight and the mean number of viable foetuses. Glyphosate did not produce a teratogenic response when administered to rats at a dosage level of 3500 mg/kg bw/day or less.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material:	Glyphosate technical	
Description:	White powder	
Lot/Batch #:	XHJ - 64	
Purity:	98.7 %	
Stability of test compound:	Not reported	
Vehicle:	0.5 % aqueous Methocel®	
Test Animals:		
Species:	Rat	
Strain:	Charles River COBS® CD®	
Source:	[redacted]	
Age:	Approx. 14 weeks when mated	
Sex:	Females	
Weight at dosing:	288 - 302 g (mean group values)	
Acclimation period:	At least 10 days	
Diet/Food:	Purina® Rodent Laboratory Chow® 5001, *ad libitum*	
Water:	Tap water, *ad libitum*	
Housing:	Individually in suspended wire mesh cages	
Environmental conditions:	Temperature:	controlled, no values reported
	Humidity:	controlled, no values reported
	Air changes:	not reported
	Light:	controlled, no values reported

B. STUDY DESIGN

In life dates: 1979-04-16 to 1979-05-12

Mating procedure: One female and one male rat of the same strain were placed together for mating. The occurrence of copulation was determined by daily inspection for a copulatory plug or by a vaginal inspection for sperm. The day that mating was detected was designated day 0 of gestation.

Animal assignment: Four test groups were employed in the study. Mated females were consecutively assigned in a block design to a control group and three treatment groups consisting of 25 rats each.

Dose selection rationale: Dose selection rationale was not provided.

Dose preparation and analyses: The appropriate amount of test item was ground with mortar and pestle prior to daily suspension in the vehicle, 0.5 % aqueous Methocel® using a tissue homogenizer. A magnetic stir bar and plate were used to ensure proper suspension during administration. Analyses of dose formulations is not reported.

Dose administration: Glyphosate was administered orally, by gavage, to 25 mated female rats per group at dose levels of 0, 300, 1000 and 3500 mg/kg bw/day from day 6 to 19 of gestation. The dose volume was 10 mL/kg bw. Dose volumes were calculated from individual body weights on gestation day 6.

Maternal observations:
Clinical observations:
Prior to treatment, the females were observed daily for mortality and overt changes in appearance and behavior. The females were observed daily for mortality and clinical signs of toxicity on days 6 through 20 of gestation. Dams not surviving to the scheduled sacrifice were necropsied in an attempt to determine the cause of death.

Body weight: Individual maternal body weights were recorded on gestation days 0, 6, 9, 12, 16 and 20.

Food consumption: Food consumption was not recorded.

Necropsy: On gestation day 20, all surviving females were sacrificed by carbon dioxide inhalation. Immediately following sacrifice, the uterus was excised and weighed and the foetuses removed. The location of viable and nonviable foetuses, early and late resorptions and the number of total implantations and corpora lutea were recorded. The abdominal and thoracic cavities and organs of the dams were examined for grossly evident morphological changes and the carcasses discarded.

Foetal observations: All foetuses were individually weighed and examined for external malformations and variations, including the palate and eyes. Each foetus was externally sexed and individually identified. Approximately one-half of the foetuses were placed in Bouin's fixative for subsequent visceral examination by razor-blade sectioning as described by Wilson. The remaining one-half of the foetuses were fixed in alcohol, macerated in potassium hydroxide and stained with Alizarin Red S by a method similar to that described by Dawson for subsequent skeletal examination.

Statistics: All statistical analyses compared the treatment groups to the control group, with the level of significance at $p<0.05$. The male to female foetal sex distribution and the number of litters with malformations were compared using the Chi-square test criterion with Yates' correction for 2 x 2 contingency tables and/or Fisher's exact probability test as described by Siegel to judge significance of differences. The number of early and late resorptions and post-implantation loss were compared by the Mann-Whitney U-test as described by Siegel and Weil to judge significance of differences. The mean number of viable foetuses, total implantations, corpora lutea and mean foetal body weights were compared

by analysis of variance (one-way classification), Bartlett's test for homogeneity of variances and the appropriate t-test (for equal or unequal variances) as described by Steel and Torrie using Dunnett's multiple comparison tables to judge significance of differences.

II. RESULTS AND DISCUSSION

A. MORTALITY

Six rats in 3500 mg/kg bw/day group died, one each on gestation days 10 and 17 and two each on gestation days 11 and 12.

B. CLINCIAL OBSERVATIONS

There were no biologically meaningful differences in appearance or behaviour attributable to treatment with the test item at 300 and 1000 mg/kg bw/day when compared to the control group. Soft stool, diarrhoea or both were noted at least once during the treatment period (in all but three rats) at 3500 mg/kg bw/day, with diarrhoea occurring primarily either prior to death or during the last days of treatment. Breathing rattles and inactivity were noted only in rats at 3500 mg/kg bw/day and red matter in the region of the nose, mouth, forelimbs or dorsal head was noted prior to death. The inactivity was observed in all rats at 3500 mg/kg bw/day each day, beginning mid-way through the treatment period, approximately l/2 to 6 hours after dosing. Inactivity was not present at subsequent dosing until the last few days of treatment when animals were inactive before and after dosing.

C. BODY WEIGHT

There were no biologically meaningful differences in mean maternal body weight gain at 300 and 1000 mg/kg bw/day when compared to the control group. However, a definite reduced mean maternal body weight gain was noted at 3500 mg/kg bw/day over the treatment period due to a mean maternal body weight loss during the first three days of treatment.

Table 5.6.2-20: Glyphosate technical: Teratology study in rats ([redacted] 1980): Mean body weights (g ± SD) and body weight gain (g)

Parameter	Glyphosate technical (mg/kg bw/day)			
	0 (control)	300	1000	3500
Mated females	25	25	25	25
No of animals included in assessment (pregnant animals)	22	20	21	17*
Body weights (g)				
Day 0	270 ± 14.4	270 ± 22.8	274 ± 15.9	261 ± 16.8
Day 6	297 ± 16.9	295 ± 22.9	302 ± 18.5	288 ± 15.8
Day 9	305 ± 17.7	303 ± 23.7	307 ± 16.5	275 ± 28.4
Day 12	318 ± 19.3	316 ± 26.2	322 ± 17.7	299 ± 31.4
Day 16	350 ± 19.1	346 ± 32.6	352 ± 21.3	326 ± 26.3
Day 20	416 ± 23.1	403 ± 47.0	416 ± 22.1	373 ± 43.1
Corrected body weight (corrected for uterus weight)	336 ± 19.6	334 ± 27.9	335 ± 17.6	311 ± 32.9
Body weight gain (g)				
Days 0 - 6	27	25	28	27
Day 6 - 9	8	8	5	-13

Day 9 - 12	13	13	15	24
Day 12 - 16	32	30	30	27
Day 16 - 20	66	57	64	47
Day 0 - 20	146	133	142	112

* Initial group size of pregnant animals was n=23, further reduced in the course of the study due to 6 mortalities

E. NECROPSY

Stomach haemorrhages were noted in two deceased rats from the 3500 mg/kg group at necropsy, however, a cause of death could not be determined for any of the six deceased dams. At sacrifice on day 20, two incidences of hydronephrosis were found, one each at 1000 and 3500 mg/kg bw/day. One incidence of splenic necrosis and adhesive peritonitis was found at 300 mg/kg bw/day at histopathological examination.

Out of 25 mated females, 22, 20, 21 and 16 had live young at day 20 in the control, and at 300, 1000 and 3500 mg/kg bw/day groups, respectively. There were no biologically meaningful differences in the mean number of viable foetuses, late or early resorptions, post-implantation loss, corpora lutea, the foetal sex distribution or mean foetal body weight at 300 and 1000 mg/kg bw/day when compared to the control group and nonviable foetuses were not present in any group. A statistically significant decrease in the mean number of total implantations and viable foetuses at 300 mg/kg bw/day dosage group was attributed to random occurrence.

At 3500 mg/kg bw/day, there were no late resorptions although a statistically significant increase in the mean number of early resorptions resulted in a slight increase in mean post-implantation loss. A statistically significant decrease in the mean number of total implantations, viable foetuses and mean foetal body weight and a slight decrease in the mean number of corpora lutea was noted in this group. Since ovulation and implantation occurred prior to treatment, the decreases in total implantation and corpora lutea were considered not to be treatment related.

F. FOETUSES

Mean litter size was statistically significantly reduced at 300 and 3500 mg/kg bw/day which was mainly due to an incidentally lower number of corpora lutea and an incidentally higher incidence of pre-implantation loss. As discussed above these differences were considered not treatment related. Mean litter size at 1000 mg/kg bw/day was similar to that of the control group.

At 3500 mg/kg bw/day, mean foetal weights were reduced in comparison with controls with differences from control values attaining statistical significance. At 300 and 1000 mg/kg bw/day mean foetal weights were similar to those of the control group. No differences in foetal sex ratios were noted between test item-treated groups and the control group.

Table 5.6.2-21: Glyphosate technical: Teratology study in rats ([redacted], 1980): Litter data

Observation	Glyphosate technical (mg/kg bw/day)			
	0 (control)	300	1000	3500
Animals assigned (mated)	25	25	25	25
Non-pregnant animals	3	5	4	2
Dams with pregnancy status not determined (intercurrent death)	0	0	0	6
Dams with total resorption	0	0	0	1
Animals with live young on Day 20	22	20	21	16
Mean no. of corpora lutea ± S.D.	15.9 ± 1.67	15.2 ± 3.30	16.1 ±1.81	14.8 ± 1.64
Mean no. of implantations ± S.D.	15.0 ± 1.11	12.1 ± 4.45**	14.8 ± 2.21	12.8 ±3.77*

Mean post-implantation loss ± S.D.	0.6 ± 090	0.2 ± 0.52	0.5 ± 0.81	1.2 ± 1.25
Mean no. of live foetuses/litter ± S.D.	14.4 ± 1.26	11.9 ± 4.36*	14.3 ± 2.08	11.5 ± 4.12*
Mean Foetal Weight (g ± S.D.)	3.5 ± 0.21	3.7 ± 0.66	3.6 ± 0.19	3.2 ± 0.34**
Sex Ratio (% male foetuses)	50.3	50.2	56.0	49.5

* Statistically significant difference from control group mean, $p<0.05$
** Statistically significant difference from control group mean, $p<0.01$

Findings in external, visceral and skeletal examination:
There were no malformations observed at 300 and 1000 mg/kg bw/day. At 3500 mg/kg bw/day, the number of litters with malformations was identical to the control group, however, several foetuses with either the anomaly classified as dwarfism or bent tails were found in single litters. As a result, an increase in the number of foetuses with malformations was noted in this group when compared to the control group. These increases were considered genetic in origin as bent tail and dwarfism occurred in several foetuses in a single litter in the historical control data. Developmental and genetic variations at 300 and 1000 mg/kg bw/day were comparable to the control group. An increase in the number of litters and foetuses with unossified sternebrae was noted in the 3500 mg/kg bw/day dosage group and was considered a developmental variation.

Table 5.6.2-22: Glyphosate technical: Teratology study in rats ([redacted], 1980): Summary of foetal malformations and developmental and genetic variations

Observation	Glyphosate technical (mg/kg bw/day)			
	0 (control)	300	1000	3500
No. of litters examined	22	20	21	16
No. of foetuses examined externally	316	237	300	196
No. of foetuses examined viscerally	155	119	150	97
No. of foetuses examined skeletally	161	118	150	99
Total % foetuses with malformations (% affected litters)	0.9 (13.9)	0 (0)	0 (0)	5.1 (16.8)
% foetuses with skeletal malformations (% affected litters)	0.3 (4.5)	0 (0)	0 (0)	4.6 (12.5)
% foetuses with visceral malformations (affected litters)	0.6 (9.1)	0 (0)	0 (0)	3.6 (12.5)
Malformations % affected foetuses (% affected litters)				
1 mm vesicle over posterior fontanelle	0 (0)	0 (0)	0 (0)	0.5 (6.3)
Brain anomaly	0.3 (4.5)	0 (0)	0 (0)	0 (0)
Dwarfism	0 (0)	0 (0)	0 (0)	1.5 (6.3)
Rib forked	0.3 (4.5)	0 (0)	0 (0)	0 (0)
Tail threadlike, no anus	0.3 (4.5)	0 (0)	0 (0)	0 (0)
Tail bent	0 (0)	0 (0)	0 (0)	3.1 (6.3)
Developmental and genetic variations % affected foetuses (% affected litters)				
27 presacral vertebrae	0 (0)	0.8 (5.3)	0 (0)	0 (0)
14th rudimentary rib(s)	11.2 (40.9)	16.1 (42.1)	16.7 (66.7)	13.1 (50.0)
7th cervical rib	0.6 (4.5)	0.8 (5.3)	0 (0)	0 (0)
Hyoid unossified	1.2 (9.1)	0 (0)	2.7 (14.3)	0 (0)

Observation	Glyphosate technical (mg/kg bw/day)			
	0 (control)	300	1000	3500
Reduced ossification of skull	0.6 (4.5)	1.7 (10.5)	0.7 (4.8)	0 (0)
Sternebrae #5 and/or #6 unossified	8.1 (36.4)	5.9 (26.3)	11.3 (38.1)	28.3 (68.8)
Other sternebrae unossified	0.6 (4.5)	0 (0)	0 (0)	6.1 (18.8)
Retrooesophgeal right subclavian	0 (0)	0 (0)	0 (0)	1.0 (6.3)
Renal papilla not developed nad/or distended ureter	1.9 (13.6)	0.8 (5.0)	2.7 (14.3)	4.1 (25.0)

III. CONCLUSIONS

Treatment with glyphosate produced signs of maternal and foetal toxicity in the 3500 mg/kg bw/day dosage group. Glyphosate did not produce a teratogenic response when administered to pregnant rats at dosage levels of 3500 mg/kg bw/day or less. Accordingly the NOAEL for maternal and embryofoetal toxicity was considered to be 1000 mg/kg bw/day.

3. Assessment and conclusion

Assessment and conclusion by applicant:

This study from 1980 is considered valid in spite of deviations to the recent OECD guideline (OECD 414, 2018).

The maternal NOAEL was set at 1000 mg/kg bw/day based on mortality, clinical signs and decreased body weight gain at 3500 mg/kg bw/day. Foetal effects were also confined to the top dose level of 3500 mg/kg bw/day. Foetal weights were significantly reduced. The mean number of viable foetuses per litter and the mean foetal weight were decreased. There was a significant increase in early resorptions causing a slight increase in total post-implantation loss.The total number of foetuses with malformations was increased at the highest dose level but the number of affected litters was identical to that in the control groups. Since the incidence and type of malformations were similar to those from historical control data, it was concluded that these findings were not related to treatment. The higher number of foetuses with unossified sternebrae in this dose group was considered an adverse effect of compound administration secondary to maternal toxicity. However, this a developmental variation not a malformation. Thus, the NOAEL for the maternal as well as for foetotoxicity was 1000 mg/kg bw/day in this study.

Assessment and conclusion by RMS:

Rabbit studies

Eight developmental toxicity studies in rabbits discussed in the previous EU evaluations are summarised below in Table 5.6.2-23 nothing where there are differences in study report and previous AIR NOAEL values and LOAEL effects. In addition, preliminary studies with non-pregnant and pregnant animals are discussed (Table 5.6.2-24) as well as other data to better characterize the adverse gastrointestinal effects of gavage administration of glyphosate acid in rabbits.

Table 5.6.2-23: Summary of developmental toxicity in rabbits

Annex Point	Study	Study type	Substance	Reference list-related category§	Result	
					NOAEL [mg/kg bw/day] {previous AIR value, where different}]	LOAEL Targets / Main effects {previous AIR noted effects}
CA 5.6.2/009	[redacted], 1996	*in vivo:* NZW: rabbit, ♀ (gavage, 0, 100, 175 and 300 mg/kg bw/day)	Glyphosate acid (Batch: P24, Purity: 95.6 %)	Valid, Category 2a	**Maternal:** 100; **Developmental:** 175	**Maternal:** diarrhoea, reduced faecal output, staining of genital area at the high and mid-dose dose **Developmental:** delayed ossification, decreased body weights at the high dose
CA 5.6.2/010	[redacted], 1996	*in vivo:* NZW: rabbit, ♀ (gavage, 0, 50, 200 and 400 mg/kg bw/day)	Glyphosate technical (Batch: H95D161A), Purity: 95.3 %)	Valid, Category 2a	**Maternal:** 200 {50}; **Developmental:** ≥ 400 {50}	**Maternal:** increased mortality, reduced body weight and diarrhoea (at the high dose), clinical signs (reduced faecal output, scours), reduced body weight at the mid-dose {reduced body weight gain} **Developmental:** no treatment-related effects, increased post implantation loss not considered dose related {increased post implantation loss considered dose-related}
CA 5.6.2/011	[redacted], 1995	*in vivo:* Japanese White rabbit, ♀ (gavage, 0, 30, 100, 300 or 1000 mg/kg bw/day)	Glyphosate technical, Code: HR-001 (Batch: T-941209, Purity: 97.56 %)	Valid, Category 2a	**Maternal:** 100; **Developmental:** ≥ 300, HDT	**Maternal:** defecation of loose stool and subsequent abortion or premature delivery and reduced body weight **Developmental:**

Table 5.6.2-23: Summary of developmental toxicity in rabbits

Annex Point	Study	Study type	Substance	Reference list-related category§	Result	
					NOAEL [mg/kg bw/day] {previous AIR value, where different}]	LOAEL Targets / Main effects {previous AIR noted effects}
						no effects
CA 5.6.2/012 CA 5.6.2/013	[redacted] 1993	*in vivo:* NZW: rabbit, ♀ (gavage, 20, 100 or 500 mg/kg bw/day)	Glyphosate technical (Batch: 60, Purity: 96.8 %)	Invalid, Category 2b	**Maternal:** 100 {20}; **Developmental:** 100	**Maternal:** increased mortality, soft stool/ liquid faeces. {increased maternal mortality at 100} **Developmental:** general signs of secondary toxicity (e.g. incomplete ossification)
CA 5.6.2/014 CA 5.6.2/015	[redacted], 1991	*in vivo:* NZW: rabbit, ♀ (gavage, 0, 50, 150 or 450 mg/kg bw/day)	Glyphosate acid (Batch: 206-FAK-25-1, Purity: 98.6 %)	Valid, Category 2a	**Maternal:** 50; **Developmental:** 150	**Maternal:** reduced food consumption, soft/liquid faeces, reduced body weight **Developmental:** increased embryo/foetal deaths and post-implantation loss {post-implantation loss and cardiac malformations at 450}
CA 5.6.2/016	[redacted] 1989	*in vivo:* NZW: rabbit, ♀ (gavage, 0, 125, 250, and 500 mg/kg bw/day)	Glyphosate technical (Batch: 38, Purity: 95 %)	Supportive, Category 2a	**Maternal:** 250; **Developmental:** 250	**Maternal:** body weights and food consumption signify-cantly reduced; 2 abortions were noted in the high dose group **Developmental:** mean number of viable im-plants significantly reduced, mean number of external, visceral and skeletal malfor-mations, and

Table 5.6.2-23: Summary of developmental toxicity in rabbits

Annex Point	Study	Study type	Substance	Reference list-related category§	Result	
					NOAEL [mg/kg bw/day] {previous AIR value, where different}]	LOAEL Targets / Main effects {previous AIR noted effects}
						mean number of variations significantly increased.
CA 5.6.2/017	[redacted], 1981	*in vivo:* NZW: rabbit, ♀ (diet, 0, 10.5, 50.7 and 255.3 mg/kg bw/day)	Glyphosate /active principle (Batch: not stated, Purity: 96.8 %)	Invalid, Category 3b	**Maternal:** 255.3; **Developmental:** 10.5 (foetal loss)	**Maternal:** body weight gain increase noted with dose. **Developmental:** litter weight decreases noted in mid and high doses in a non-dose responsive manner. Foetal loss noted in mid and high doses. No statistical methods employed.
CA 5.6.2/018	[redacted], 1980 (dose range-finder)	*in vivo:* Dutch Belted: rabbit, ♀ (gavage, 125, 250, 500, 1250 and 2500 mg/kg bw/day)	Glyphosate technical (Batch: XHJ-64, Purity: 100 %)	Supportive, Category 1	**Maternal:** 250; **Developmental:** 250	**Maternal:** severe maternal toxicity ≥500. Dose-responsive clinical signs of gastro-intestinal disturbances (liquid stools or diarrohea); reduced days to mortality with increasing dose. **Developmental:** no full-term pregnancies ≥500 due to mortality except for one dam at 500, which aborted early.
CA 5.6.2/019	[redacted], 1980	*in vivo:* Dutch Belted: rabbit, ♀ (gavage, of 75, 175 and 350 mg/kg bw/day)	Glyphosate technical material (Batch: XHJ-64, Purity: 98.7 %)	Valid, Category 2a	**Maternal:** 75; **Developmental:** 350 {175}	**Maternal:** diarrhoea and soft stool, and increased mortality at the high dose **Developmental:** no treatment-related effects.

Table 5.6.2-23: Summary of developmental toxicity in rabbits

Annex Point	Study	Study type	Substance	Reference list-related category§	Result	
					NOAEL [mg/kg bw/day] {previous AIR value, where different}]	LOAEL Targets / Main effects {previous AIR noted effects}
						{insufficient number of surving dams and therefore litters, to confirm 350 as overall offspring NOAEL}

§ The category describNOes the acceptability/reliability of the study within the AIR 5 submission. This category does not describe hazard classification.

The previous EU Annex I Renewal review concluded that the NOAEL for maternal and developmental effects in rabbits was 50 mg/kg bw/day ([redacted] (1991) (CA 5.6.2/014 and 5.6.2/015), with maternal LOAEL of 150 mg/kg bw/day; [redacted] (1996) (CA 5.6.2/010) maternal and developmental LOAEL of 200 mg/kg bw/day) and that effects on the foetuses were only observed in the presence of marked maternal toxicity. This NOAEL was then used as the basis for the acceptable daily intake (ADI) and acceptable operator exposure level (AOEL) by the BfR and EFSA, and then also for an acute reference dose (ARfD) by EFSA.

Overall the previous EU evaluation determined that glyphosate was not teratogenic in rabbits. Three additional studies have been included in this submission. The results from these studies are consistent with the data that has been previously reviewed, the pattern of maternal toxicity is consistent and the effects on the foetuses were only observed in the presence of maternal toxicity and no teratogenicity was observed.

Rabbits were more sensitive to oral gavage dosing than other species. In rabbits, glyphosate exposure via oral gavage led to clinical signs of toxicity in does consistent with gastro-intestinal disturbances. Clinical signs observed included diarrhoea/soft faeces, reduced faecal output, reduced body weights, reduced food consumption and increased mortality. Table 5.6.2-23 details maternal toxicity observed following glyphosate treatment via oral gavage. These effects are consistent with gastro-intestinal stasis (ileus) likely caused by the mucosal membrane irritation potential of glyphosate acid. Rabbits (caecotrophs) are particularly sensitive to disruption of the gastro-intestinal tract. Stress and other environmental factors can lead to the normal muscular contractions of the stomach and intestines being greatly diminished, which in turn leads to disruption of the normal intestinal/ceacum bacterial flora. It is likely that the bolus administration of low pH glyphosate acid stresses the does as well as leads to the irritation of mucosal membrane of the rabbit gastro-intestinal tract. Consequently the associated stress leads to gastro-intestinal stasis. The gross necropsy signs observed in maternal animals in the studies by [redacted] (1995) (CA 5.6.2/011), [redacted] (1996) (CA 5.6.2/010) and [redacted] (1996) (CA 5.6.2/009), such as hair like boluses in the stomach, fluid filled large intestines and gas distension in the lower gastrointestinal tract are indicative of gastro-intestinal stasis. These findings appear to be relevant to only hindgut fermenters as it is not seen in rats or dogs following administration of an oral bolus dose.

Table 5.6.2-24: Summary of maternal toxicity in glyphosate acid developmental toxicity in rabbits and preliminary studies in pregnant and non-pregnant rabbits

Study Strain (Reference)	Dose level (mg/kg bw/day)	Maternal Mortality #	Diarrhoea/ loose faeces #	Reduced faecal output #	Body weight effect	Necropsy findings	Number of dams with live young or litters at Day 29 #
Preliminary, Japanese White ([redacted], 1995)	1000	6/6	6/6	not recorded	Remarkably decreased		0/6
	300	0/6	1/6	not recorded	Lower than control		5/6
Definitive, Japanese White ([redacted], 1995)	300	1/18	4/17	0/17	Lower than control	Erosion in the stomach, hair bolus in stomach, watery contents in large intestine/caecum	15/18
Preliminary, non-pregnant NZW ([redacted] 1996)	500 (1 day) then 1000 (4 days)	3/3	3/3	not recorded	Severe body weight loss	Relaxed fecal output, reduced food consumption, anus stained with fecal mterial, ptosis and subdued behaviour and mortality	Not reported
	500 (9 days)	0/3	3/3	3/3	Body weight loss	Fecal staining around anus	Not reported
Preliminary, pregnant NZW ([redacted], 1996)	400	2/6	4/6	2/6	Reduced body weight gain		All 4/6 pregnant, 2/6 aborted or bleeding from vagina
	200	0/6	0/6	0/6	Reduced body weight gain		All 4/6 pregnant
NZW ([redacted], 1996)	400	2/18	10/16	2/18	Initial loss and then statistically significant lower body weight gain than control	Fluid filled large intestines, haemorrhage, ulceration and sloughing of the stomach, duodenum congested and	16/18

Table 5.6.2-24: Summary of maternal toxicity in glyphosate acid developmental toxicity in rabbits and preliminary studies in pregnant and non-pregnant rabbits

Study Strain (Reference)	Dose level (mg/kg bw/day)	Maternal Mortality #	Diarrhoea/ loose faeces #	Reduced faecal output #	Body weight effect	Necropsy findings	Number of dams with live young or litters at Day 29 #
						colon, rectum and appendix gas distended.	
	200	1/18*	0/16	2/18	Lower body weight gain than control		16/18
NZW ([redacted], 1996)	300	2/20	19/20	9/20	Reduction in maternal body weight gain	Hair-like substance in the stomach	17/20
	175	2/20	11/20	9/20	Reduction in maternal body weight gain	-	17/20
Preliminary, Dutch Belted ([redacted] 1980)	2500	5/5	5/5	not recorded			0/5
	1250	5/5	5/5	not recorded			0/5
	500	5/5	5/5	not recorded			0/5
	250	0/5	3/5	not recorded			5/5
Definitive, Dutch Belted ([redacted], 1980)	350	10/16	16/16	not recorded	No effect	-	6/16
	175	2/16	slight increase in incidence	not recorded	No effect	-	11/16
MTD, NZW ([redacted], 1991, CHV 40/901303)	1000 (7 days)	0/2	2./2	not recorded			Not reported
	750 (13 days)	0/2	2/2	not recorded			Not reported
	500 (13 days)	0/2	2/2	not recorded			Not reported

Table 5.6.2-24: Summary of maternal toxicity in glyphosate acid developmental toxicity in rabbits and preliminary studies in pregnant and non-pregnant rabbits

Study Strain (Reference)	Dose level (mg/kg bw/day)	Maternal Mortality #	Diarrhoea/ loose faeces #	Reduced faecal output #	Body weight effect	Necropsy findings	Number of dams with live young or litters at Day 29 #
Preliminary, NZW ([redacted], 1991, CHV 39/901303)	625	2/6	6/6	6/6			4/6
	250	0/6	6/6	6/6			6/6
	100	0/6	4/6	2/6			6/6
Definitive, NZW ([redacted], 1991, CHV 45/901303)	450	1/20	13/20	12/20	No effect	-	13/20
	150	0/16	5/16	11/16	No effect	-	15/16
NZW ([redacted] 1993)	500	8/15	12/15	0/15	Statisticaly significant lower body weights than controls	-	6/15
NZW ([redacted], 1989)	500	0/15	0/15	0/15	Statisticaly significant lower body weight gain	-	12/15

- Not reported / not applicable

Strong evidence that maternal toxicities noted in rabbit developmental toxicity studies dosed via oral gavage are related to general gastro-intestinal disturbance (to which rabbits are especially sensitive, as noted above), rather than systemic toxicity following repeated exposure, is the complete absence of systemic toxicity noted in three repeated dose dermal toxicity assays up to equivalent systemic exposures in the same species (see Table 5.3.3-1). Reported gross necropsy observations, organ weights, organ pathology, hematology and clinical chemistry in these repeat-dose studies confirm an absence of specific target organ toxicity following repeated exposure in rabbits. An important consideration is that glyphosate is essentially unmetabolised in mammals and therefore systemic doses provide the opportunity to evaluate for specific target organ toxicity following repeated exposure, irrespective of the route of exposure.

[redacted] (1982) (CA 5.3.3/008) noted no effects up to the highest dose tested in a repeated dose rabbit 21–day dermal toxicity study, 5000 mg/kg bw/day (dermal). In order to understand the equivalent systemic glyphosate dose in these rabbits, [redacted] (2012) (CA 5.8.2/014) measured dermal absorption *in vitro* through rabbit skin and determined that the dermal dosing regimen in [redacted] (1982) (CA 5.3.3/008)

resulted in 2.66 % glyphosate acid available as a systemic dose, i.e. 133 mg/kg bw/day. Applying the glyphosate RAR gastrointestinal absorption value of 20% oral bioavailability[22], the systemic dose of 133 mg/kg bw/day resulting from a dermal dose of 5000 mg/kg bw/day, would be equivalent to an oral dose of 665 mg/kg bw/day; significant toxicity would be expected following an oral dose at this level in rabbits. Furthermore, systemic glyphosate is excreted nearly exclusively through the kidneys, with no biliary excretion and therefore, no gastrointestinal exposure in repeat dose dermal toxicity studies.

Clinical signs of gastrointestinal irritation manifesting as diarrhea and loos stools in rabbits dose orally by gavage in these studies resulted in nutritional compromise and mortality. Lagomorphs, hind gut fermenting herbivorous small mammals (including rabbits), practice coprophagy (consume their own faeces) in order to maintain balanced nutrition. This is a widely known fact and is documented in the scientific literature going back over 130 years;

> "L'ingestion des crottes est un phénomène physiologique. Elle aurait pour but de soumettre les aliments à une seconde élaboration digestive et à une nouvelle absorption. **Cet acte est indispensable à l'entretien de la vie des léporidés**" (page 223 conclusions, Morot, 1882)[23].
>
> "Ingestion of droppings is a physiological phenomenon. It would aim to submit food to a second digestion and a new absorption. **This act is essential to the maintenance of life in rabbits and hares**" (English translation page 223 conclusions, Morot, 1882).

Published research over the last century elucidates that the practice of coprophagy is vital to the rabbit accessing the necessary nutrition to thrive and survive. Rabbits produce two types of fecal pellets on a diurnal cycle; hard pellets by day and small soft nutritious pellets by night (Pickard & Stevens, 1972)[24]. Additional opportunity to absorb nutrients is afforded in the practice of rabbits eating the small soft pellets produced at night, by directly removing them from the anus and swallowing them whole. These pellets are high in nitrogen, protein, sulfur and vitamins. The nutritional significance of coprophagy is especially crucial with reduced feed intake, wherein consumption of up to 100% of the soft feces produced may be required to supply essential dietary needs (Sloave & Brand, 1991)[25]. Research from over 60 years ago noted that the soft feces consumed by rabbits are significantly higher in four vitamins than the hard pellets produced at day; levels of niacin and riboflavin at 3 to 4-fold higher, pantothenic acid 6-fold higher and vitamin B12 2 to 3-fold higher in soft feces than hard (Kulwich *et al.*, 1953)[26].

Published results from research conduced over 40 years ago demonstrated that preventing coprophagy in rabbits resulted in a substantial reduction in body weight gain (Stevens, 1977)[27]. These results were of no great surprise, given earlier research showed that preventing coprophagy resulted in decreased digestibility of dry matter, decreased protein digestibility and decreased nitrogen utilisation in the rabbit (Thacker & Brandt, 1955)[28]. Subsequently it was shown that the soft feces contain enzymes which aid in the digestion of proteins (Camara & Prieur, 1984)[29].

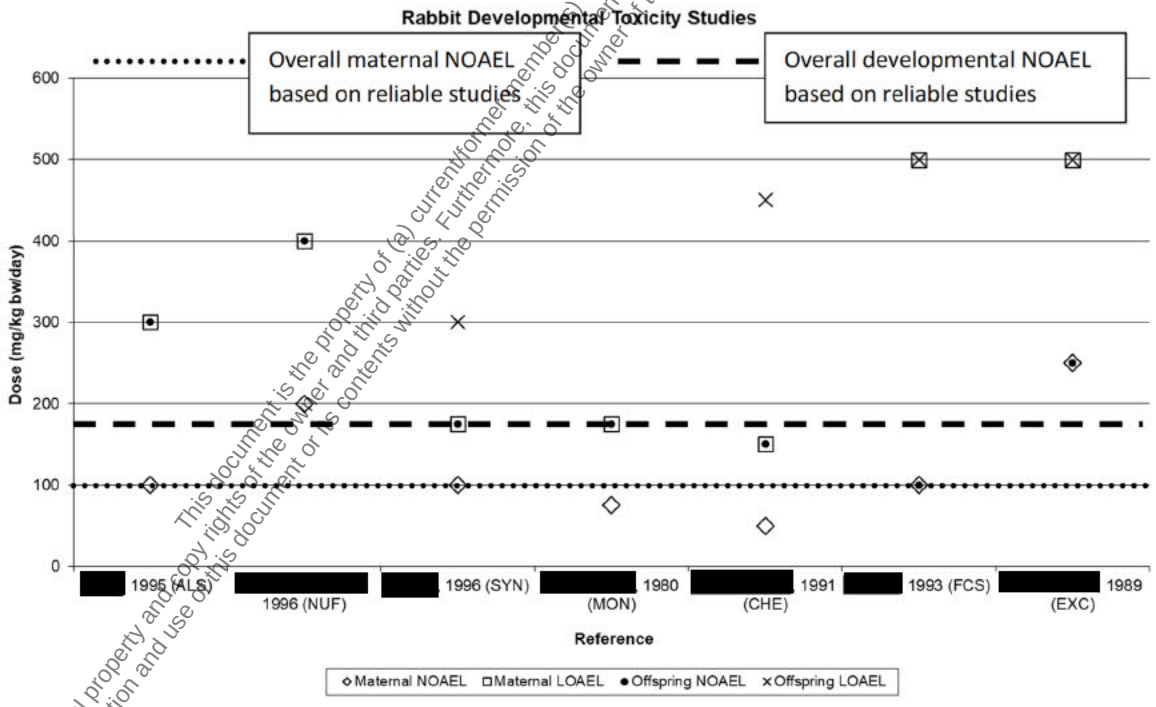
The rabbit studies (including maximum tolerated dose, pilot and definitive studies) summarised in the Table 5.6.2-24 note a high incidence of diarrhea and loose stools where maternal mortality is recorded. As a consequence, the essential practice of coprophagy was not possible (soft pellets could not form due to diarrhea), leading to nutritional compromise of these oral gavaged rabbits.

[22] EFSA (2015) 13(11):4302
[23] Morot MC (1882). Des Pelotes Stomacales des Léporidés. Mem. Soc. Cent. Med. Vet. 12 (1), 139-239.
[24] Pickard DW and Stevens CE (1972). Digesta flow through the rabbit large intestine. Amer. J. Physiol. 1972: 115 (222), 121-136.
[25] Sloave O and Brand CD (1991). Coprophagy in Animals: A Review. Cornell Vet. 81 (4), 357-364.
[26] Kulwich R, Struglia L and Pearson PB (1953). The effect of coprophagy on the excretion of B vitamins by the rabbit. J. Nutr. 49, 639-645.
[27] Stevens AG (1977). Digestibility and coprophagy in the growing rabbit. Proc. of the Nutr. Soc. 36, 4A.
[28] Thacker EJ and Brandt CS (1955). Coprophagy in the rabbit. J. Nutr. 55, 375-387.
[29] Camara VM and Prieur DJ (1984). Secretion of the colon isozyme of lysozyme in association with cecotrophy in rabbits. Amer. J. Physiol. 247G, 19-23.

Gastrointestinal irritation manifesting as diarrhea and loose stools in these rabbits dosed orally by gavage, resulted in nutritional compromise and mortality. This hypothesis is consistent with the mortality noted in the latter portion of the developmental toxicity studies, particularly at the high doses. Such severe effects are not observed in the multiple repeat-dose dermal toxicity studies in the same species with higher systemic exposures to glyphosate, where no specific target organ toxicity was identified during gross necropsy or microscopic examinations of organs. This confirms the hypothesis that the maternal effects observed in rabbit developmental toxicity studies are not evidence of systemic toxicity and are not relevant to humans.

The current European ADI for glyphosate is based on a 50 mg/kg bw/day NOAEL in two rabbit studies ([redacted] (1991) (CA 5.6.2/014 and 5.6.2/015); [redacted] (1996) (CA 5.6.2/010)). However, the large glyphosate data set with multiple rabbit developmental toxicity studies clearly demonstrates an overall maternal NOAEL of 100 mg/kg bw/day and offspring NOAEL of 175 mg/kg bw/day (Figure 5.6.2-~~1~~2). However, given the discussion above the rabbit maternal toxicity findings are clearly not relevant to humans for three simple reasons. Firstly, humans are not exposed to bolus doses of glyphosate acid in their diet, and therefore are not subjected to the irritating effects seen in rabbit gastrointestinal tracts. Secondly, the maternal toxicity in rabbit developmental toxicty studies is not due to subchronic or chronic exposures. Thirdly, humans are not coprophagic; we obtain our nutrients through a balanced diet rather than nutrient recycling via the consumption of faeces.

Figure 5.6.2-2: Results and overall maternal and developmental NOAELs of developmental toxicity studies in rabbits



The AOEL is a health-based exposure level to protect workers from systemic toxicity. In the previous Annex 1 Renewal the AOEL was based on the rabbit maternal toxicity endpoint, discussed above, and the GRG believe this endpoint is not appropriate because it was not established based on systemic toxicity. Rather the maternal effects seen at ≥150 mg/kg bw/day (Figure 5.6.2-1) are the consequence of gastrointestinal disturbances following bolus dosing of an acidic material and at higher doses, and the inability to perform coprophagy which over time results in malnutrition.

A recent analysis of acute reference dose (ARfD) setting in Europe (Moxon, 2020)[30], notes there are a number of potentially errantly assigned ARfDs, particularly where the endpoint selection is based on developmental toxicity studies. Moxon (2020) (CA 5.6.2/009) notes three relevant toxicological effects that may be based on a developmental toxicity, as described by the JMPR (2002)[31].

(i) Early effects noted in repeat dose studies: the effects noted at the overall maternal LOAEL for do not appear early in the study.
(ii) Reproductive/developmental effects: the overall offspring LOAEL of 300 mg/kg bw/day (Table 5.6.2-23) and previous RAR offspring LOAEL of 200 mg/kg bw/day ([redacted] (1996) (CA 5.6.2/010) are in the presence of maternal toxicity, and the overall reproductive/developmental NOAEL is 175 mg/kg bw/day. Moxon (2020) points out that such effects in the presence of maternal toxicity are of questionable relevance.
(iii) Direct effects on the gastrointestinal tract, where considered relevant to human exposures: bolus dosing via gavage is not representative of human dietary exposures.

Moxon (2020) also notes that unreliable studies should not be used over and above reliable definitive toxicity studies, and therefore the [redacted] (1993) (CA 5.6.2/012 and CA 5.6.2/013) study, summarised below should be disregarded in this case. In addition, with the lack of human relevance for rabbit gastrointestinal effects which result in the inability of rabbits to perform coprophagy to maintain nutritional balance, the acute reference dose (ARfD) from the previous EFSA conclusion, does not appear to be justified.

1. Information on the study

Data point:	**CA 5.6.2/009**
Report author	[redacted]
Report year	1996
Report title	Glyphosate acid: Developmental toxicity study in the rabbit
Report No	[redacted]/P/5009
Document No	Not reported
Guidelines followed in study	OECD 414 (1981), EEC B.31 (1988),US-EPA 83-3
Deviations from current test guideline (OECD 414, 2018)	Dosed from day of implantation through the period of organogenesis, rather than through to day prior to necropsy
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Glyphosate acid was administered by gavage to three groups of 20 mated New Zealand White rabbits each, at doses of 100, 175 and 300 mg/kg bw/day from gestation day 8-20 (mating = day 1). A further group of 20 animals was exposed to the vehicle (deionised water) to serve as control.

[30] Moxon M Strupp C Aggarwal M Odum J Lewis R Zedet S Mehta J. An analysis of the setting of the acute reference dose (ARfD) for pesticides in Europe. *Regul. Toxicol. Pharmacol.* 113 (2020) 104638.
[31] JMPR, 2002. Further Guidance on Derivation of an Acute RfD, Pesticide Residues in Food – 2002, Report on the JMPR, vol. 178 FAO Plant Production and Protection Paper, pp. 4-8 2002.

Individual clinical observations, body weight and food consumption were recorded during the study. The females were killed on day 30 of gestation, examined macroscopically for external and internal malformation. The uteri were examined number of corpora lutea, implantation number, position and type, foetal weights, foetal sex, external appearance and internal visceral anomalies/abnormalities were recorded. All live foetuses were preserved, processed and subsequently examined for skeletal anomalies with the heads of half the offspring preserved and examined for visceral anomalies.

Administration of 175 or 300 mg/kg bw/day was associated with dose-related maternal toxicity. This toxicity was manifested as signs of diarrhoea, reduction in faecal output as a result of reduced food consumption and a corresponding reduction in body weight. There was no maternal toxicity attributable to the administration of 100 mg/kg bw/day. At the high dose level, when maternal toxicity was seen, a reduction in mean foetal weight and very minor alterations in foetal ossification were evident. There was no effect on the number of foetuses or their survival and there was no evidence of teratogenicity.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material:

Identification:	Glyphosate acid
Description:	White solid
Lot/Batch #:	P24
Purity:	95.6 %
Stability of test compound:	The stability of the test substance was confirmed for the study period.
Vehicle:	Deionised water
Test Animals:	
Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Not reported
Sex:	Females (time-mated)
Weight at dosing:	approximately 3.8 kg
Acclimation period:	At least 4 days
Diet/Food:	Harlan Teklad 9603TRB rabbit diet, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in mobile rabbit units
Environmental conditions:	Temperature: 17 ± 2 °C Humidity: 55 ± 15 % Air changes: 25-30/hour 12 hours light/dark cycle

B. STUDY DESIGN

In life dates: 1996-01-01 to 1996-02-09

Animal assignment and treatment:
Eighty time-mated females were supplied. Sexually mature, virgin females were paired with stud males. The day of copulation was designated Day 1 of gestation. The females were delivered to [redacted] at or before Day 3 of gestation and were allocated randomised to treatment groups. Groups of 20 time-mated New Zealand White female rabbits received 0, 100, 175 or 300 mg/kg bw/day test substance by gavage (2 mL/kg bw) from gestation Day 8-20. The dose levels were chosen based on results of a preliminary dose finding.

Dose formulation and analysis
For each dose level an appropriate amount of deionised water was added to a weighed amount of glyphosate acid (adjusted for purity). Each preparation was thoroughly mixed and subdivided into aliquots. Fresh aliquots were used for each day of the study. Two preparations were made per concentration (i.e. 0, 50, 87.5 and 150 mg/mL). The dosing preparations were stored at room temperature. Representative samples of each dosing preparation were analysed prior to being used for dosing to verify the achieved concentration of glyphosate acid in the vehicle. Samples were taken for the determination of homogeneity at 50 and l50 mg glyphosate acid/mL (low and high dose levels).
The chemical stability of glyphosate acid in the vehicle was determined by re-analysis of the lowest and highest concentrations of the dosing preparations after an interval of 40 days.
Dose formulations were shaken prior to dosing, and during dosing as required.

Clinical observations
A check for clinical signs of toxicity, ill-health or behavioural changes was made once daily during the pre- and post-dosing periods and twice daily (before and after dosing) during the dosing period.

Body weight
Individual body weights were recorded on arrival, on Day 4, prior to dosing on days 8 to 20 and on days 23, 26 and 30 of gestation.

Food consumption
Food consumption of females was recorded on days 4-8, days 8-11, days 11-14, days 14-17, days 17-20, days 20-23, days 23-26 and days 26-30 of gestation.

Sacrifice and pathology
All rabbits at scheduled termination on day 30 and any requiring euthanasia during the study were killed by an overdose of 200 mg/mL sodium pentobarbitone solution given as i.v. injection. All animals were subjected to an examination *post mortem*. This involved an external observation and an examination of the thoracic and abdominal viscera. The pregnancy status of each animal was determined. Where there was no clear evidence of implantation, the uterus was removed and stained with ammonium polysulphide to determine whether or not implantation had occurred. For pregnant animals the intact gravid uterus (minus ovaries and trimmed free of connective tissue) was removed and weighed. The ovaries, uterus and contents where then examined. Number of corpora lutea, number and position of implantations, number of live foetuses, foetus weight and early and late intrauterine deaths were determined for each sacrificed doe.

Developmental parameters
After weighing the foetuses were killed with an intracardiac injection of approximately 0.1 mL of 200 mg/mL pentobarbitone sodium solution. An external examination of each foetus was made together with an examination of the oral cavity. All foetuses were then examined internally for visceral abnormalities, sexed, eviscerated and fixed in 70 % industrial methylated spirits. After approximately 24 h the head of each foetus was cut along the fronto-parietal suture line and the brain was examined for macroscopic abnormalities. The carcasses were then returned to 70 % industrial methylated spirits for subsequent processing and staining with Alizarin Red S. The remaining stained foetal skeletons were examined for abnormalities and the degree of ossification was assessed.

Statistics
Data relating to those animals which were non-pregnant and animals that died intercurrently were excluded

from the statistical analysis. Maternal body weight during the dosing and post dosing periods was considered by analysis of covariance on initial (day 8) body weight. Maternal food consumption during the dosing and post dosing periods, the numbers of implantations and live foetuses per female, gravid uterus weight, litter weight, mean foetal weights per litter and mean *manus* and *pes* scores per litter were considered by ANOVA. Maternal performance data, the proportion of foetuses with each individual *manus* and *pes* score, the proportion of foetuses with each defect and the proportion of litters with each defect were considered by Fisher's Exact Test. Pre-implantation loss, post-implantation loss, early intra-uterine deaths, late intra-uterine deaths, male foetuses, major external visceral defects, minor external/visceral defects, external visceral variants, major skeletal defects, minor skeletal defects and skeletal variants were analysed as follows:
1) Percentages were analysed by ANOVA following double arcsine transformations of Freeman and Tukey (1950),
2) the proportion of foetuses and, with the exception of male foetuses, the proportion of litters affected were considered by Fisher' s Exact Test.

All analyses were carried out in SAS (1989). For Fisher's Exact Tests the proportion in each treated group was compared to the control group proportion. Analyses of variance and covariance allowed for the replicate structure of the study design. Least-squares means for each group were calculated using the LSMEAN option in SAS PROC GLM. Unbiased estimates of differences from control were provided by the difference between each treatment group least-squares mean and the control group least-squares mean. Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean using a Student's t-test, based on the error mean square in the analysis.
All statistical tests were two sided.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS

The concentrations of glyphosate acid in the dosing formulations were within 12% of the target concentrations. The homogeneity and stability of the test substance in the dosing formulations was satisfactory.

B. FOOD CONSUMPTION

During the dosing period, does receiving 175 or 300 mg/kg bw/day showed significantly reduced food consumption compared to the controls.

Table 5.6.2-25: Glyphosate acid: Teratology study in the rabbit ([redacted], 1996): Mean food consumption (in g/rabbit/day) during gestation

	Day of gestation	Dose level in mg/kg bw/day			
		0 (Control)	100	175	300
	4 - 8	155	144	139	160
During Dosing	8 - 11	183	171	158*	149**
	11 - 14	172	162	137**	131**
	14 - 17	172	163	123**	98**
	17 - 20	177	168	143*	115**
Post Dosing	20 - 23	185	176	180	172
	23 - 26	158	160	179	179
	26 - 30	137	148	166*	175*

* Significantly different from control at $p<0.05$
** Significantly different from control at $p<0.01$

C. MORTALITY

The incidence of intercurrent deaths was 1, 2, 2 and 2 in the control, 100, 175 and 300 mg/kg/ bw/day groups, respectively.

In the post-dosing period, one doe in the control group showed weight loss, reduced food consumption, signs of diarrhoea, mucus in the faeces, few faeces and staining in the genital area. This animal aborted on day 30. Changes in the stomach and caecum were observed *post mortem*.

In the low dose level group, one doe showed slight loss of body weight and reduced food consumption between days 4 and 8 (i.e. prior to the onset of dosing) and this response continued into the dosing period, until the animal aborted its litter on day 19. Examination *post mortem* noted the presence of a mass in the right inguinal region of the abdominal cavity. A second animal in this group aborted its litter on day 25 having shown weight loss and reduced food consumption from day 14.

At the intermediate dose level, one doe was killed for humane reasons on day 23 having shown loss of body weight and reduced food consumption from day 4 on. By day 23, the animal had become thin and subdued and all uterine implantations were found to be dead. A second animal in this group aborted its litter on day 22 having shown slight weight loss from day 14 and reduced food consumption from day 4. At the high dose level, two animals aborted their litters on days 24 and 23, respectively. Both animals showed a reduction in food consumption from day 11 and body weight loss from day 11/13. A hair-like substance was found in the stomachs of both animals at examination *post mortem*.

D. CLINICAL OBSERVATIONS

In the high dose level group, there was an increased incidence of animals producing few faeces, with signs of diarrhoea or with staining in the genital area, in comparison with the control group. The production of few faeces and signs of diarrhoea were also of increased incidence in does of the intermediate dose group. There were no clinical effects observed in rabbits treated at a dose level of 100 mg/kg bw/day (see table below).

Table 5.6.2-26: Glyphosate acid: Teratology study in the rabbit ([redacted], 1996): Observed clinical signs during the dosing period

Clinical sign	Number of rabbits affected in dose group			
	Control	Low	Intermediate	High
	(0 mg/kg be/day)	(100 mg/kg bw/day)	(175 mg/kg bw/day)	(300 mg/kg bw/day)
Blood on tray	0	2	2	1
Cold	0	0	1	0
Dry sores 1 or more areas	0	1	0	0
Ears torn	0	2	1	1
Eye opaque	0	1	0	0
Few faeces on tray	3	3	9	9
Mucus in faeces	1	0	0	0
No faeces on tray	0	1	2	3
Scabs in 1 or more areas	4	6	3	3
Signs of diarrhoea	4	5	11	19
Staining in genital area	2	2	3	11
Subdued behaviour	0	0	1	0
Thin	0	0	1	2
Urine coloured	0	1	1	0
Wet sores in 1 or more areas	2	0	1	0

E. BODY WEIGHT

Administration of 300 mg/kg bw/day was associated with a reduction in maternal body weight gain. The statistical different observed body weight development at 175 mg/kg bw/day was due to differences in body weights at the begin of the study. All animals except one of the high dose group showed signs of recovery in the post-dosing period. The reduction in food consumption was therefore accompanied by a corresponding reduction in body weight. In the low dose level group, body weight gain was comparable to controls throughout the study period (see table below).

Table 5.6.2-27: Glyphosate acid: Teratology study in the rabbit ([redacted], 1996): Mean body weight development (in g) during gestation

		Dose level in mg/kg bw/day			
		0 (Control)	100	175	300
Animals per group		17	18	17	17
Day of gestation	8	3924	3771	3822	3815
	9	3845	3837	3834	3823
	10	3857	3863	3856	3830
	11	3885	3873	3874	3854
	12	3894	3879	3877	3856
	13	3917	3905	3902	3880
	14	3942	3932	3930	3875
	15	3975	3982	3939	3896
	16	4020	4031	3959	3907*
	17	4049	4053	3982	3923*
	18	4063	4051	3990	3914**
	19	4085	4061	4005	3927**
	20	4088	4059	3995	3926**
	23	4177	4118	4049*	3951**
	26	4236	4210	4169	4093*
	30	4313	4294	4256	4183

* Significantly different from control at $p < 0.05$
** Significantly different from control at $p < 0.01$

F. PATHOLOGY

Necropsy

There were no macroscopic findings that were considered to be related to the administration of glyphosate acid.

Observations on the ovary and uterus

No treatment related effects were evident in the study.

In the control, low, intermediate and high dose level groups 17, 18, 17, and 17 females, respectively, survived to termination of the main study and were proven to be pregnant. The number and distribution of females that were not pregnant indicate that there were no treatment-related effects on pregnancy rates. Litter size at caesarean necropsy was comparable in all treatment groups.

G. DEVELOPMENTAL PARAMETERS

Number and viability of foetuses

The proportion of foetuses that were male was statistically significantly increased in the intermediate dose level group, in comparison with the control group. In the absence of a dose-related trend, this finding was

considered incidental to the administration of glyphosate acid. There was no adverse effect of glyphosate acid on the number or survival of the foetuses in utero.

Foetal body weights

There was a statistically significant reduction in mean foetal weight in the high dose level group, in comparison with the control group. This difference was considered attributable to two litters for which the mean pup weight was particularly low. These two litters were from females which had the most severe reductions in body weight. Thus, the reduction in foetal weight is considered to be a consequence of maternal welfare rather than a direct effect of glyphosate acid on the foetuses. There was no effect of 100 or 175 mg/kg bw/day on mean foetal weight.

Table 5.6.2-28: Glyphosate acid: Teratology study in the rabbit ([redacted], 1996): Intergroup comparison of litter data

Observation		Glyphosate acid (mg/kg bw/day)			
		0 (control)	100	175	300
# Animals Pregnant		18	20	19	19
# Intercurrent deaths		1	2	2	2
Gravid Uterine Weight (g) (mean ± S.D.)		580 ± 117	520 ± 121	493 ± 110	505 ± 119
Mean Placental Weight (mg)					
No. of corpora lutea (mean ± S.D.)		10.8 ± 2.2	11.0 ± 1.6	11.1 ± 1.3	11.2 ± 1.4
No. of implantations (mean ± S.D.)		9.7 ± 2.1	9.0 ± 1.8	9.1 ± 2.5	9.8 ± 1.9
No. of Live Foetuses (mean ± S.D.)		8.4 ± 1.8	8.2 ± 2.2	7.9 ± 2.2	8.5 ± 2.3
Implanation Loss (% mean ± S.D.)	Pre	10.7 ± 11.0	18.2 ± 11.1	18.1 ± 20.8	12.8 ± 11.9
	Post	11.7 ± 12.0	9.5 ± 16.7	12.1 ± 9.7	13.6 ± 16.6
Intra Uterine Deaths (% mean ± S.D.)	Early	6.2 ± 9.7	7.5 ± 17.0	8.1 ± 8.1	11.0 ± 16.0
	Late	5.5 ± 10.4	1.9 ± 4.5	4.0 ± 4.9	2.5 ± 8.3
Mean Litter Weight (g) ± S.D.		371 ± 75	350 ± 88	333 ± 79	337 ± 91
Mean Foetal Weight (g) ± S.D.		44.4 ± 4.3	43.3 ± 3.9	43.2 ± 5.7	40.7* ± 7.8
Sex Ratio		0.41	0.46	0.58**	0.45

* Significantly different from control at $p<0.05$
** Significantly different from control at $p<0.01$

External, visceral and skeletal examination

The number of foetuses with major defects was 3/143 (2/17 litters), 1/147 (1/18 litters), 0/135 (0/17 litters) and 2/144 (2/17 litters) in the control, 100, 175 and 300 mg/kg bw/day groups, respectively. Neither the type nor incidence of major defects provided evidence for an adverse effect of glyphosate acid. The proportion of foetuses with minor external visceral defects was similar for all groups, including the control. There were no significant differences in litter incidences for minor external/visceral defects noted. Consideration of the specific defects provided no evidence for an adverse effect of glyphosate acid (see tables below).

The proportion of foetuses with minor skeletal defects was statistically significantly increased in the 100 and 300 mg/kg bw/day groups, in comparison with the control group, but not in the 175 mg/kg bw/day group. Evaluation of the specific defects noted an increased incidence of foetuses in the high dose level group with partially ossified transverse processes on the 7th cervical vertebra (8 foetuses in 2 litters), unossified transverse processes on the 7th lumbar vertebra (14 foetuses in 4 litters) or partially ossified 6th sternebra (16 foetuses in 7 litters). None of the specific minor defects were statistically significantly increased in the low or intermediate dose level groups. None of the foetuses were found to have an

external/visceral variant.
The proportion of foetuses with skeletal variants was statistically significantly increased in the high dose level group, in comparison with the control group. Evaluation of the specific variants noted a slight, but not statistically significant, increase in the incidence of foetuses in this group with partially ossified odontoids (62 foetuses in 15 litters) or with 27 pre-sacral vertebrae (37 foetuses in 12 litters).
The slightly higher mean *manus* score observed in the high dose level group, in comparison with the control group, was due to a slight reduction in ossification as shown by the increase in incidence of foetuses scoring 4 or 5. A similar response was apparent from the *pes* scores. The slight reduction in ossification was influenced by the low weight foetuses. There were no treatment-related alterations in foetal ossification in the 100 or 175 mg/kg bw/day groups.

Table 5.6.2-29: Glyphosate acid: Teratology study in the rabbit ([redacted], 1996): Summary of the type and incidence of major defects

Major foetal defects	Number of foetuses affected in dose group*			
	Control	Low	Intermediate	High
	(0 mg/kg bw/day)	(100 mg/kg bw/day)	(175 mg/kg bw/day)	(300 mg/kg bw/day)
Heart single ventricle, ventricle walls thickened, aorta enlarged, pulmonary artery reduced	0/143	1/147	0/135	1/144
Encephalocoele (gross malformation of the skull)	0/143	0/147	0/135	1/144
Cebocephaly, internal hydrocephaly, maxillae fused and shortened, aorta enlarged, persistent truncus arteriosus	1/143	0/147	0/135	0/144
Shortened upper and lower jaws, cleft lip, cleft palate, nares absent, forepaws flexed (right extremely, left slight)	1/143	0/147	0/135	0/144
Reduced number of lumbar vertebrae (25 pre-sacral vertebrae)	1/143	0/147	0/135	0/144

* number affected / total number

Table 5.6.2-30: Glyphosate acid: Teratology study in the rabbit ([redacted], 1996): Summary of the type and incidence of major defects (litter incidences)

Major foetal defects	Number of litters affected in dose group*			
	Control	Low	Intermediate	High
	(0 mg/kg bw/day)	(100 mg/kg bw/day)	(175 mg/kg bw/day)	(300 mg/kg bw/day)
Heart single ventricle	0/17	1/18	0/17	1/18
aorta enlarged	1/17	1/18	0/17	1/18
pulmonary artery reduced	0/17	1/18	0/17	1/18
Encephalocoele (gross malformation of the skull)	0/17	0/18	0/17	1/18
Cebocephaly, internal hydrocephaly, maxillae fused and shortened, Shortened upper and lower jaws, cleft lip, cleft palate, nares absent	1/17	0/18	0/17	0/18
persistent truncus arteriosus	1/17	0/18	0/17	0/18
forepaws flexed (right extremely,	1/17	0/18	0/17	0/18

left slight)				
Reduced number of lumbar vertebrae, 25 pre-sacral vertebrae	1/17	0/18	0/17	0/18

* number affected / total number

Table 5.6.2-31: Glyphosate acid: Teratology study in the rabbit ([redacted], 1996): Incidence of foetal malformations and variations in rabbits treated with glyphosate acid

Foetal findings	Dose level (mg/kg bw/day)			
	0	100	175	300
No. of litters examined	17	18	17	17
No. of foetuses examined	143	147	135	144
Skeletal malformations				
Total no. of foetuses with major defects	3	0	0	1
Total no. of litters with major defects	2	0	0	1
Percentage of litters with major defects (%)	11.8	0.0	0.0	5.9
Total no. of foetuses with minor defects	58	82*	59	79*
Total no. of litters with minor defects	16	18	16	17
Percentage of litters with minor defects (%)	94.1	100	94.1	100
Skeletal variations				
Total no. of foetuses affected	119	129	116	132*
Total no. of litters affected	17	18	17	17
Percentage of litters affected (%)	100	100	100	100
External and visceral findings				
No. of foetuses with major defects	2	1	0	2
No of litters with foetuses with major defects	2	1	0	2
Percentage of litters with foetuses with major defects (%)	11.8	5.6	0.0	11.8
No. of foetuses with minor defects	12	7	9	11
No of litters with foetuses with minor defects	8	5	8	7
Percentage of litters with foetuses with minor defects (%)	47.1	27.8	47.1	41.2

* Statistically significant from control ($p<0.05$)

Table 5.6.2-32 Glyphosate acid: Teratology study in the rabbit ([redacted], 1996): Intergroup comparison of *manus/pes* assessment

Observation	Glyphosate acid (mg/kg bw/day)			
	0	100	175	300
Number of Litters	17	18	17	17
MANUS Scores				
Score 1	0	0	0	1
Score 2	25	20	19	19
Score 3	107	113	110	96
Score 4	10	13	6	21
Score 5	1	1	0	7
Mean *MANUS* Score per litter (± S.D.)	2.88 ± 0.36	2.97 ± 0.27	2.89 ± 0.23	3.05 ± 0.57
PES Scores				
Score 1	134	135	131	116**
Score 2	9	12	4	28**

Mean *PES* Score per litter (± S.D.)	1.07 ± 0.14	1.08 ± 0.05	1.03 ± 0.05	1.18 ± 0.32

** Significantly different from control at $p < 0.01$

III. CONCLUSIONS

The oral administration of glyphosate acid to time-mated rabbits by gavage at a maximum dose level of 300 mg/kg bw/day from Gestation Day 8-20 resulted maternal toxicity at 175 and 300 mg/kg bw/day. Administration of 300 mg/kg bw/day was associated with a reduction in mean foetal weight and minor alterations in foetal ossification. There was no effect on the number of foetuses or their survival and there was no evidence for teratogenicity. Therefore the 'No Observed Adverse Effect Level' (NOAEL) was considered to be 100 mg/kg bw/day for maternal toxicity. The NOAEL for developmental toxicity was considered to be 175 mg/kg bw/day.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study is considered valid, though treatment duration covered the period from implantation through organogenesis rather than through to the day prior to necropsy, as recommended in the current OECD 414 guideline.

The oral administration of glyphosate acid to time-mated rabbits by gavage at a maximum dose level of 300 mg/kg bw/day from Gestation Day 8-20 resulted in maternal toxicity at 175 and 300 mg/kg bw/day. There were no treatment-related effects on pregnancy or foetuses at any dose level that could not be attributed to maternal toxicity.

Therefore the NOAEL was considered to be 100 mg/kg bw/day for maternal toxicity. The NOAEL for developmental toxicity was considered to be 175 mg/kg bw/day.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	**CA 5.6.2/010**
Report author	[redacted]
Report year	1996
Report title	Glyphosate technical: Oral gavage teratology study in the rabbit
Report No	434/020
Document No	Not reported
Guidelines followed in study	OECD 414 (1981), JMAFF 59 NohSan 4200 (1985), US-EPA 83-3 (1984)
Deviations from current test guideline (OECD 414, 2018)	Dosed from day of implantation through the period of organogenesis, rather than through to day prior to necropsy
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised	Yes

testing facilities	
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

In an initial maximum tolerated repeat dose study, 434/019 Part II, appended to the final report 434/020, three non-pregnant rabbits were dosed at 500 for one day, then stepped up to 1000 mg/kg bw/day for four more days. Severe clinical signs of body weight loss, relaxed fecal output, reduced food consumption, anus stained with fecal amterial, ptosis and subdued behaviour and mortality were noted; one animal was found dead and the remaining two terminated in extremis. Then a repeat dose tolerability study three non-pregnant rabbits dosed daily for nine days at 500 mg/kg bw/day. All animals displayed significant body weight loss and reduced food consumption. Therefore the dose of 500 mg/kg bw/day was assumed too high for the preliminary dose range finding study.

In the preliminary dose rangex inding study, 434/019 Part I, appended to the final report 434/020, groups of six pregnant does were dosed with 0, 50, 200 or 400 mg/kg bw/day glyphosate. In high dose animals clinical observations included reduced body weight gain, reduced food consumption, 4/6 presented loose stools and 2/6 were terminated in extremis. Similar but less severe effects were noted at 200 mg/kg bw/day and no maternal effects at 50 mg/kg bw/day. No treatment effects on uterine or foetal parameters examined were noted across all doses.

In the definitive study, glyphosate technical was administered by gavage to three groups of 18 mated New Zealand White rabbits each, at doses of 50, 200 and 400 mg/kg bw/day from gestation day 7-19 (mating = day 0). A further group of 18 animals was exposed to the vehicle to serve as control.
Individual clinical observations, body weight and food consumption were recorded during the study. The females were killed on day 29 of gestation, examined macroscopically for external and internal malformation. The uteri were examined. The number of corpora lutea, implantation number, position and type, foetal weights, external appearance and internal visceral anomalies/abnormalities were recorded. All live foetuses were preserved, processed and subsequently examined for skeletal anomalies with the heads of half the offspring preserved and examined for visceral anomalies.

At the high dose level there was evidence of treatment-related effects resulting in one treatment-related death. Clinical signs of toxicity, particularly scours, reduced faecal output and diarrhoea, reduced body weight gain and reduced food consumption were seen. At the intermediate dose level similar, but less severe, effects were seen on body weight gain. At the low dose level, no treatment related effects were observed. Thus, the only dose-responsive clinical observation was gastrointestinal output (reduced fecal output or loose stools/diarrhoea). Animal deaths were 1, 0, 1 and 2 across increasing dose group; deaths in the control and mid-dose group were attributable to dosing technical errors, but at least one of the high dose deaths was considered treatment-related. There were no treatment-related effects on the uterine or foetal parameters examined in any dose group.

The oral administration of glyphosate technical to time-mated rabbits by gavage from gestation day 7-19 resulted maternal toxicity at 200 and 400 mg/kg bw/day. There were no treatment-related effects on pregnancy or foetuses at any dose level.

a) MATERIALS AND METHODS

A. MATERIALS

Test Material:

Identification:	Glyphosate technical
Description:	White powder

Lot/Batch #:	H95D161A
Purity:	95.3 %
Stability of test compound:	The dose formulation was stable for at least 1 h.
Vehicle:	1% carboxymethyl cellulose
Test Animals:	
Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	17 - 19 weeks
Sex:	Females (time-mated)
Weight at dosing:	2.7 - 4.1 kg
Acclimation period:	At least 4 days
Diet/Food:	SQC Standard Rabbit Diet (SDS Ltd., Witham, Essex, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in stainless steel cages with grid floor
Environmental conditions:	Temperature: 20 ± 3 °C Humidity: 50 ± 20 % Air changes: 15/hour 12 hours light/dark cycle

B. STUDY DESIGN

In life dates: 1995-10-13 - 1995-12-12

Animal assignment and treatment in the preliminary study:
Twenty-four time-mated females were supplied. Sexually mature, virgin females were paired with stud males. The day of copulation was designated ay 0 of gestation. The females were delivered to [redacted] at or before ay 3 of gestation and were allocated randomised to treatment groups. Groups of 6 mated New Zealand white female rabbits received 0, 50, 200 or 400 mg/kg bw/day test substance in 1 % carboxymethyl cellulose by gavage (5 mL/kg bw) from gestation day 7-19. The dose levels were chosen based on results of a preliminary dose finding study with 6 female nulliparous rabbits, where administration of 500 or 1000 mg/kg bw resulted in toxicity signs (scours, fluid filled caecum, stomach ulceration, body weight loss, reduced food consumption). Based on these findings dose levels of ≥ 500 mg/kg bw were considered to be too high for a prolonged study.

Animal assignment and treatment in the main study:
Seventy-two time-mated females were supplied as described for the preliminary study (see above). Groups of 18 mated New Zealand white female rabbits received 0, 50, 200 or 400 mg/kg bw/day test substance in 1% carboxymethyl cellulose by gavage (5 mL/kg bw) from gestation day 7-19.

Dose formulation and analysis
For each dose level, the test material was suspended daily in 1 % carboxymethyl cellulose by weighing the required amount into a glass jar and adding vehicle to make the appropriate final volume. Homogeneity was assured by mixing the formulation with a homogeniser. The concentration, stability and homogeneity of the test material were analysed. The formulation was stable for at least 1 h.

Clinical observations
A check for clinical signs of toxicity, ill-health or behavioural changes was made twice daily (before and after dosing) during the dosing period.

Body weight
Individual body weights were recorded on day 3, 7, 10, 13, 16, 19, 22, 25 and 29 of gestation.

Food consumption
Food consumption of females was recorded on days 3 to 7, days 7 to 10, days 10-13, days 13-16, days 16-19, days 19-22, days 22-25 and days 25-29 of gestation.

Sacrifice and pathology
Females were euthanatised by an i.v. injection of an overdose of sodium pentobarbitone into the auricular vein on day 29 of gestation, examined for macroscopic abnormalities and subjected to caesarean sectioning. The ovaries and uteri were removed, weighed and then examined for the number of corpora lutea and for the number and position of implants and dead or live foetuses. Resorptions and foetal deaths were classified into implantation sites, placental remnants, and macerated foetuses according to the difference in developmental stage at which deaths had occurred. Foetal sex, external and internal foetal appearance and foetal weight was determined. After examination of the ovaries and conceptuses, each female was necropsied.

Developmental parameters
The foetuses were killed by intrathoracic injection of sodium pentobarbitone. All foetuses were dissected and examined for visceral abnormalities macroscopically. The heads of alternate foetuses were removed and identified using an indelible marker and placed in Bouin‘s fixative. After a minimum of 14 days, the heads were transferred to 90 % industrial methylated spirits (IMS) in distilled water and examined for visceral anomalies under a low power binocular microscope (Van Julsingha and Bennett 1977). All foetuses were identified using colour coded wires and placed in 70 % IMS in distilled water. The foetuses were eviscerated, processed and the skeletons stained with alizarin red (Dawson 1926). The foetuses were examined for skeletal development and anomalies.

Statistics in the main study
Female body weight change (relative to day 7 of gestation) and food consumption were analysed statistically by one-way analysis of variance with the Bonferroni multiple comparison test followed by pair wise analysis of control values against treated group values using Students ‘t‘ test where appropriate. All foetal parameters, skeletal development, group incidence of specific visceral and skeletal anomalies were analysed statistically by Kruskall-Wallis non parametric analysis of variance followed by pair wise analysis of control values against treated values using the Mann-Whitney U - test where appropriate.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS
The test substance was detected at the levels of 81-102 % of the target concentrations in each dosing solution.

B. FOOD CONSUMPTION
In the preliminary study, significantly reduced food consumption was observed while administering in the high dose level of 400 mg/kg/day (days 7 to19 of gestation). This observation was confirmed in the main study. At the high dose level, there was a reduction in food consumption during the dosing period compared to controls (days 10 to 13, $p<0.05$; days 13 to 19, $p<0.01$). No other significant changes were observed in the remaining groups during the main study.

C. MORTALITY

In the preliminary study, two does were killed *in extremis* in the high dose group, one had aborted foetuses and the other was bleeding from the vagina. No mortalities occurred at any dose up to 400 mg/kg/day in the preliminary study.

In the main study, two rabbits were found dead or moribund at the high dose level. One female was found dead prior to dosing on day 19 of treatment. One female was killed *in extremis* on day 20 of treatment. Clinical observations noted at this time included hunched posture, lethargy, ptosis, hypothermia and blood on the litter tray. At the intermediate dose level, one female was found dead after dosing on Day 16 of treatment. Necropsy findings of reddened lungs, a fluid filled thorax and test material in thoracic cavity are consistent with mal-dosing. At the low dose level, no mortalities occurred. One female was found dead two minutes after dosing in the control group. Necropsy findings of blood in thorax, inflated appearance of lungs and a large area of congestion on the right caudal lobe are consistent with mal-dosing.

D. CLINICAL OBSERVATIONS

In both the preliminary and the main study, the clinical signs were in general the same. There was a toxicologically significant increase in the incidence of clinical observations, particularly scours, reduced faecal output and diarrhoea at the high dose level (400 mg/kg bw/day). Observations of lethargy, ptosis, hunched posture, hypothermia and blood on tray were noted for one animal of the main study killed *in extremis*.
At 200 mg/kg bw/day, vaginal bleeding and blood on tray were noted for one animal of the main study. Scours were also noted in animals at 200 and 50 mg/kg bw/day as well as in the control group, but the incidence and duration were not as severe as at the high dose level (see table below). No other treatment-related observations were evident.
Thus, for the findings observed at doses below 400 mg/kg bw/day, a clear dose-response could not be established.

Table 5.6.2-33: Glyphosate technical: Teratology study in the rabbit ([redacted], 1996): Observed clinical signs during the dosing period

Clinical sign	Number of rabbits affected in dose group#			
	Control (0 mg/kg bw/day)	Low (50 mg/kg bw/day)	Intermediate (200 mg/kg bw/day)	High (400 mg/kg bw/day)
Scours	5/14 (4)	10/18 (0)	7/16 (2)	16/16 (2)
Reduced faecal output	1/14 (4)	1/18 (0)	2/16 (2)	6/16 (2)
Diarrhoea	0/14 (4)	1/18 (0)	0/16 (2)	10/16 (2)
Diuresis	0/14 (4)	0/18 (0)	1/16 (2)	0/16 (2)
Blood on tray	0/14 (4)	0/18 (0)	1/16 (2)	1/16 (2)
Noisy respiration	0/14 (4)	0/18 (0)	1/16 (2)	1/16 (2)
Lethargy	0/14 (4)	0/18 (0)	0/16 (2)	1/16 (2)
Ptosis	0/14 (4)	0/18 (0)	0/16 (2)	1/16 (2)
Hunched posture	0/14 (4)	0/18 (0)	0/16 (2)	1/16 (2)
Hypthermia	0/14 (4)	0/18 (0)	0/16 (2)	1/16 (2)
Anal staining	0/14 (4)	0/18 (0)	0/16 (2)	1/16 (2)
Subdued behaviour	0/14 (4)	0/18 (0)	0/16 (2)	1/16 (2)
Vaginal bleeding	0/14 (4)	0/18 (0)	1/16 (2)	0/16 (2)

x/y: number affected / total number of animals in group
Figures in parentheses represent the number of animals having no grossly observable conceptus.

E. BODY WEIGHT

In the preliminary, study a toxicologically significant decrease in body weight gain from Day 13 to 19 *post*

coitum was evident at the high and intermediate dose levels.
Likewise a reduction in group mean body weight gain from Days 9 to 29 *post coitum* was observed in the high dose level group during the main study. The difference in group mean body weight change compared to controls was statistically significant (P<0.05 to 0.01) from Days 13 to 29 *post coitum.* Also in the intermediate dose level group a slight reduction (although not statistically significant) in group mean body weight gain from Day 9 to Day 29 *post coitum* was noted. In the low dose level group body weight gain was comparable to controls throughout the study period (see table below).

Table 5.6.2-34: Glyphosate technical: Teratology study in the rabbit ([redacted], 1996): Mean body weight gain during gestation

Dose level (mg/kg bw)	No. of animals	Body weight change (g) at Day (relative to Day 7)						
		10	13	16	19	22	25	29
0 (Control)	14	29	95	202	260	314	375	409
50	18	12	75	158	223	278	325	395
200	15	-11	54	143	198	263	309	294
400	15	-33	-45*	11**	21**	96**	153**	250*

* Significantly different from control at p<0.05.
** Significantly different from control at p<0.01.

F. PATHOLOGY

Necropsy

The macroscopic necropsy findings of the two does of the high-level dose group that died or were killed *in extremis* included fluid filled large intestines, haemorrhage, ulceration and sloughing of the stomach, duodenum congested and colon, rectum and appendix gas distended. These findings indicate that the test material may affect the gastrointestinal tract. The animal killed *in extremis* at this level also had both uterine horns containing blood and dead foetuses in the uterus. This may be a result of maternal toxicity. All other necropsy findings were not treatment-related.

Observations on the ovary and uterus

No treatment related effects were evident in both the preliminary and the main study.

In the control, low, intermediate and high dose level groups 14, 18, 15, and 15 females, respectively, survived to termination of the main study and were proven to be pregnant. The number and distribution of females that were not pregnant indicate that there were no treatment-related effects on pregnancy rates. Litter size at caesarean necropsy was comparable in all treatment groups.

G. DEVELOPMENTAL PARAMETERS

Number and viability of foetuses

The litter size at caesarean section was comparable in all treatment groups. In the high dose level group, there were slight, but not statistically significant, increases in late foetal deaths and post implantation loss, mainly due to one animal that had nine late deaths, resulting in a post implantation loss of 69.2 %. This was therefore considered not to be a treatment-related effect. At 200 mg/kg bw/day, there were statistically significant increases (p<0.05) in total foetal deaths and post implantation loss. These increases were caused by a slight, but not statistically significant, rise in early foetal deaths. At this dose level, there was no rise in late foetal deaths, as seen at the high level. Since there is no dose response observed, early foetal deaths, late foetal deaths, and total foetal deaths (the sum of early plus late foetal deaths), increases in total foetal deaths and post-implantation loss at 200 mg/kg bw/day were considered not to be treatment-related.

Table 5.6.2-35: Glyphosate technical: Teratology study in the rabbit ([redacted], 1996): Intergroup comparison of litter data

Observation		Glyphosate acid (mg/kg bw/day)			
		0 (control)	50	200	400
# Animals Pregnant at Necropsy		14	18	15	15
No. of corpora lutea (mean ± S.D.)		10.9 ± 2.2	10.5 ± 2.4	10.7 ± 2.1	11.5 ± 1.8
No. of implantations (mean ± S.D.)		9.5 ± 2.5	9.1 ± 2.3	8.9 ± 2.5	10.3 ± 2.3
No. of Live Foetuses (mean ± S.D.)		9.1 ± 2.5	8.7 ± 2.4	7.9 ± 2.5	8.9 ± 2.6
Embryonic/Foetal Deaths	Early	0.21 ± 0.43	0.22 ± 0.55	0.87 ± 1.06	0.47 ± 0.92
	Late	0.14 ± 0.53	0.11 ± 0.32	0.13 ± 0.35	0.93 ± 2.28
	Total	0.36 ± 0.63	0.33 ± 0.77	1.00* ± 1.00	1.40 ± 2.35
Implantation Loss (% mean ± S.D.)	Pre	12.5 ± 18.2	13.6 ± 9.4	16.4 ± 15.5	9.3 ± 12.5
	Post	3.7 ± 6.5	3.6 ± 8.5	11.5* ± 11.4	12.1 ± 18.6
Total Litter Weight (g) ± S.D.		372.0 ± 92.6	334 ± 75.1	321.5 ± 79.2	337.0 ± 84.4
Mean Foetal Weight (g) ± S.D.		41.5 ± 5.5	39.4 ± 5.6	41.7 ± 4.5	38.2 ± 5.2
Sex Ratio		0.56	0.51	0.54	0.51

* Significantly different from control at $p < 0.05$.

Foetal body weights

No statistically significant differences were noted in the mean foetal body weights between the control group and the treated groups. Mean total litter weights were comparable in all treatment groups.

External, visceral and skeletal examination

At the high dose level, there was one litter with one foetus with major malformations. This foetus was found to have spina bifida and clubbed and malrotated hind limbs. At the intermediate dose level, two foetuses of two different litters had major malformations. One foetus had retinal infolding and a haemorrhage in the retinal layer, the other acephaly, small kinked tail, bilateral forelimb flexure, interrupted aorta and an intraventricular septal defect. At skeletal examination, this foetus was found to have multiple rib and vertebral column abnormalities. At the low dose level, three foetuses of two different litters had major abnormalities. In one litter, one foetus had forked ribs with a displaced vertebral centrum. In another litter, one foetus had a small eye with retinal infolding and aphakia. A second foetus from this litter had nostrils close together, and a thin nasal septum not attached at posterior pole near the front of the nasal passages. In the control group, there were two foetuses from two different litters with major abnormalities. One foetus had gastroschisis and the other foetus had an extra vertebral arch resulting in scoliosis.

These findings were considered to be within the range of normal variation for this species. There were no treatment-related effects on the degree of skeletal development.

Table 5.6.2-36: Glyphosate technical: Teratology study in the rabbit ([redacted], 1996): Incidence of foetal malformations and variations in rabbits treated with glyphosate acid

Foetal findings	Dose level (mg/kg bw/day)			
	0	50	200	400
No. of litters examined	14	18	15	15
No. of foetuses examined	128	157	119	134
Skeletal malformations				
Total no. of foetuses with skeletal malformations	1	0	1	0

Total no. of litters with skeletal malformations	1	0	1	0
Percentage of litters with skeletal malformations (%)	7.1	0.0	6.7	0.0
Skeletal variations				
Total no. of foetuses with skeletal variations	43	48	39	49
Total no. of litters with skeletal variations	13	18	15	15
Percentage of litters with skeletal variations (%)	92.8	100	100	100
External and visceral findings				
No. of litters examined	14	18	15	15
No. of foetuses examined	128	157	119	134
No of litters with anomalous foetuses	2	5	2	3
Percentage of litters with anomalous foetuses (%)	14.3	27.8	13.3	20
No. of litters with major malformations	2	2	2	1
Percentage of litters with malformed foetuses (%)	14.3	11.1	13.3	6.7

III. CONCLUSIONS

The oral administration of glyphosate technical to pregnant rabbits by gavage from gestation day 7-19 resulted maternal toxicity at 200 and 400 mg/kg bw/day. There were no treatment-related effects on pregnancy or foetuses at any dose level. Therefore the 'No Observed Adverse Effect Level' (NOAEL) was considered to be 50 mg/kg bw/day for maternal toxicity. The NOAEL for developmental toxicity was considered to be 400 mg/kg bw/day. It was considered that the test material had no teratogenic potential at dose levels up to 400 mg/kg bw/day.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study is considered valid, though treatment duration covered the period from implantation through organogenesis rather than through to the day prior to necropsy, as recommended in the current OECD 414 guideline.

The oral administration of glyphosate technical to pregnant rabbits by gavage from gestation Day 7-19 resulted maternal toxicity at 200 and 400 mg/kg bw/day. Therefore the 'No Observed Adverse Effect Level' (NOAEL) was considered to be 50 mg/kg bw/day for maternal toxicity. There were no treatment-related effects on pregnancy or foetuses at any dose level. The NOAEL for developmental toxicity was considered to be 400 mg/kg bw/day.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	**CA 5.6.2/011**
Report author	[redacted]
Report year	1995
Report title	HR-001: A Teratogenicity Study in Rabbits
Report No	[redacted] 94-0153
Document No	M-301383-01-1

Guidelines followed in study	OECD 414 (1981), JMAFF 59 NohSan 4200 (1985), US-EPA 83-3 (1984)
Deviations from current test guideline (OECD 414, 2018)	Dosed from day of implantation through the period of organogenesis, rather than through to day prior to necropsy
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

In the preliminary study, [redacted] 94-0140, appended to the final report [redacted] 94-0153, groups of six pregnant does were dosed with 0, 30, 100, 300 or 1000 mg/kg bw/day glyphosate. All high dose animals presented loose stools and died. All animals at 300 mg/kg bw/day survived, one animal presented loose stools, subsequently aborted and was found dead.

In the definitive study, glyphosate was administered by gavage to three groups of 18 artificially inseminated Japanese White rabbits each, at doses of 10, 100 and 300 mg/kg bw/day from gestation day 6-18 (artificial insemination = day 1). A further group of 18 animals was exposed to the vehicle to serve as control.

No adverse effects related to test substance treatment were observed in any animals of the low and mid dose group. In the high dose group, defecation of loose stool and subsequent abortion or premature delivery was considered to be related to test substance treatment with regard to the results of the preliminary study.

Mean maternal body weights and body weight gains on days 16-24 of gestation in the high dose group decreased slightly although the differences from controls were not statistically significant.

Examinations at caesarean sectioning demonstrated no significant differences in the gravid uterine weights and the numbers of corpora lutea and implants between the control and the treated groups. The mean number of live foetuses, mean percent incidences of resorptions and foetal deaths, foetal sex ratios, mean foetal body weights, and mean placental weights in the treated groups were comparable to those in the control group.

Teratological examinations demonstrated no test substance-related external, visceral and skeletal abnormalities in any foetuses in any treated groups.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material:

Identification:	Glyphosate technical, Code: HR-001
Description:	White crystal
Lot/Batch #:	T-941209
Purity:	97.56 %
Stability of test compound:	Not reported
Vehicle:	Purified water with 0.5% carboxymethylcellulose

Test Animals:

Species:	Rabbit
Strain:	Japanese White rabbits Kbl:JW, SPF
Source:	[redacted]
Age:	18 weeks (females)
Sex:	Females (males were used for mating only)
Weight at dosing:	3.32 - 4.08 kg
Acclimation period:	10 days
Diet/Food:	GC4 (Oriental Yeast Co., Ltd.), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in aluminium cages with wire-mesh floors.
Environmental conditions:	Temperature: 22 ± 2 °C Humidity: 55 ± 10 % Air changes: 15/hour 12 hours light/dark cycle

B. STUDY DESIGN

In life dates: 1995-03-31 to 1995-06-09

Animal assignment and treatment:
In a teratogenicity study groups of 18 Japanese White female rabbits received doses of 0, 10, 100 and 300 mg/kg bw/day test substance in carboxymethylcellulose by gavage from Gestation day 6-18 after artificial insemination performed on 12 or 16 females each day for 5 consecutive days. The dose levels were chosen based on results of a preliminary teratogenicity study.

Diet preparation and analyses
For each dose level, dosing solutions were prepared two times during the study by suspending the test substance in purified water with the aid of 0.5 % sodium carboxymethylcellulose. For each dose level dosing solutions were analysed for concentration of the test substance before use.

Clinical observations
A check for clinical signs of toxicity, ill-health or behavioural changes was made once daily during the pre- and post-dosing periods and twice daily (before and after dosing) during the dosing period.

Body weight
Individual body weights were recorded on day 0, 6-18, 24 and 27 of gestation. Body weight gains were calculated by subtracting the body weight value on Day 0 of gestation from each value determined on days 6 through 27 of gestation. Adjusted weights were also calculated by subtracting the gravid uterine weight from the body weight on day 27 of gestation.

Food consumption
Food consumption of females was determined on alternate days from day 0 to day 26 of gestation and on days 26 to 27 of gestation. In each interval, daily food consumption (g/rabbit/day) was calculated for each female by dividing values of total food consumption by the number of days.

Sacrifice and pathology
Females were euthanatised by an injection of an overdose of a pentobarbital sodium solution into the

auricular vein on day 27 of gestation and subjected to caesarean sectioning.
The ovaries and uteri were removed, weighed and then examined for the number of corpora lutea and for the number and position of implants and dead or live foetuses. Resorptions and foetal deaths were classified into implantation sites, placental remnants, and macerated foetuses according to the difference in developmental stage at which deaths had occurred. When uterine implants were not grossly apparent, the uteri were stained with 10 % ammonium sulfide solution to detect very early resorptions. After examination of the ovaries and conceptuses, each female was necropsied.

Developmental parameters
Live foetuses and their placentas were individually weighed. Live foetuses were uniquely identified by litters. Then they were euthanatised by an intraperitoneal injection of a pentobarbital sodium solution and examined for external abnormalities. The eyes were examined for alterations after removing the palpebral skin. The sex of the foetuses was determined by observation of the gonads.
After these examinations, each foetus was examined for visceral abnormalities. Then the thoracic and abdominal organs were removed and preserved in 10 % neutral- buffered formalin along with the ovaries and placentas. The remaining skeletons were fixed in 70 % isopropanol, stained with alizarin red S and cleared in 70 % glycerin for examination of skeletal abnormalities. After examination, skeletal specimens were stored.

Statistics
The following statistical tests were used to estimate significance of differences between the control group and the treated groups. The data on body weights, adjusted body weights, body weight gains, and food consumption of maternal rabbits, numbers of corpora lutea, implants, and live foetuses, and weights of gravid uteri, foetuses and placentas were evaluated as follows: Equality of variances was first evaluated by Bartlett's test. When group variances were homogeneous, a parametric analysis of variance in one-way classifications was used to determine if any statistical differences exist among groups. If the analysis of variance was significant, Dunnett's t-test or Scheffe's multiple comparison test was performed to detect any statistically significant differences between the treated groups and their corresponding controls. When Bartlett's test indicated that the variances were not homogeneous, Kruskal-Wallis test was used for detecting any statistical differences among groups and if significant, Dunnett-type mean rank test or Scheffé-type mean rank test was performed to detect statistical differences between the treated groups and their corresponding controls. Fisher's exact probability test was used for the data on the incidences of clinical and gross pathological findings in maternal rabbits, incidences of maternal rabbits having foetuses with malformations and variations, incidences of foetal malformations and variations, and foetal sex ratio, and Mann-Whitney's U-test for the data on the percent incidences of resorptions and foetal deaths.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS
The test substance was detected at levels of 95-105 % of the target concentrations in each dosing solution. Analyses for homogeneity indicated that the dose preparations were homogeneous. The test substance was stable in the 0.5 % carboxymethylcellulose solution for at least 14 days after preparation.

B. FOOD CONSUMPTION
Mean food consumption in the treated groups was comparable to that in the control group throughout the study period.

C. MORTALITY
One rabbit in the high dose group died on day 20 of gestation without showing any clinical signs. Females with resorptions only had no grossly observable conceptus but had implantation sites in the uteri. These

animals were excluded form the statistical evaluation.

Table 5.6.2-37: Glyphosate technical: Teratology study in the rabbit ([redacted], 1995): Survival and pregnancy status in maternal rabbits

Dose level (mg/kg bw/day)	No. of females mated	No. of females pregnant	No. of females died or killed	No. of females aborted	No. of females survived	No. of females with resorptions only	No. of females with live foetuses
0	18	18	0	0	18	0	18
10	18	18	0	2	16	1	15
100	18	18	0	0	18	2	16
300	18	18	1	2	15	1	14

D. CLINICAL OBSERVATIONS

During the treatment period one animal each showed hair loss (forelimb) and scab on the auricle, respectively in low and mid dose group, respectively (see table below). In the high dose group four animals showed loose stool and two showed soiled fur in the perianal region that was considered to be an alteration caused by defecation of loose stool. The incidence of loose stool was significantly high when compared with the control.

During the post-dosing period, two and one animal in the control group showed loose stool and red material on the tray, respectively. In the low dose group, hair loss (forelimb) was found in one animal and loose stool in another. Besides these findings, one dam aborted on day 20 of gestation, and another one prematurely delivered on day 27 of gestation. In the mid dose group only one animal showed hair loss in the lower abdominal region. In the high dose group, two animals out of four, that had shown loose stool during the dosing period, still showed this alteration, and one animal out of these two aborted on day 26 of gestation. Although loose stool disappeared from the two other dams, the first prematurely delivered on day 27 of gestation and the second had hair loss (dorsal region).

Considering the results of the preliminary study, defecation of loose stool and subsequent abortion or premature delivery observed in the highest dose group were considered to be related to test substance treatment.

Table 5.6.2-38: Glyphosate technical: Teratology study in the rabbit ([redacted], 1995): Observed clinical signs during the dosing period

Clinical sign	Number of rabbits affected in dose group[#]			
	Control (0 mg/kg bw/day)	Low (10 mg/kg bw/day)	Mid (100 mg/kg bw/day)	High (300 mg/kg bw/day)
No abnormalities detected	18/18 (0)	16/17 (1)	15/16 (2)	13/17 (0)
Hair loss	0/18 (0)	1/17 (0)	0/16 (0)	0/17 (0)
Scab on the auricle	0/18 (0)	0/17 (0)	1/16 (0)	0/17 (0)
Soiled fur in the perianal region	0/18 (0)	0/17 (0)	0/16 (0)	2/17 (0)
Loose stool	0/18 (0)	0/17 (0)	0/16 (0)	4/17 (1)*

[#] x/y: number affected / total number of animals in group

* Significantly different from control at $p<0.05$.

Figures in parentheses represent the number of animals having no grossly observable conceptus. These animals were excluded from statistical evaluation.

E. BODY WEIGHT

Mean body weights of animals in the low and mid dose group were comparable to those in the control group. In the high dose group, although differences from controls were not statistically significant, the mean values on days 15-24 of gestation were somewhat lower than those in the control group.

Table 5.6.2-39: Glyphosate technical: Teratology study in the rabbit ([redacted], 1995): Body weight gain (g) during gestation

Dose level		Body weight on gestation day														
mg/kg bw/day		0-6	0-7	0-8	0-9	0-10	0-11	0-12	0-13	0-14	0-15	0-16	0-17	0-18	0-24	0-27
0	Mean	95	113	110	119	122	145	166	183	193	193	192	203	212	260	286
	SD	40	57	65	55	63	64	95	115	132	149	160	165	172	244	278
	No.	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18
10	Mean	104	119	117	135	145	173	195	225	225	234	241	256	250	335	351
	SD	40	48	53	60	64	74	81	84	97	118	126	140	139	186	151
	No.	17	17	17	17	17	17	17	17	17	17	17	17	17	16	15
100	Mean	129	143	139	156	162	200	213	253	270	280	290	305	309	383	442
	SD	67	74	74	101	90	114	104	109	107	111	111	112	120	160	174
	No.	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16
300	Mean	116	122	121	125	121	151	157	176	175	164	134	145	147	193	322
	SD	62	75	87	95	91	115	143	176	200	224	244	256	286	363	216
	No.	17	17	17	17	17	17	17	17	17	17	17	17	17	16	14

F. PATHOLOGY

Necropsy

Necropsy of maternal animals aborted, prematurely delivered or found dead on the study noted no abnormalities in the rabbits in the low dose group. In the high dose group, the aborted rabbit had yellow-coloured adipose tissue, a hair bolus in the stomach, watery contents in the large intestine and accentuated lobular pattern in the liver. The prematurely delivered rabbit in the high dose group had soiled fur in the perianal region, erosion in the stomach, a hair bolus in the stomach, and watery contents in the caecum. In the dead rabbit, pale liver and ascites (red) in the abdominal cavity were found; however, the cause of death was not known.

Gross pathological findings observed in animals which survived to termination of the study were: hair loss in the lower abdominal or dorsal region in one animal in each of the mid and high dose groups; hair bolus in the stomach in one animal each of the control and low dose groups. The occurrence of these gross pathological findings was low, and considered to be unrelated to test substance treatment.

Observations on the ovary and uterus

In the control, low, mid and high dose groups, 18, 16, 18, and 15 females, respectively, survived to termination of the study and were proven to be pregnant. However, one, two and one females in the low, mid and high dose group, respectively, had no grossly observable conceptus while implantation sites were detected by uterine staining with a 10 % ammonium sulfide solution, indicating very early resorptions; all data from these females were excluded from subsequent calculations.

Examinations of uterine contents demonstrated no abnormalities in all groups including the control. Mean gravid uterine weights and mean numbers of corpora lutea and implants were comparable between the control and the treated groups.

Table 5.6.2-40: Glyphosate technical: Teratology study in the rabbit ([redacted], 1995): Intergroup comparison of maternal performance

Observation	Glyphosate acid (mg/kg bw/day)

	0 (control)	10	100	300
# Animals mated	18	18	18	18
# Animals Pregnant	18	18	18	18
# Pregnancy status (non-pregnant)	0	0	0	0
# Pregnant does died or killed in extermis	0	0	0	1
# Does aborted	0	2	0	2
# Litters	18	16	18	15
Gravid Uterine Weight (g) (mean ± S.D.)	407±111	454±127	488±118	416±155
Mean Placental Weight (mg)	4928	5156	4717	4697
No. of corpora lutea (mean ± S.D.)	10.2±2.0	11.7±2.2	12.1±2.0	10.1±2.3
No. of implantations (mean ± S.D.)	8.5±2.8	9.8±2.9	10.4±2.9	8.6±3.3
No. of Live Foetuses (mean ± S.D.)	7.8±2.4	8.7±3.2	9.4±2.7	8.0±3.2
% of Foetal resorption and death	7.1	13.8	8.7	6.5
Males Mean Foetal Weight (g) ± S.D.	35.8±8.1	37.3±5.4	36.7±3.3	36.2±5.4
Females Mean Foetal Weight (g) ± S.D.	35.7±6.7	36.1±5.1	36.0±3.9	34.9±4.4
Sex Ratio	0.49	0.53	0.49	0.47

G. DEVELOPMENTAL PARAMETERS

Number and viability of foetuses

No statistically significant differences were noted in the mean number of live foetuses and mean percent incidences of resorptions and foetal deaths between the control group and the treated groups.

Sex ratio, foetal body weights and placental weights

No statistically significant differences were noted in the sex ratios, mean foetal body weights, mean placental weights, mean number of live foetuses, and mean percent incidences of resorptions and foetal deaths between the control group and the treated groups.

External, visceral and skeletal examination

No statistically significant differences were noted in the incidences of maternal animals having foetuses with external, visceral and/or skeletal malformations in the low and mid dose groups when compared with the controls. In the high dose group, the number of litters with malformations was significantly higher than that in the control group (see table below). This increased malformation rate was due to an increase in skeletal malformations, as no external or visceral malformations were noted in foetuses from the high dose group. This was considered to be a sporadic alteration rather than the test substance treatment-related alteration because the types of skeletal malformations observed were inconsistent. Further, a dose-response in the number of foetuses showing skeletal malformations was not evident across dose groups.

With regard to variations, the incidence of total no. of litters with skeletal variations in the 100 mg/kg bw/day group was significantly higher than that in the control group (see table below). This high value was due to a significantly high incidence (87.5 % of litters, 27.3 % of the foetuses) of lumbar ribs in this dose group when compared with the control (72.2 % of litters, 16.4 % of foetuses). The total litter incidence for skeletal variations in the 100 mg/kg bw/day group was 100 %. However, the increased incidence of lumbar ribs in the 100 mg/kg bw/day group was considered to be a sporadic alteration because the value was within the historical control range (8.1-35.0 % of examined foetuses), and because no such increase was observed in the 300 mg/kg bw/day group (13.4 %).

Table 5.6.2-41: Glyphosate technical: Teratology study in the rabbit ([redacted], 1995): Observed clinical signs during the dosing period

Dose level (mg/kg bw/day)	No. of pregnant females examined	No. of females having anomalous foetuses (%)	No. of foetuses examined	Observations		
				No. of foetuses with external malformations (%)	No. of foetuses with visceral malformations (%)	No. of foetuses with skeletal malformations (%)
0	18	1	140	0 (0.0)	0 (0.0)	1 (0.7)
10	15	3	130	0 (0.0)	1 (0.8)	4 (3.1)
100	16	3	150	2 (1.3)	3 (2.0)	6 (4.0)
300	14	5*	112	0 (0.0)	0 (0.0)	5 (4.5)

* Significantly different from control at p<0.05.

Table 5.6.2-42: Glyphosate technical: Teratology study in the rabbit ([redacted], 1995): Incidence of foetal malformations and variations

Foetal findings	Dose level (mg/kg bw/day)			
	0	10	100	300
Malformations				
No. of litters examined	18	15	16	14
No. of foetuses examined	140	130	150	112
No of litters with anomalous foetuses	1	3	3	5*
Percentage of litters with malformations (%)	5.5	20.0	18.8	35.7
Skeletal malformations				
Fusion of the frontal/parietal bones	0	1	0	2
Fissure of the parietal bone	0	0	3	0
Hypoplasia of the interparietal bone	0	1	0	0
Splitting of the parietal bones	0	0	3	1
Shortening of the nasal/frontal/mandibular bones	0	0	1	0
Hemivertebra	1	0	0	2
Unilateral ossification centre of the thoracic/lumbar vertebral bodies	0	1	0	0
Bifurcation of the ribs	1	0	0	0
Sternal cleft	0	0	1	0
Splitting of the sternebrae with sternocostal joint displacement	0	2	0	0
Total no. of foetuses with skeletal malformations	1	4	6	5
Percentage of foetuses with skeletal malformations (%)	0.7	3.1	4.0	4.5
Total no. of litters with skeletal malformations	1	3	2	5
Percentage of litters with skeletal malformations (%)	5.6	20.0	12.5	29.4
Variations				
No. of litters examined	18	15	16	14
No. of foetuses examined	140	130	150	112
No of litters with anomalous foetuses	16	14	16	8*
Percentage of litters with variations (%)	88.9	93.3	100	57.1
Skeletal variations				
No. of foetuses examined	140	130	150	112
27 presacral vertebrae	4	1	4	3
27 presacral vertebrae with 13th ribs	12	9	15	12
Cervical ribs	1	3	1	1
Lumbar ribs	23	19	41*	15
Extra ossification centre anterior to the 1st sternebra with costal cartilage joining	0	0	0	1
Total no. of foetuses with skeletal variations	40	32	61*	31
Total no. of litters with skeletal variations	16	12	16	8
Percentage of litters with skeletal variations (%)	88.9	80	100	57.1

* Significantly different from control at p<0.05.

III. CONCLUSIONS

Study conclusion:
The oral administration of glyphosate to artificially inseminated rabbits by gavage from gestation day 6-18 resulted in treatment-related changes at 300 mg/kg bw/day. Therefore the 'No Observed Adverse Effect Level' (NOAEL) was considered to be 100 mg/kg bw/day for maternal toxicity. No developmental toxic effects were observed. The NOAEL for offspring was considered to be 300 mg/kg bw/day.

1. Assessment and conclusion

Assessment and conclusion by applicant:

The study is considered acceptable, though treatment duration covered the period from implantation through organogenesis rather than through to the day prior to necropsy, as recommended in the current OECD 414 guideline. The NOAEL for maternal and developmental effects was 100 mg/kg bw/day and 300 mg/kg bw/day, respectively.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	**CA 5.6.2/012**
Report author	[redacted]
Report year	1993
Report title	Teratogenicity study in rabbits – Test compound: Glyphosate technical
Report No	TOXI: 884-TER-RB
Document No	not available
Guidelines followed in study	OECD 414 (1981)
Deviations from current test guideline (OECD 414, 2018)	Dosed from day of implantation through the period of organogenesis, rather than through to day prior to necropsy
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Invalid
Category study in AIR 5 dossier (L docs)	Category 2b

CA 5.6.2/013
1. Information on the study

Data point:	**CA 5.6.2/013**
Report author	[redacted]
Report year	1993

Report title	Amendment to final report - Teratogenicity study in rabbits – Test compound: Glyphosate technical (FSG 03090 h/05 March 1990)
Report No	TOXI: 884-TER-RB
Document No	not available
Guidelines followed in study	OECD 414 (1981)
Deviations from current test guideline (OECD 414, 2018)	Dosed from day of implantation through the period of organogenesis, rather than through to day prior to necropsy
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Invalid
Category study in AIR 5 dossier (L docs)	Category 2b

2. Full summary

This developmental toxicity study in rabbits is not clearly or accurately documented and does not provide appropriate interpretation of the study findings. Groups of presumed mated female New Zealand White rabbits were administered once daily by gavage, glyphosate (batch No.: 60, purity 96.8 %) in 0.5 % carboxymethylcellulose (dose volume 2 mL/kg bw) at dose levels of 0 (vehicle control), 20, 100 or 500 mg/kg bw/day from day 6 to day 18 of pregnancy (mating = day 0). Dose volumes were calculated for individual animals from day 6 and adjusted according to body weight daily. The day of mating was considered as day 0. Dose levels were based on the findings of preliminary studies. All animals were observed twice daily for onset and duration of signs of toxicity and for mortality. All animals in the experiment that died, were killed moribund or killed at termination on day 28 were subjected to *post mortem* gross pathological examination. Individual body weights of dams were recorded on days 0, daily from days 6 – 18 and on day 28 of gestation.

The ovaries and uteri were examined to determine the number of corpora lutea, implantations, the number of dead/abnormal/live foetuses, the number and distribution of embryonic and /foetal resorptions. Foetuses were examined for individual foetal weight and foetal abnormalities, sex and visceral organ malformations by foetal necropsy/modified Wilson's technique. The eviscerated and skinned foetuses were stained with Alizarin Rad-S for skeletal evaluation.

There were 4/16 and 8/15 mortalities at dose levels of 100 and 500 mg/kg bw/day, respectively. The deaths at 500 mg/kg bw/day were accompanied by clinical signs, including the increased incidence of soft liquid/diarrhoea or mucoid faeces, reduction in feed consumption and reduction in body weight gain during the treatment period. However, clinical signs were not evident for the 100 mg/kg bw/day dose group and only one incidence of soft faeces was recorded at 100 mg/kg bw/day. Further, a number of lung and tracheal findings in the dams at gross necropsy indicated possible gavage errors to which the deaths at this dose may be attributed.

Glyphosate technical was considered not to be teratogenic in this developmental toxicity study in rabbits. The incidence of one visceral effect, dilated heart, was increased at the highest test dose and was present at lower dose levels, but there were too few foetuses present in the high dose group to corroborate a dose-response relationship. Further, foetal findings at the highest test dose were observed in the presence of extensive maternal toxicity. Mortality and clinical signs of toxicity at 500 mg/kg bw/day included reduced feed consumption and soft faeces and reduced body weight gain during the dosing period one incidence of complete resorptions.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material:

Identification:	Glyphosate technical
Description:	Odourless white crystals
Lot/Batch #:	60
Purity:	96.8 %
Stability of test compound:	Stable over 2 years at ambient temperature
Vehicle:	0.5 % w/v carboxymethylcellulose
Test Animals:	
Species:	Rabbit
Strain:	Female New Zealand White
Source:	[redacted]
Age:	Approximately 6 months and above (at the start of study)
Sex:	Females (males were used for mating only)
Weight at dosing:	Females: >2500 g (group mean values)
Acclimation period:	At least 10 days.
Diet/Food:	Pelleted rabbit diet, supplied by M/S Lipton India Ltd, Bangalore (composition and feed analysis reports were provided) was provided, *ad libitum*
Water:	Protected deep bore well drinking water, treated via activated charcoal filter and UV in Aquaguard on-line water filter-cum-purifier provided, *ad libitum*
Housing:	Individually in 3-tier all aluminium cages with wire mesh bottom and common self-draining litter trays.
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 40 – 70 % Air changes: 10 – 15/h Natural lighting supplemented with fluorescent lighting 12 hours light/dark cycle

B. STUDY DESIGN

In life dates: not reported

Animal assignment and treatment:
In a developmental toxicity study, groups of presumed mated female New Zealand White rabbits were administered once daily by gavage, glyphosate (batch no.: 60, purity 96.8 %) in 0.5 % carboxymethylcellulose (dose volume 2 mL/kg bw) at dose levels of 0 (vehicle control), 20, 100 or 500 mg/kg bw/day from day 6 to day 18 of pregnancy. Dosages for individual animals were calculated from day 6 and adjusted daily according to body weight. Dose levels were based on the findings of preliminary studies.

In a preliminary dose-range finding study, one male rabbit/dose group was administered glyphosate

technical dissolved in 0.5 % carboxymethylcellulose (dose volume 2 mL/kg bw) by gavage at dose levels of 0 (control), 10, 20, 50, 500 or 1000 mg/kg bw/day for 13 days. Doses of ≥ 500 mg/kg bw/day resulted in loss in body weight and in feed intake and the 1000 mg/kg bw/day test animal died on day 9 of treatment.

In a second dose-range finding study, one pregnant rabbit was administered 500 mg/kg bw/day glyphosate from day 6 to 18 of gestation and the findings compared with that of 20 historical control animals. Caesarean section and terminal necropsy was performed on day 28. There were no signs of toxicity from the treatment; body weight gain was greater (26 % more than the historical control mean) but notable apparent treatment-related changes were substantial reduction in feed intake (34 % of historical control mean) and reduced litter size in the test female (4) compared with the historical control mean (7).

Observations
All animals were observed twice daily for onset and duration of signs of toxicity and for mortality. All animals in the experiment that died, were killed moribund or killed at termination were subjected to *post mortem* gross pathological examination. Tissues with gross lesions were preserved for histopathological examination as necessary.

Body weight
Individual body weights were recorded on days 0, daily from days 6 – 18 and on day 28 of gestation.

Food consumption and compound intake
Food consumption was recorded on days of weighing throughout gestation.

Sacrifice and pathology
On day 28 of pregnancy, all surviving dams were subjected to *post mortem* examinations and pups were delivered by Caesarean section. The ovaries and uteri were excised and weighed and maternal and foetal data were recorded. The maternal data determined were pregnant/non-pregnant, uterine weight, the number of corpora lutea, the number of implantations, the number of embryonic and foetal resorptions. The foetal data recorded were the number of dead/abnormal/live foetuses, individual foetal weight and sex.

Litter parameters
All the foetuses were examined for external, visceral and skeletal abnormalities employing appropriate techniques. Live young were euthanised with ether and visceral organs examined by a modified Wilson technique. Skeletal assessments were performed after appropriate preparation including staining in Alizarin Red. Structural changes were presented as variants, minor and major malformations.

Statistics
Statistical methods employed included the following. Maternal body weight and weight gain, feed intake, number of corpora lutea, number of implantations and mean foetal weight were analysed by Bartlett‘s test followed by ANOVA and Dunnett’s test. Day ‘0’and absolute body weight data were compared by the Paired Student’s ‘t‘ test. The number and percent embryonic resorptions and foetal resorptions, the number of dead foetuses, the number of abnormal foetuses and percentage pre-implantation and post-implantation loss by Mann Whitney test. Litter size was by Student ‘t‘ test. The sex ratio, number of dams with any resorptions, number of dams with all resorptions and incidence of malformations were analysed by Chi-square test.

The statistical analysis and comparison of individual treatment groups with control value were done at 5 % probability level and the results were designated as significantly higher (+) / lower (-) than control value at $P \leq 0.05$.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS

Not reported.

B. FOOD CONSUMPTION

During the dosing period, feed consumption was significantly reduced (31 %) in females receiving the 500 mg/kg bw/day dose compared to the controls (see table below). Feed consumption during the post-treatment period did not show significant intergroup differences.

Table 5.6.2-43: Glyphosate technical: Teratology study in the rabbit ([redacted] 1993): Summary of food consumption (mean ± SD)

Parameter	Historical positive control#	Dose Group (mg/kg bw/day)			
		0 (control)	20	100	500
Food consumption (g/rabbit/day)					
No of dams included in assessment	7	20	13	12	6
Day 0 – 6 (Pre-treatment)	105 ± 21.6	114 ± 31.8	88 ± 23.4*	125 ± 14.7	118 ± 25.4
Day 6 – 19 (Treatment)	70 ± 36.5*	103 ± 26.5	109 ±19.4	102 ±31.5	71 ± 56.9*
Day 19 – 28 (Post treatment)	129 ± 12.5	109 ± 38.7	135 ± 34.4	107 ± 40.1	105 ±25.4
Day 0 - 28	96 ± 22.5	107 ±27.3	113 ± 18.2	108 ± 24.4	92 ± 37.9

Treatment with Acetylsalicylic acid (ASA) at 200 mg/kg bw (treatment: day 6-18; post-treatment: day 18-28)
* Significantly lower than controls by Dunnett's test P≤0.05

C. MORTALITY

The four and eight deaths observed in the mid- and high-dose group were considered to be treatment-related by the study director. However, the two confirmed misdosings in the control, the absence of signs of toxicity at 100 mg/kg bw/day and the absence of mortality in this dose range in the considerably high number of parallel studies shed serious doubt on a relation to treatment at this dose level. Further, various findings at gross necropsy were noted in the lungs and trachea for the 100 and 500 mg/kg bw/day dose groups; these findings suggest possible gavage errors, which could be responsible for some of the deaths observed at these doses and and are not appropriately discussed in the report. The number of deaths clearly not attributable to gavage dosing errors for each dose group fall within the range of historical control deaths for this laboratory.

Table 5.6.2-44: Glyphosate technical: Teratology study in the rabbit ([redacted] 1993): Summary of mortality in dams

Parameter	Dose Group (mg/kg bw/day)			
	0 (control)	20	100	500
Mated females	26	17	16	15
Dead during treatment	1*	0	4 (1)	5 (2)
Died post-treatment	1*	0	0	3
Total number of deaths (historical control range 1-4)	2 (2)	0	4 (1)	8 (5)
% mortality	7.7	0.0	25.0	53.3

* Animal died due to wrong gavaging
Parentheses indicate number of deaths with necropsy reporting lung/trachea findings suggestive of gavage errors, such as spillage of gavage fluid into the trachea and lungs.

D. CLINICAL OBSERVATIONS

Signs of toxicity were observed at the 500 mg/kg bw/day dose group and were predominantly

gastrointestinal effects, which included soft stool/liquid faeces and soft stool with mucus. Further signs of toxicity were rales, weakness, dyspnoea and ocular discharge.

Table 5.6.2-45: Glyphosate technical: Teratology study in the rabbit ([redacted] 1993): Summary of relevant clinical signs in dams

Parameter / clinical sign	Dose Group (mg/kg bw/day)			
	0 (control)	20	100	500
Mated females	26	17	16	15
Pregnant at termination	20	13	12	6
Rales	1	0	0	3
Soft stool with mucus	0	0	0	2
Soft stool/liquid faeces	0	0	1	12
Weak	0	0	0	2
Ocular discharge	0	0	0	1
Dyspnoea	0	0	0	1

E. BODY WEIGHT

No treatment-related and dose-related significant changes were observed in maternal body weight and body weight gain between the control, low- and mid-dose groups. In the high-dose group, initial body weight and body weights at the different time intervals were significantly lower than in the control group.

Table 5.6.2-46: Glyphosate technical: Teratology study in the rabbit ([redacted] 1993): Summary of maternal body weight data (mean ± SD)

Parameter	Historical positive control#	Dose Group (mg/kg bw/day)			
		0 (control)	20	100	500
Number of dams pregnant at termination		20	13	12	6
Mean body weights (kg)					
Day 0		3.1 ± 0.5	2.8 ± 0.4	3.0 ± 0.2	2.6 ± 0.6*
Day 6		3.2 ± 0.5	3.0 ± 0.4	3.0 ±0.2	2.8 ± 0.7*
Day 18		3.2 ± 0.5	3.1 ± 0.5	3.1 ± 0.3	2.8 ± 0.7
Day 28		3.3 ± 0.5	3.3 ± 0.4	3.3 ±0.3	3.0 ± 0.7*
Day 28 (body weight – uterine weight)	2.7*	3.0	3.0	2.9	2.7
Mean body weight gain (kg)					
Day 0 – 6 (Pre-treatment)		0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.0 ± 0.1	0.1 ± 0.1
Day 6 – 18 (Treatment)		-0.1 ± 0.2	0.1 ± 0.1	0.1 ± 0.2	0.1 ± 0.1	0.0 ± 0.2
Day 18 – 28 (Post-treatment)		0.2 ± 0.2	0.1 ± 0.1	0.2 ± 0.2	0.2 ± 0.1	0.2 ± 0.2
Day 0 – 28 (Throughout gestation)		0.3 ± 0.2	0.2 ± 0.3	0.5 ± 0.2**	0.3 ± 0.1	0.3 ± 0.3

Treatment with Acetylsalicylic acid (ASA) at 200 mg/kg bw/day
* Significantly lower than controls by Dunnett's test P≤0.05
** Significantly higher than controls by Dunnett's test P≤0.05

F. PATHOLOGY

Necropsy

Gross examination of dams at *post mortem* did not identify any treatment-related effects. However, various findings were noted in the lungs and trachea for the 100 and 500 mg/kg bw/day dose groups which suggest possible gavage errors and issues with animal husbandry; 2/2, 0/0, 1/4, and 5/8 deaths attributable to dosing errors at 0, 20, 100 and 500 mg/kg bw/day, respectively. There include gavage needle puncture/damage, and spillage of gavage fluid into the trachea and lungs.

Observations on the ovary and uterus
A total of 20, 13, 12 and 6 pregnant females survived to termination and 134, 80, 78 and 28 foetuses were recorded for the 0 (control), 20, 100 and 500 mg/kg bw/day dose groups, respectively, and were included in the assessment. Litter size at caesarean necropsy was comparable in all treatment groups. Total litter loss (complete resorptions) was recorded for one female in the 500 mg/kg bw/day dose group; otherwise, the incidence of dams with any resorptions did not show any treatment-related differences.

There were no significant intergroup differences in the mean numbers of corpora lutea, pre-implantation and post-implantation losses and resorptions (embryonic and foetal).

Table 5.6.2-47: Glyphosate technical: Teratology study in the rabbit ([redacted] 1993): Summary of maternal observations

Parameter	Historical positive control#	Dose group (mg/kg bw/day)			
		0 (control)	20	100	500
Mated females	12	26	17	16	15
Total number of deaths	4	2 (2)	0	4(1)	8 (5)
Pregnant at termination	7	20	13	12	6
Mean number of corpora lutea ± SD	9 ± 2.3	11 ± 2.8	10 ±2.4	10 ± 1.9	9 ± 2.0
Mean number of implantations ± SD	8 ±1.8	8 ± 2.0	8 ± 1.5	9 ± 1.8	6 ± 2.4
Total number of embryonic resorptions (%)	6 (11)	10 (7)	11 (11)	11 (11)	9 (24)
Total number of foetal resorptions (%)	2 (4)	8 (5)	7 (7)	13 (13)	1(3)
Total number of pre-implantation loss (%)	10 (19)	72 (48)	28 (29)	20 (20)	14 (37)
Total number of post-implantation loss (%)	8 (15)	18 (12)	18 (18)	24 (24)	10 (26)
Number of dams with any resorptions (%)	2 (29)	12 (60)	11 (85)	9 (75)	2 (33)
Dams with complete resorptions (%)	1 (14)	0 (0)	0 (0)	0 (0)	1 (17)

\# Treatment with acetylsalicylic acid (ASA) at 200 mg/kg bw
Parentheses indicate number of deaths with necropsy reporting lung/trachea findings suggestive of gavage errors

G. DEVELOPMENTAL PARAMETERS
Number and viability of foetuses
Because of the large number of maternal deaths at 500 mg/kg/day (and thus, the reduced number of total litters), the total number of foetuses was substantially less in this dose group compared to the other dose groups. However, the mean litter size, the mean numbers of abnormal, dead or live foetuses and the sex ratios of foetuses did not show any significant treatment-related differences. Glyphosate also did not cause an increase in the number of foetal deaths in utero (see table below).

Foetal body weights
Although foetal body weights in the 20 and 100 mg/kg/day dose groups were reported to be significantly different from control, the weights were increased, the changes were less than 10 % of control values and no dose-response across treatment groups was evident. Thus, the foetal body weight differences observed in these two dose groups are biologically inconsequential with respect to adverse effects.

Table 5.6.2-48: Glyphosate technical: Teratology study in the rabbit ([redacted] 1993): Mean litter data at caesarean section

	Historical positive control#	Dose group (mg/kg bw/day)			
		0	20	100	500
Mated females	12	26	17	16	15
Total number of deaths	4	2	0	4	8
Pregnant at termination	7	20	13	12	6

Number of litters	6	20	13	12	5
Total number of foetuses	46	134	80	78	28
Mean litter size	8	7	6	7	6
Abnormal foetuses (%)	0 (0)	1 (1)	2 (3)	0	0
Dead foetuses (%)	0 (0)	0 (0)	1 (1)	1 (1)	0 (0)
Post-implantation loss (%)	8 (15)	18 (12)	18 (18)	24 (24)	10 (26)
Number of live foetuses	46	133	77	77	28
Mean weight of live foetuses (g ± SD)	29 ± 1.4	32 ± 5.3	35 ± 3.7*	35 ± 2.4*	33 ± 4.9
Sex ratio (Male : Female)	1 : 1.3	1 : 0.7	1 : 1.2	1 : 1.2	1 : 1.8

# Treatment with acetylsalicylic acid (ASA) at 200 mg/kg bw
SD = standard deviation
* Significantly higher than controls by Dunnett's test P≤0.05

External, visceral and skeletal examination

The incidence of major external malformations did not identify any treatment-related differences; further, none of the external malformations occurred in the highest dose group.

Visceral examination noted no significant treatment-related incidences of minor malformations or variants. Major visceral malformations primarily affected the heart, but occurred in single incidences and showed no dose-response. The exception was dilated heart, which was reported in four foetuses of 3 litters in the 20 mg/kg bw/day dose group, 4 foetuses (3 + 1) from 2 litters of the 100 mg/kg bw/day dose group and all foetuses (4) of one litter and one foetus of another litter at the 500 mg/kg bw/day (Statistically significant $P \leq 0.05$). The terminology used to describe the heart malformations in this study is different from that typically employed in teratology research (e.g., dilated heart, seal-shaped heart). Consequently, what is meant by the description "dilated heart" is not well defined and not documented with photographs or retained tissue sections or slides. How this malformation might relate to others reported in the heart (i.e., dilated left or right ventricle, seal-shaped heart, cardiomegaly) is not clear. Further, because too few foetuses were available for examination in the high dose group, it cannot be determined whether these defects exhibited a true dose-related increase. It is important to note, however, that only 2 litters exhibited major visceral malformations in the high dose group. Additionally, these findings were found in the presence of extensive maternal toxicity, evidenced by reduced food consumption and body weight gains in the few animals that survived this dose level, clinical signs, and substantial deaths.

Major, minor and skeletal malformations did not show any clear treatment-related findings and appeared to be incidental.

Table 5.6.2-49 Glyphosate technical: Teratology study in the rabbit ([redacted] 1993): Summary of relevant external, visceral and skeletal findings (litter data)

Foetal findings	HC Data #	Dose level (mg/kg bw/day) 0	20	100	500
No. of litters examined	6	20	13	12	5
No. of foetuses examined	46	133	79	77	28
Minor external malformations					
Percentage of small foetuses (%)	--	0	0	1.3	0
Litter incidence (%)	--	0	0	8.3	0
Major external malformations					
Percentage of foetuses with upper cleft palate (%)	0	0.8	2.5	0	0
Litter incidence (%)	0	5	15	0	0
Percentage of foetuses with forelimb arthrogryposis	--	0	0	1.3	0
Litter incidence (%)	--	0	0	8.3	0
Percentage of foetuses with multiple malformations	0	0.8	2.5	0	0
Litter incidence (%)	0	5	15	0	0
Percentage of foetuses with major malformations (%)	0	1.5	2.5	1.3	0
Litter incidence (%)	--	10	7.7	8.3	0

Foetal findings	HC Data #	Dose level (mg/kg bw/day) 0	20	100	500
Major visceral malformations					
Percentage of foetuses with dilated heart (%)	--	0	5.1*	5.2*	17.9*
Litter incidence (%)	--	0	23.1	16.7	40.0
Percentage of foetuses with anencephaly (%)	0	0.8	0	0	0
Litter incidence (%)	0	5.0	0	0	0
Percentage of foetuses with heart–seal shaped (%)	0	0.8	0	0	0
Litter incidence (%)	0	5.0	0	0	0
Percentage of foetuses with cardiomegaly & sealed heart (%)	--	0	0	1.3	0
Litter incidence (%)	--	0	0	8.3	0
Percentage of foetuses with dilated ventricle (left) (%)	--	0	0	1.3	0
Litter incidence (%)	--	0	0	8.3	0
Percentage of foetuses with dilated ventricle (right) (%)	--	0	0	0	3.6
Litter incidence (%)	--	0	0	0	20
Percentage of foetuses with persistent truncus arteriousus (%)	--	0.8	0	0	0
Litter incidence (%)	--	5.0	0	0	0
Percentage of foetuses with gallbladder absent (%)	--	0	0	0	3.6
Litter incidence (%)	--	0	0	0	20
Percentage of foetuses with liver (median) haematoma (%)	--	0	0	0	3.6
Litter incidence (%)	--	0	0	0	20
Minor skeletal malformations					
No. of foetuses with extra 13th rib		0	1	2	1
Percentage of foetuses with extra 13th rib	8.7**	0	1.3	2.6	3.6*
Litter incidence (%)	--	0	7.7	16.7	20
Major skeletal malformations					
Percentage of foetuses major malformations (%)	10.9	8.3	6.3	0*	3.6
Litter incidence (%)	50	20	23.1	0	20

Historical positive control data (--: no data available)
* Significantly different from control at $p < 0.05$.
** Significantly different from control by Contingency test ($P \leq 0.05$)

III. CONCLUSIONS

Glyphosate technical was considered not to be teratogenic in this developmental toxicity study in rabbits. The incidence of one undefined visceral effect, "dilated heart", is not consistent with identification of visceral malformations described in Appendix 35 of the report (Protocol for Visceral Evaluation), which identifies four separate visceral malformations within this cluster of findings. This group of malformations was increased at the highest test dose and was present at lower dose levels, but there were too few foetuses present in the high dose group to corroborate a dose-response relationship. Further, foetal findings at the highest test dose were observed in the presence of extensive maternal toxicity that exceeded guideline recommendations for a high dose. The NOAEL for maternal toxicity was 100 mg/kg bw/day based on mortalities at dose levels of ≥ 100 mg/kg bw/day. Mortality and clinical signs of toxicity including reduced feed consumption and soft faeces and reduced body weight gain during the dosing period one incidence of complete resorptions at the 500 mg/kg bw/day dose level. The NOAEL for foetotoxicity and teratogenicity was ≤ 20 mg/kg bw/day based on occurrence of several types of malformations all grouped together under "dilated hearts".

2. Assessment and conclusion

Assessment and conclusion by applicant:

The study is considered invalid due to numerous weaknesses including a small number of litters for

examination (low pregnancy rate at all dose levels, lethality in mid and high dose dams), high apparent gavage error rate resulting in mortality, and other reporting deficiencies. The maternal mortality rate for deaths not attributable to garage errors falls within the range of historical control mortalities from this laboratory. The percentage of foetuses with 'dilated heart' was significantly increased at all dose levels. However, the absolute number of affected foetuses and litters is quite small and did not show a marked difference between the treated groups. Unfortunately, the reporting of "dilated heart" in the study report is not consistent Appendix 35 "Protocol for Visceral Evaluation" in this report, which notes four different categories of dilated heart; (i) dilation of the ventricle of the olfactory bulb, (ii) dilation of the lateral ventricle, (iii) dilation of the third ventricle, and (iv) dilation of the fourth ventricle. Given the reporting is not consistent with the detail of the protocol, a low level of confidence is placed in the reporting of these different endpoints.The diagnosis 'dilated heart' was not defined in this study report, does not follow "Appendix 35 Protocol for Visceral Evaluation", and neither criteria used for this diagnosis nor measurements of the heart were provided. In addition, this group of reported malformations lacks a clear dose-response noting that litters, not foetuses, are the experimental unit in developmental toxicity studies; dilated heart foetuses (litters) were 0 (0), 4 (3), 4 (2), and 5 (2) across 0, 20, 100 and 500 mg/kg bw/day, respectively. The inconsistency of malformation terminology in this study report when compared both with other studies and this laboratory's own visceral evaluation terminology in Appendix 35, suggests that interpreting this group of malformation types within this study is not appropriate or justified. Given the inaccuracies in reporting of heart findings and lack of dose-response, the offspring NOAEL in disregarding these endpoints, should be 100 mg/kg bw/day, due to the high dose proving to be well in excess of a limit dose. Similarly, the maternal NOAEL is 100 mg/kg bw/day, since the maternal mortality at this dose is within the range of historical controls for this laboratory. However, little if any weight should be placed on this study due to its many shortcomings. The applicant believes this study should be disregarded as unreliable for the above mentioned reasons.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	**CA 5.6.2/014**
Report author	[redacted]
Report year	1991
Report title	The Effect of Glyphosate on Pregnancy of the Rabbit (Incorporates Preliminary Investigations)
Report No	CHV 45 & 39 & 40/901303
Document No	Not reported
Guidelines followed in study	OECD 414 (1981), US EPA 83-3
Deviations from current test guideline (OECD 414, 2018)	Dosed from day of implantation through the period of organogenesis, rather than through to day prior to necropsy
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities[1,2]	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

1. Information on the study

Data point:	**CA 5.6.2/015**
Report author	[redacted]
Report year	1991
Report title	Historical control data: Control Indiviual Incidence Major Anormalities (Interfauna Rabbit 1989)
Report No	CHV 45 & 39 & 40/901303
Document No	Not reported
Guidelines followed in study	OECD 414 (1981), US EPA 83-3
Deviations from current test guideline (OECD 414, 2018)	Dosed from day of implantation through the period of organogenesis, rather than through to day prior to necropsy
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities[1,2]	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

In the preliminary study Part III, CHV/40, included with the definitive study report, CHV/45/901303, groups of two non-pregnant does were dosed with 1000 mg/kg bw/day glyphosate for seven days and 750 or 500 mg/kg bw/day for 13 days. Liquid/soft faeces were noted for all animals.

In the preliminary study Part II, CHV/39, included with the definitive study report, CHV/45/901303, groups of six pregnant does were dosed with 0, 100, 250 or 625 mg/kg bw/day glyphosate on gestation days 7-19. Signs of reaction to treatment were restricted to gastro-intestinal disturbances and consisted of a dosage-related increase in the incidence of females showing soft/liquid faeces, and two high dose deaths.

In a definitive developmental toxicity study, CHV/45/901303, groups of 16 - 20 time-mated female New Zealand White rabbits were administered glyphosate acid in 1% methylcellulose once daily by gavage at dose levels of 0 (vehicle control), 50, 150 or 450 mg/kg bw/day from day 7 to day 19 of pregnancy (mating = day 0). All animals were observed daily for clinical signs and mortality, and body weights and food consumption were measured on days 1, 7, 9, 11, 15, 20, 24 and 29 of gestation. On day 29 of gestation, the does were sacrificed and a gross necropsy was performed. The ovaries and uteri were examined to determine the number of corpora lutea, the number and distribution of live young, the number and distribution of embryonic and /foetal deaths, individual foetal weight and foetal abnormalities. All live foetuses were examined for external, visceral and skeletal abnormalities.
Observations recorded included one death at 450 mg/kg bw/day following abortion. Reduced food intake during the treatment period and reduction in body weight gain from days 11 – 19 of pregnancy were noted at 150 and more pronounced at 450 mg/kg bw/day. Clinical signs included a dose-related increase in the number of females showing soft/liquid faeces (gastrointestinal disturbances) and slight reductions in food consumption and body weight at 150 and 450 mg/kg bw/day.

Glyphosate was not teratogenic in this developmental toxicity study in rabbits.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material:

Identification:	Glyphosate acid
Description:	White solid
Lot/Batch #:	206-JAK-25-1
Purity:	98.6 %
Stability of test compound:	Stable over the duration of the study
Vehicle:	1 % methylcellulose
Test Animals:	
Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	11-24 weeks (on delivery)
Sex:	Female
Weight at dosing:	Females: 3582 – 3709 g (group mean values)
Acclimation period:	7 days
Diet/Food:	SQC Standard rabbit diet (Special Diet Services Ltd., Essex, UK), *ad libitum*
Water:	Mains drinking water, *ad libitum*
Housing:	Individually in metal cages equipped with sheet stainless steel sides, back and top, a stainless steel wire front and an aluminium perforated floor panel; individual undercage plastic trays lined with absorbent paper
Environmental conditions:	Temperature: 19 ± 1 °C Humidity: 49 ± 15 % Air changes: Not recorded Natural lighting supplemented with artificial lighting from 07 – 21:00 hours

B. STUDY DESIGN

In life dates: 14 -12-1989 – 02-03-1990

Animal assignment and treatment:
In a developmental toxicity study, groups of 16 - 20 time-mated female New Zealand White rabbits were administered glyphosate in 1 % methylcellulose (dose volume 5 mL/kg) once daily by gavage at dose levels of 0 (vehicle control), 50, 150 or 450 mg/kg bw/day from day 7 to day 19 of pregnancy. Dose volumes were calculated for individual animals on day 7 and adjusted according to body weight on days 9, 11 and 15. The day of mating was considered as day 0. Dose levels were based on the findings of a preliminary study.

Dosing formulations were prepared daily and administered within 3 hours of preparation.

Observations

All animals were regularly handled and observed daily for overt changes or signs of reaction to treatment. Animals that died or were killed for animal welfare reasons were weighed and subjected to *post mortem* examination.

Body weight
Individual body weights were recorded days 1, 7, 9, 11, 15, 20, 24 and 29 of gestation.

Food consumption and compound intake
Food consumption was recorded on days of weighing throughout gestation.

Sacrifice and pathology
On day 29 of pregnancy all surviving does were subjected to *post mortem* examinations for congenital abnormalities and gross pathological changes in maternal organs.
The ovaries and uteri were examined to determine the number of corpora lutea, the number and distribution of live young, the number and distribution of embryonic and /foetal deaths, individual foetal weight and foetal abnormalities. Embryonic/foetal deaths were classified as early, late or abortions.

Litter parameters
Live young were examined for external, visceral and skeletal abnormalities employing appropriate techniques. Live young were killed by intrathoracic injection of pentobarbitone sodium then weighed and dissected for examination of visceral abnormalities. Where appropriate, suspected abnormalities were further examined by alternative procedures such as microdissection and histopathology to clarify initial observations. Pups were fixed in industrial methylated spirit, the heads sliced along the line of the frontoparietal suture and the brain examined for abnormalities before clearing and staining by the modified Dawson technique of the carcasses for skeletal examination. Structural changes were presented as malformations, anomalies or variants.

Statistics
Two-tailed tests for significance were performed on litter data only, and significance at 1% and 5% were reported. Mean values of litter size, pre and post-implantation loss, litter weight, mean foetal weight and the incidence of anomalous offspring were analysed by the Kruskal-Wallis test. Intergroup comparisons were made by the non-parametric equivalent of the Williams' test following a significant h-statistic. Where 75% of the values for a given variable consisted of one value, a Fisher's exact test was used.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS
The analysis of the dosing formulations taken at the first dosing showed the mid- and high-dose group to be within 6% of the nominal dose whilst the low-dose group was 19 % below the nominal dose; however, a renalysis on day 19 showed the concentration to be 5 % above the nominal dose.

B. FOOD CONSUMPTION
During the dosing period, females receiving 150 and 450 mg/kg bw/day showed reduced food consumption compared to the controls. A slight reduction was evident from days 11 – 19 at 150 mg/kg bw/day (approximately 12% compared with controls) and throughout the treatment period for the 450 mg/kg bw/day dose group (6-17% during days 7-19).

Table 5.6.2-50: Glyphosate technical: Teratology study in the rabbit ([redacted], 1991): Summary of mean food consumption (g/rabbit/day)

	Dose Group (mg/kg bw/day)			
	0 (control)	**50**	**150**	**450**
Mated females	19	19	16	20

	Dose Group (mg/kg bw/day)			
	0 (control)	50	150	450
No. of animals included in assessment	18	12	15	13
Food consumption (g/rabbit/day) during				
Days 1-6	142	143	141	152
Days 7-8	143	154	150	135
Days 9-10	146	148	148	132
Days 11-14	153	149	134	129
Days 15-19	148	151	131	123
Days 20-23	142	154	149	149
Days 24-28	131	143	153	166

C. MORTALITY

There was one death in the 450 mg/kg bw/day dose group on day 20 following signs of abortion on day 19 and signs of gastrointestinal disturbance, manifested as soft/liquid faeces, severe reduction in food consumption and body weight loss from the onset of treatment. Two other deaths (a broken hind leg and an incidence of congenital abnormality) were unrelated to the treatment and were eliminated from the study assessment.

D. CLINICAL OBSERVATIONS

Clinical signs included a dose-related increase in the number of females showing soft/liquid faeces (gastrointestinal disturbances) and signs of lack of appetite (off feed/reduction in food consumption) at 150 and 450 mg/kg bw/day (see below).

Table 5.6.2-51 Glyphosate technical: Teratology study in the rabbit ([redacted], 1991): Summary of maternal performance and relevant clinical signs

Parameter	Dose Group (mg/kg bw/day)			
	0 (control)	50	150	450
Mated females	19	19	16	20
Not pregnant	0	6	1	5
Number of does with live young or litters at day 29	18	12	15	13
Clinical signs#				
Off-feed	8	6	10	9
Reduced faecal output	9	8	11	12
Soft/liquid faeces	0	2	5	13

Only animals with live young included

E. BODY WEIGHT

A slight reduction in body weight gain was noted from day 11 of pregnancy to termination of treatment in the 150 and 450 mg/kg bw/day dose groups, which coincided with the reduction in food consumption during the same period (see table below).

Table 5.6.2-52Glyphosate technical: Teratology study in the rabbit ([redacted], 1991): Summary of body weight data (group means)

Parameter	Dose Group (mg/kg bw/day)			
	0 (control)	50	150	450
Mated females	19	19	16	20

No of animals included in assessment	18	12	15	13
Body weights (g) at				
Day 1	3538	3524	3568	3658
Day 7	3582	3604	3624	3709
Day 9	3589	3639	3637	3732
Day 11	3601	3653	3661	3743
Day 15	3742	3804	3779	3833
Day 20	3770	3831	3775	3835
Day 24	3844	3927	3849	3965
Day 29	3999	4084	3975	4103

F. PATHOLOGY

Necropsy

Gross examination of does at *post mortem* did not identify any treatment-related effects.

Observations on the ovary and uterus

A total of 18, 12, 15 and 13 pregnant females survived to termination and 163, 104, 112 and 95 foetuses were recorded for the 0 (control), 50, 150 and 450 mg/kg bw/day dose groups respectively. Litter size at caesarean necropsy was comparable in all treatment groups. Total litter loss was recorded for one female of the 450 mg/kg bw/day dose group which aborted on day 19 and died and also for one female at 50 mg/kg bw/day. One female at 150 mg/kg bw/day aborted 1/9 foetuses.

There were no significant intergroup differences in the numbers of corpora lutea, implantations, pre-implantation loss, foetal sex ratios or foetal weights. There was a statistically significant increase in embryo/foetal death and post-implantation loss at all exposure levels. The study investigators questioned the biological significance of these findings for several reasons: 1) No dose-response pattern was evident, 2) the control value was at the lower end of the historical control range, while those of the exposed groups were at the higher end, and 3) the values in all groups were within or slightly above the historical control range. The latter two statements are supported by the historical control data provided in the study report (page 32). Although embryo/foetal death was within the historical control range, post-implantation loss was above the historical control values in the high-dose group, and both of these parameters were statistically significant ($p<0.01$) at the high dose.

Table 5.6.2-53 Glyphosate technical: Teratology study in the rabbit ([redacted], 1991): Summary of the maternal and litter parameters (group mean values)

Parameter	Dose Group (mg/kg bw/day)				Historical control range (mean value)
	0 (control)	50	150	450	
No. of mated females	19	19	16	20	--
No. not pregnant	0	6	1	5	--
No. of does with live young or litters at Day 29	18	12	15	13	--
Corpora lutea	11.5	12.4	11.7	11.3	9.0 – 12.9 (11.2)
Implantations	9.7	10.5	9.0	9.2	7.0 – 11.1 (9.5)
Pre-implantation loss	14.6	15.4	23.4	18.8	2.3 – 26.1 (15.1)
Early embryonic deaths	0.4	0.9	0.9	0.5	0.3 – 1.1 (0.6)
Late embryonic deaths	0.2	0.9	0.5	1.3**	0.1 – 1.3 (0.7)
Abortions	0.0	0.0	0.1	0.0#	0.0 – 0.1 (0)
Total embryonic deaths	0.6	1.8*	1.5*	1.8**	0.6 – 2.0 (1.2)
Post-implantation loss (%)	5.7	19.5*	15.3*	21.0**	6.5 – 17.5 (12.9)
Live young	9.1	8.7	7.5	7.3	6.1 – 9.5 (8.3)
Litter weight (g)	389.5	370.6	320.5	315.0	281.9 – 402.2 (352.9)

Mean foetal weight (g)	43.9	43.3	44.0	44.5	41.4 – 47.6 (44.1)
Sex (% males)	55.3	55.8	57.6	53.8	--

* Statistically significant by Kruskal –Wallis 'H' test P<0.05
** Statistically significant by Kruskal –Wallis 'H' test P<0.01
# Fisher exact test follow-up by intergroup comparison with control was not statistically significant P>0.05

G. DEVELOPMENTAL PARAMETERS

Number and viability of foetuses

There were 18, 12, 15 and 13 viable litters at 0, 50, 150 and 450 mg/kg bw/day, respectively. The concurrent control showed low mean values for embryonic deaths and post implantation losses when compared with historical control values. When compared with these historical data as noted above, mean values in the treated groups were within the expected range; therefore, it was concluded that no adverse effect on foetal survival was attributed to glyphosate.

Foetal body weights

There was a dose-related reduction in mean foetal weight on a litter basis in all treated groups (not statistically significant) compared with the control; however, the mean individual foetal weight was not affected.

External, visceral and skeletal examination

Malformations were slightly increased in the 150 and 450 mg/kg bw/day dose groups compared to controls and appeared to be associated with an apparent increase in malformations of the thoracic region. However, neither the incidence nor the percentage of malformed foetuses was outside the historical control range and the values were not statistically different from concurrent control values. Several of the cardiovascular malformations that were observed, particularly in the high-dose group, occurred in the same animals and are related to a single morphogenetic mechanism (i.e. displacement of the developing aorticopulmonary septum), which is likely to adjust during the first two weeks of postnatal life. These related findings, which often cluster together, included dilated/narrow aorta and narrow/dilated pulmonary artery; interventricular septal defect; and disproportionately sized right and left ventricles. These findings were observed (often in clusters) in the historical control data that were provided by the conducting laboratory. Individual presentation of these malformations in tables when the malformations occurred together in the same foetus and are due to the same mechanisms and artificially inflates the sense that there is a much stronger cardiac effect than is actually present.

The cardiac malformation observed with greatest frequency in this study was interventricular septal defect. The number of foetuses and litters with ventricular septal defects were 1, 1, 1 and 4 in the 0, 50, 150 and 450 mg/kg bw/day dose groups, respectively. Comparison of the historical control data (see table below) shows that the heart findings (when presented on a percent individual and/or litter incidence basis) were slightly outside of the historical background range from 13 studies conducted during the same period. However, the disparity in values is a consequence of the small numbers of litters in the study report. If the data are displayed as a fraction (rather than a percentage), then the number of litters affected were 1/18, 1/12, 1/15, and 4/13 in the 0, 50, 150, and 450 mg/kg/day dose groups, respectively. The historical control range is 0/19 – 3/13. Thus, the findings at the high dose are barely outside of the historical control range. Further, they were observed in conjunction with clear signs of maternal toxicity (reduced food consumption, body weight gains and increased clinical signs).

The other cardiovascular finding found in this study not related to the morphogenetic mechanism involving formation of the spiral septum is retroesophageal right subclavian artery. This finding was also observed regularly throughout the historical period. It is not uncommon and is oftentimes an inconsequential anatomical difference in vascular arrangement. At autopsy this condition is found in 0.5 – 2.0 % of subjects.

The malformations of the cranial region, the lumbar and the lumbar/sacral regions did not show any treatment-related trend and are considered to be incidental. The incidences of anomalies and variants did not suggest any treatment relationship. The incidence of foetuses with reduced ossification did not show

any dose-relationship; however, lower foetal weights were observed for the 450 mg/kg bw/day dose group with reduced ossification.

The observed foetal malformations and anomalies are summarised in the following table.

Table 5.6.2-54Glyphosate technical: Teratology study in the rabbit ([redacted], 1991): Summary of foetal examination

Parameter	Dose Group (mg/kg bw/day)				Historical control range or x/y ◊ (mean)
	0 (control)	50	150	450	
Number of does with live young or litters at Day 29	18	12	15	13	--
Mean foetal weight (g)	43.9	43.3	44.0	44.5	41.4 – 47.6 (44.1)
Sex (% males)	55.3	55.8	57.6	53.8	--
Malformations					--
Total number of foetuses examined	163	104	112	95	1511
No. of malformed foetuses	3	3	5	6	51
%	1.9	5.8	4.3	5.9 (F)	0.7 – 5.9 (3.8)
Number of Affected Litters	3	3	3	5	43/188
%	16.67	25	20	38.5	22.9
Thoracic region malformations					--
No. of foetuses with interventricular septal defect	1	1	1	4	10/1511
%	0.6	1.0	0.9	4.2	0.66
Litter incidence	1	1	1	4	10/188
%	5.56	8.3	6.67	30.8	5.32
Foetuses with enlarged left, reduced right ventricles	0	0	0	2	2/1511
%	0.0	0.0	0.0	2.1	0.13
Litter incidence	0	0	0	2	2/188
%	0	0	0	15.4	1.10
Foetuses with retro-oesophageal right subclavian artery	0	0	3	2	7/1511
%	0.0	0.0	2.7	2.1	0.46
Litter incidence	0	0	1	1	7/188
%	0	0	6.6	7.6	3.72
Foetuses with narrow/dilated aortic arch/pulmonary trunk/arterial trunk	1	1	1	3	8/1511
%	0.6	1.0	0.9	3.2	0.52
Litter incidence	1	1	1	3	8/188
%	5.56	8.3	6.67	23.1	4.25
Anomalies					--
Total number of foetuses examined[#]	160	101	107	89	--
No. of foetuses with gross/visceral anomalies	9	14	14	6	--
%	6.4	19.5	12.9	9.6 (K)	--
No. of foetuses with skeletal anomalies	21	13	14	11	--
%	11.7	17.7	12.5	10.1 (K)	--
No. of foetuses with reduced ossification	7	4	5	4	--
%	4.4	4.0	4.7	4.5	--
Mean foetal weight of foetuses with reduced ossification (g)	37.9	43.6	37.7	26.1	--

[#] Total number at caesarean section
[##] Number ± SD; historical control without SD
◊ number affected / total number examined
[#] Malformed foetuses are excluded

Parameter	Dose Group (mg/kg bw/day)				Historical control range or x/y (mean)
	0 (control)	50	150	450	

(F) Fisher's exact test applied, not statistically significant (P>0.05)
(K) Kruskal-Wallis 'H' statistic, not significant (P>0.05)
-- no data

III. CONCLUSIONS

Study conclusion:
Glyphosate was not teratogenic in this developmental toxicity study in rabbits. The NOAEL for maternal toxicity was 50 mg/kg bw/day based on clinical signs of toxicity including reduced feed consumption and body weight gain and soft/liquid faeces during the dosing period. The NOAEL for foetotoxicity was 150 mg/kg bw/day based on statistically significantly increased embryo/foetal deaths and post implantation loss. The NOAEL for teratogenicity was 450 mg/kg bw/day.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study is considered acceptable, though treatment duration covered the period from implantation through organogenesis rather than through to the day prior to necropsy, as recommended in the current OECD 414 guideline. In this study, there was a significant increase in embryonic death and post-implantation loss in treated groups compared to controls, however without a clear dose-relationship. Regarding the post-implantation losss, values for the low and high dose groups are outside the historical control range. The cardiac malformation observed with greatest frequency in this study was the interventricular septal defect. At 450 mg/kg bw/day this effect was outside the historical control range (4.2 % compared to 0.66 % in historical controls). Taken into account the high post implantation loss at the same dose level, the incidence of additionally cardiac malformation may be covered. At mid dose level, foetuses with an higher incidence of retrooesophageal right subclavian artery were reported. However, this effect has to be considered equivocal, because no clear-dose relationship could be established.
In conclusion, the NOAEL for maternal toxicity is considered 50 mg/kg bw/day based on slightly reduced food consumption, slightly reduced body weight gain and soft/liquid faeces at 150 mg/kg bw/day. The NOAEL for developmental toxicity is considered to be 150 mg/kg bw/day based on the post implantation loss, late embryonic death and an increase in cardiac malformations at 450 mg/kg bw/day.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	**CA 5.6.2/016**
Report author	[redacted]
Report year	1989
Report title	Rabbit Teratology Study with Glyphosate Technical
Report No	1086

Document No	Not reported
Guidelines followed in study	OECD 414 (1981)
Deviations from current test guideline (OECD 414, 2018)	Group size smaller than required, dosed from day of implantation through the period of organogenesis, rather than through to day prior to necropsy, no uterine weight reported, no maternal necropsy findings reported. Deviations from the current version of OECD 414 (2018) are basically due to the fact that the study was aligned to an older version of the OECD test guideline 414.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities[1,2]	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

This developmental toxicity study in rabbits is limited in its extent of documentation. Glyphosate Technical was administered by gavage to three groups of 15 successfully mated New Zealand albino rabbits each, at doses of 125, 250, and 500 mg/kg bw/day from gestation day 6-18 (mating = day 1). A further group of 15 animals was exposed to the vehicle to serve as control.

No adverse effects related to test substance treatment were observed in any animals of the low and mid dose group. Mean maternal body weights and food consumption were lower in the high dose group, however clinical signs of systemic toxicity were not observed at any dose. In the high dose group, two cases of total abortion were considered to be related to test substance treatment.

The mean number of viable implants (foetuses) per litter was lower in the high dose group and the mean number of external, visceral and skeletal malformations as well as the mean number of variations was higher in the high dose group compared to the control group. No differences in the examined developmental parameters were found in the low and mid dose groups, although a dose-related increase in the numbers of malformations and variations was noted.

II. MATERIALS AND METHODS

A. MATERIALS

Test Material:

Identification:	Glyphosate technical
Description:	White amorphous powder
Lot #:	38
Purity:	95 %
Stability of test compound:	Not reported
Vehicle:	0.1 % gum acacia in water
Test Animals:	
Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	24 - 28 weeks

Sex:	Females	
Weight at dosing:	1.50 – 2.00 kg	
Acclimation period:	6 days	
Diet/Food:	Pelleted rabbit feed supplied by Lipton India Ltd., Bangalore, India	
Water:	Tap water, *ad libitum,* supplied in polypropylene bottles by Maharashtra Industrial Development Corpn., New Bombay	
Housing:	Individually in stainless steel cages equipped with food and water dispensers and stainless steel grate at bottom	
Environmental conditions:	Temperature:	20 ± 3 °C
	Humidity:	30 to 70 %
	Air changes:	not reported
	12 hours light/dark cycle	

B. STUDY DESIGN

In life dates: 1989-07-03 to 1989-11-02

Animal assignment and treatment:
Groups of 15 New Zealand White female rabbits received doses of 0, 125, 250 and 500 mg/kg bw/day test substance in 0.1% gum acacia in water by gavage from gestation day 6-18 after successful mating with adult vigorous males. The day of mating was taken as the 1st day of pregnancy.

Diet preparation and analyses
For each dose level, dosing solutions were prepared in 0.1 % gum acacia in water as vehicle.

Clinical observations
A check for clinical signs of toxicity, ill-health or behavioural changes was made twice daily (before and after dosing) during the dosing period.

Body weight
Individual body weights were recorded on days 0, 6, 12, 18, 23, and 29 (at necropsy). Changes in body weight were calculated and recorded as group maternal weight changes for the periods of days 0-6 (pre-exposure), 6-12, 12-18, 18-23, 23-29 and 18-29 (post-exposure observation period).

Food consumption
Food consumption was recorded on days 0, 6, 12, 18, 23, and 29.

Sacrifice and pathology
Females were euthanatised by carbon dioxide asphyxiation on day 29 of gestation and examined for any abnormalities that would affect pregnancy.
The ovaries and uteri were removed, the uteri were weighed, and the ovaries were examined for the number of corpora lutea and uteri for the number and position of implants and dead or live foetuses. Uteri from non-gravid females were placed in 10% ammonium sulfide solution for detection of early resorptions.

Developmental parameters
Each rabbit foetus was removed from the uterus and was killed by injection of pentobarbitone. All live foetuses were weighed and examined for external malformations including cleft palate and variations. All live foetuses were examined for thoracic and visceral abnormalities, and each foetus was sexed. Following visceral examination, all foetuses were eviscerated and processed for skeletal staining with Alizarin Red S.

All foetuses were decapitated and heads were fixed in Bouin's solution for examination of craniofacial structures.

Statistics
Not reported.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS
Not reported.

B. FOOD CONSUMPTION
Mean food consumption in the low and mid dose groups was comparable to that in the control group throughout the study period. Significantly lower food consumption (~17% lower mean food consumption compared to control, low or mid dose group) was observed in the high dose group starting with the day of treatment throughout the rest of the observation period.

Table 5.6.2-55: Glyphosate technical: Teratology study in the rabbit ([redacted], 1989): Food consumption (mean ± SD)

Parameter		Dose Group (mg/kg bw/day)			
		0 (control)	125	250	500
Food consumption (g/rabbit/day)					
No of dams included in assessment		15	15	15	15
Day 0 – 6	(Pre-treatment)	92.3 ± 8.55	97.2 ± 6.56	96.7 ± 6.73	100.1 ± 6.32
Day 6 – 12	(Treatment)	98.7 ± 6.53	99.5 ± 5.76	100.7 ± 6.30	82.7 ± 6.61
Day 12 – 18	(Treatment)	98.6 ± 6.62	98.4 ± 4.34	94.4 ± 4.58	80.9 ± 4.87
Day 18 – 29	(Post treatment))	192.5 ± 7.69	202.5 ± 8.93	205.3 ± 7.07	162.1 ± 12.03

C. MORTALITY
None of the rabbits died during the study period.

D. CLINICAL OBSERVATIONS
No toxic symptoms were observed in any of the animals during the study.

E. BODY WEIGHT
Mean body weights of animals in the low and mid dose group were comparable to those in the control group. In the high dose group, the mean maternal weight increase was lower for each of the observation periods between days 12-29 compared to controls, but no statistical comparison was provided in the report.

Table 5.6.2-56: Glyphosate technical: Teratology study in the rabbit ([redacted], 1989): Maternal body weight changes (mean ± SD)

Parameter		Dose Group (mg/kg bw/day)			
		0 (control)	125	250	500
Body weight change (g)					
No of dams included in assessment		15	15	15	15
Day 0 – 6	(Pre-treatment)	166.5 ± 3.35	160.5 ± 12.15	153.6 ± 19.91	156.3 ± 9.15
Day 6 – 12	(Treatment)	14.0 ± 5.41	8.0 ± 5.09	13.0 ± 7.06	10.1 ± 7.08
Day 12 – 18	(Treatment)	61.7 ± 21.07	73.0 ± 18.11	69.5 ± 18.45	40.0 ± 16.15

Day 18-29	(Post exposure)	116.7 ± 39.56	125.9 ± 25.51	109 ± 22.44	68.9 ± 15.99

F. PATHOLOGY

Necropsy

No abnormalities that could affect pregnancy were reported at maternal necropsy.

Observations on the ovary and uterus

Two animals of the high dose group aborted.

Table 5.6.2-57: Glyphosate technical: Teratology study in the rabbit ([redacted], 1989): Gestational parameters and foetal weights

Gestational parameter	Dose level (mg/kg bw/day)			
	0	125	250	500
No. of mated females	15	15	15	15
No. of early deliveries	0	0	0	0
No. of abortions	0	0	0	2
No. of females without foetuses	0	0	0	2
No. nonpregnant at termination	2	1	1	3
No. of litters	13	14	14	12
Mean no. of corpora lutea per doe ± SD	10.0 ± 1.69	10.1 ± 1.60	10.3 ± 1.44	9.8 ± 1.57
Mean no. of total implants per litter ± SD	9.0 ± 1.20	9.3 ± 1.33	9.4 ± 1.12	8.5 ± 1.05
Mean % pre-implantation loss ± SD	21.3 ± 32.4	14.9 ± 24.09	14.7 ± 24.38	13.1 ± 6.34
Mean no. of viable implants per litter ± SD	7.3 ± 3.10	8.0 ± 2.59	8.0 ± 2.59	5.2 ± 3.03
Mean no. of non-viable implants per litter ± SD	0.07 ± 0.26	0.13 ± 0.35	0.27 ± 059	1.4 ± 2.20
Mean no. of early resorptions per litter ± SD	1.7 ± 3.22	1.1 ± 2.53	1.0 ± 2.56	1.9 ± 2.43
Sex ratio (% males) ± SD	44.4 ± 20.17	49.2 ± 6.90	49.7 ± 11.45	50.1 ± 13.00
Mean foetal body weight per litter ± SD	40.6 ± 16.6	47.1 ± 0.95	47.5 ± 1.38	48.7 ± 1.87

G. DEVELOPMENTAL PARAMETERS

Number and viability of foetuses

The mean number of viable implants (foetuses) per litter was lower in the high dose group, and accordingly, the mean number of non-viable implants (foetuses) per litter was greater in the high dose group, but no statistical comparisons were provided in the report.

Sex ratio, foetal body weights and placental weights

No differences were noted in the sex ratios, mean foetal body weights, mean number of corpora lutea per dose, mean number of total implants per litter, mean percentage of pre-implantation loss, and mean number of early resorptions between the control and the treated groups. In the high dose group, two dams had no live foetuses due to abortions. However, stasticial analyses were provided in the report.

External, visceral and skeletal examination

No difference was noted in the incidences of maternal animals having foetuses with external, visceral and/or skeletal malformations in the low and mid dose groups when compared with the controls. In the high dose group, the incidences of external, visceral and skeletal malformations were higher than that in the control group. With regard to the heart malformations, 0, 1, 1, and 2 interventricular septal defects were observed in the 0, 125, 250, and 500 mg/kg bw/day dose groups.

A similar pattern was seen in the variations observed externally, viscerally and skeletally; in the high dose group, the total number of observed variations was higher than those of the control, low or mid dose groups.

The increase in malformations and variations observed in the high dose group occurred in the presence of maternal toxicity (reduced food consumption and body weight gains). Further, this was at a dose (500 mg/kg bw/day) that caused significant toxicity, including mortality, in another rabbit developmental study. However, stasticial analyses were not provided in the report.

Table 5.6.2-58: Glyphosate technical: Teratology study in the rabbit ([redacted], 1989): Incidence of foetal malformations and variations

Foetal findings	Dose level (mg/kg bw/day)			
	0	125	250	500
Malformations				
No. of litters examined	13	14	14	12
No. of foetuses examined	109	113	120	78
No of litters with malformations	3	6	10	12
% of litters with malformations	23.08	42.86	71.43	100
No. of foetuses with malformations	3	6	10	20
% of foetuses with malformations	2.75	5.31	8.33	25.64
Number of foetuses (litters) with external malformations				
Tail abnormal	1 (1)	1 (1)	2 (2)	3 (2)
Low-set ears	0 (0)	1 (1)	1 (1)	2 (1)
Total external malformations	1	2	3	3
Total external malformations (%)	0.92	1.77	2.50	3.85
Number of foetuses (litters) with visceral malformations				
Ventricular septal defect	0 (0)	1 (1)	1 (1)	2 (2)
Postcaval lung lobe absent	0 (0)	1 (1)	2 (2)	4 (3)
Kidney(s) absent	1 (1)	2 (2)	2 (2)	6 (4)
Total visceral malformations	1	4	5	12
Total visceral malformations (%)	0.92	3.54	4.17	15.38
Number of foetuses (litters) with skeletal malformations				
Rudimentary rib (no. 14)	1 (1)	0 (0)	2 (2)	5 (2)
Total skeletal malformations	1	0	2	5
Total skeletal malformations (%)	0.92	0.00	1.67	6.41
Variations				
No. of foetuses examined	109	113	120	78
Total no. of observed variations	26	30	49	93
Number of foetuses (litters) with external variations				
Tail blunt tipped	1 (1)	0 (0)	3 (2)	5 (4)
Number of foetuses (litters) with visceral variations				
Irregular rugae on palate	0 (0)	2 (1)	3 (2)	2 (2)
Lateral ventricles of cerebrum dilated	0 (0)	2 (2)	2 (2)	6 (4)
Right ventricle small than normal	1 (1)	3 (2)	3 (2)	5 (3)
Globular heart	2 (2)	0 (0)	3 (2)	5 (4)
Incomplete separation of lung lobes	1 (1)	2 (1)	2 (1)	4 (2)
Parietal foetal atelectasis	0 (0)	1 (1)	1 (1)	1 (1)
Liver irregular shape	0 (0)	2 (1)	2 (2)	6 (4)
Kidney(s) globular shape	0 (0)	0 (0)	2 (1)	5 (3)
Number of foetuses (litters) with skeletal variations				
Cervical centra 1-3 and/or 4 bilobed	1 (1)	0 (0)	1 (1)	2 (2)
Anterior arch of the atlas poorly ossified	2 (1)	2 (1)	1 (1)	4 (2)
Anterior arch of the atlas split	0 (0)	0 (0)	2 (1)	3 (1)
Extra thoracic centrum and arch	1 (1)	3 (2)	2 (1)	5 (3)
Thoracic centrum only one ossification centre	1 (1)	0 (0)	1 (1)	3 (2)
Thoracic centra fused	2 (1)	1 (1)	1 (1)	2 (1)
Extra ribs on thoracic centra and arch 13 bilateral	1 (1)	0 (0)	3 (2)	5 (4)
Sternebra 6 poorly ossified	2 (1)	1 (1)	2 (1)	4 (2)
Sternebra(e) split	2 (1)	2 (1)	1 (1)	5 (3)
Sternebra(e) unossified	3 (2)	1 (1)	3 (2)	6 (4)
Pubis, poorly ossified	3 (2)	2 (2)	3 (1)	4 (3)
Some ossification in knee area	1 (1)	0 (0)	3 (2)	4 (3)

Table 5.6.2-58: Glyphosate technical: Teratology study in the rabbit ([redacted], 1989): Incidence of foetal malformations and variations

Foetal findings	Dose level (mg/kg bw/day)			
	0	125	250	500
Skull bones poorly ossified	1 (1)	3 (2)	2 (1)	2 (2)
Frontal, hole in bone	0 (0)	1 (1)	2 (2)	2 (2)
Reduced number of caudal segments	1 (1)	2 (2)	1 (1)	3 (2)

III. CONCLUSIONS

The oral administration of glyphosate to mated rabbits by gavage from gestation day 6-18 resulted in treatment-related changes at 500 mg/kg bw/day. Therefore the NOAEL for reprotoxic and non-reprotoxic effects was considered to be 250 mg/kg bw/day. Considering the significantly reduced food consumption and body weight gain at 500 mg/kg bw/day, the maternal NOAEL is 250 mg/kg bw/day.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The NOAEL for maternal and developmental toxicity is considered to be 250 mg/kg bw/day based on reduced food consumption and body weight gain at 500 mg/kg bw/day in does. Developmental effects were visible as foetolethality and several malformations (external, visceral, skeletal) at high dose levels: total number of foetuses per litter with malformations was higher in the groups receiving the mid and high dose lever, but without statistical significance. However, it remains unclear, whether statistical analysis of the data had been performed at all. Ventricular septal defects were noted in 2 out of 12 litters in the high dose group (control incidence 0/13 litters). The higher number of further visceral malformations at the top dose level was due to absent kidneys and postcaval lung lobes. Because no individual data are provided it is not identifiable, whether the malformations described were confined to single foetuses or if the foetuses were multiple malformed.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	**CA 5.6.2/017**
Report author	[redacted]
Report year	1981
Report title	Teratological investigation of glyphosate in rats and rabbits
Report No	Not reported
Document No	Not reported
Guidelines followed in study	Not stated (pre-guideline)
Deviations from current test guideline (OECD 414, 2018)	Dosed from day of implantation through the period of organogenesis, rather than through to day prior to necropsy, group size smaller than required, foetal visceral examination apparently not performed, no data on results of foetal skeletal examination, no statistical methods described or used.

Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities[1,2]	No (GLP was not compulsory at the time of study conduct)
Acceptability/Reliability:	Invalid
Category study in AIR 5 dossier (L docs)	Category 3b

2. Full summary

In a developmental toxicity study, groups of 14-16 time-mated female New Zealand White rabbits were administered glyphosate acid from days 6 to 19 of gestation as a dietary admix. Concentrations of glyphosate in the diet are not stated, but admix of the test item was reported to result in test item intakes of 10.5, 50.7 and 255.3 mg/kg bw/day, respectively. Does were sacrificed in a CO_2 chamber on day 28 of gestation. After opening the uterine horns the total number of implantations, of live, dead and resorbed embryos/foetuses were counted. The mean litter and foetal weights, gestational growth, the mean body weight gain were calculated. During necropsy heart, kidney, spleen and lungs were prepared and fixed in 4 % neutral formalin. For skeletal examination the laboratory's modification of the Staples and Schnell's method was used.

Treatment with glyphosate had no effect on the behaviour and appearance of the animals. Body weight was higher in the treated groups than in the controls. No macroscopic changes were noted in the control and treated does. Glyphosate was not teratogenic in this study. Gross and histopathological examination at scheduled necropsy indicated no abnormalities. Foetal loss was increased in mid and high dose and litter weight was decreased. No other findings were noted.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material:

Identification:	Glyphosate /active principle
Description:	White odourless adhesive powder
Lot/Batch #:	Not reported
Purity:	96.8 %
Stability of test compound:	Not reported
Vehicle:	Rabbit diet
Test Animals:	
Species:	Rabbit
Strain:	New Zealand White
Source:	Not reported
Age:	Not reported
Sex:	Female
Weight at dosing:	3560 – 3924 g (group mean values)
Acclimation period:	Not stated
Diet/Food:	Normal standard food (LATI), *ad libitum* (before and after the treatment period)
Water:	Not stated

Housing:	Individually, in wire-floor cages	
Environmental conditions:	Temperature:	21 ± 1 °C
	Humidity:	65 ± 5 %
	Air changes:	Not reported
	Light regime	Not reported

B. STUDY DESIGN

In life dates: Not stated

Animal assignment and treatment:
In this developmental toxicity study, groups of 14 - 16 time-mated female New Zealand White rabbits were administered glyphosate in the diet from day 6 to day 19 of gestation. Concentrations of glyphosate in the diet are not stated, but admix of the test item was reported to result in test item intakes of 10.5, 50.7 and 255.3 mg/kg bw/day, respectively.
Food pellets were prepared freshly from food sizings of 10 x 30-40 mm size by means of MOM type machine /Simon-Hessen kind. Food was stored at -20 °C till use.

Clinical observations
Not stated.

Body weight
Individual body weights were recorded on days 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 and 28 of gestation.

Food consumption and compound intake
Not reported.

Sacrifice and pathology
Does were sacrificed in a CO_2 chamber on day 28 of gestation. During necropsy, heart, kidney, spleen and lungs were prepared and fixed in 4 % neutral formalin..

Litter parameters
After opening the uterine horns the total number of implantations, of live, dead and resorbed embryos/foetuses were counted. The mean litter and foetal weights, gestational growth, the mean body weight gain were calculated.

Statistics
Not reported.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS
Not reported.

B. FOOD CONSUMPTION
Not reported.

C. MORTALITY
No mortality occurred during the study.

D. CLINICAL OBSERVATIONS
Not reported.

E. BODY WEIGHT
Absolute body weights during gestation were similar across groups. Body weight gains were higher in the treated groups than in the control (see table below).

Table 5.6.2-59: Glyphosate technical: Teratological investigation of glyphosate in rats and rabbits ([redacted], 1981): Summary of body weight data (group means)

Parameter	Dose Group (mg/kg bw/day)			
	0 (control)	11	51	255
Mated females	14	15	14	16
No of animals included in assessment	14	15	14	16
Body weights (g) at				
Day 6	3495	3392	3170	3391
Day 10	3560	3504	3319	3592
Day 16	3719	3668	3462	3720
Day 26	3924	3839	3679	3964
Body weigt gain day 6-26 (g)	426	452	516	578

F. PATHOLOGY
Necropsy
No macroscopic changes were noted in the control and test item-treated does.

Observations on the ovary and uterus
A total of 14, 15, 14 and 16 pregnant females survived to termination and 106, 125, 93 and 119 live foetuses were recorded for the 0 (control), 11, 51 and 255 mg/kg bw/day dose groups respectively. There were no substantial intergroup differences in the numbers of implantations. The incidence of dead foetuses was higher in the mid and high dose than in the controls. The percentage of foetal loss was higher in the mid and high dose than in the controls but without clear dose response. No total litter loss was recorded in any group. Litter size at caesarean necropsy was comparable in all treatment groups.

Table 5.6.2-60: Glyphosate technical: Teratological investigation of glyphosate in rats and rabbits ([redacted], 1981): Summary of litter parameters

Parameter	Dose Group (mg/kg bw/day)			
	0 (control)	11	51	255
No. of mated females	14	15	14	16
Mean No. of implantations/dam	7.64	8.40	7.07	8.00
Mean No. of dead foetuses/dam	0.07	0.07	0.43	0.56
Foetal loss (%)	0.93	0.79	6.06	7.03
Mean No. of live foetuses/dam	7.57	8.33	6.64	7.44
Mean litter weight (g)	273	283	240	254
Mean foetal weight (g)	36.51	34.33	36.47	34.40
Externally abnormal foetuses	0	0	0	0

G. DEVELOPMENTAL PARAMETERS
Number and viability of foetuses

There were 14, 15, 14 and 16 viable litters at 0, 11, 51 and 255 mg/kg bw/day, respectively. There were no substantial intergroup differences in the number of live foetuses. The number of dead foetuses per dam was slightly increased in the mid and high dose group.

Foetal body weights
Mean foetal weights were considered not affected by treatment. But mean litter weights were decreased at 51 and 255 mg/kg bw/day, although a dose response was lacking and no statistical comparisons were undertaken.

External, visceral and skeletal examination
No externally abnormal foetuses were noted in any group. No data on results of skeletal or visceral examinations are provided.

III. CONCLUSIONS

Treatment with glyphosate had no effect on the behaviour and appearance of the animals. Body weight was higher in the treated groups than in the controls. No macroscopic changes were noted in the control and treated does. Glyphosate was not teratogenic in this study. Gross and histopathological examination at scheduled necropsy indicated no abnormalities. Foetal loss and was increased in the mid and high dose, but no statistical analyses were undertaken. Litter weight decreases in a non-dose responsive manner were noted. No other findings were noted.

3. Assessment and conclusion

Assessment and conclusion by applicant:

This study is very poorly reported and with major deviations from the recent OECD 414 (treatment duration shorter than required, group size smaller than required, no data on visceral or skeletal examination, no statistics).

Treatment with glyphosate had no effect on the behaviour and appearance of the animals. Body weight was higher in the treated groups than in the controls. No macroscopic changes were noted in the control and treated does. Glyphosate was not teratogenic in this study. Gross and histopathological examination at scheduled necropsy indicated no abnormalities. Foetal loss and litter weight were increased in the mid and high dose, with no statistical analyses undertaken. No other findings were noted. This study is considered invalid.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	**CA 5.6.2/018**
Report author	[redacted]
Report year	1980
Report title	Technical Glyphosate: Pilot teratology study in rabbits
Report No	401-055
Document No	Not reported
Guidelines followed in study	Not applicable for this pilot study
Deviations from current test	Not applicable

guideline	
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No (pre-GLP study)
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

In this pilot study technical glyphosate was administered by gavage to groups of 5 pregnant Dutch Belted rabbits each, at doses of 125, 250, 500, 1250 and 2500 mg/kg bw/day from gestation day 6-27 (insemination = day 0). A further group of five animals was exposed to the vehicle (1% aqueous Methocel®) to serve as control. Thus, a step dose-response for mortality was demonstrated with no substantial adverse effects at 250 mg/kg bw/day, yet severe body weight loss and 1/5 does with sponeaneous abortion (terminated in extremis) and death of the remaining 4/5 does at 500 mg/kg bw/day. Earlier time to mortality was dose-dependent at 500, 1250 and 2500 mg/kg bw/day.
Individual clinical observations and body weights were recorded during the study. The animals were either killed on day 28 of gestation, or after they had aborted during the study period. All sacrificed animals or animals found dead were subjected to a gross necropsy. The uteri were examined for number of early and late resorptions and total implantations as well as the number of viable and non-viable foetuses. The number of corpora lutea was also recorded. Foetuses were not examined.
There were no biologically meaningful differences in appearance or behaviour attributable to treatment with the test item at 125 and 250 mg/kg bw/day when compared to the control. One rabbit at 500 mg/kg bw/day group aborted. The four remaining rabbits in this group and all rabbits at 1250 and 2500 mg/kg bw/day died.
There were no biologically meaningful differences in mean maternal body weights at 125 and 250 mg/kg bw/day compared to the control. A severe body weight loss during the treatment period was noted at 500 mg/kg bw/day. A clear dose-response for clinical signs of loose stool/diarrhoes onset, as well as treatment-related increase in severity of these clinical signs across all doses, and as dose-related earlier death at doses greater than 250 mg/kg bw/bay. There were no biologically meaningful differences in the mean number of corpora lutea, total implantations, post-implantation loss or viable foetuses at 125 and 250 mg/kg bw/day. Due to lack of signs of maternal toxicity at 250 mg/kg bw/day and severe maternal toxicity at 500 mg/kg bw/day, a high dose level between 250 and 500 mg/kg bw was considered suitable for the pivotal teratology study.

The oral administration of glyphosate acid to pregnant rabbits by gavage from gestation day 6-27 did not result in maternal toxicity at 125 and 250 mg/kg bw/day; there were no treatment-related effects on pregnancy or foetuses at these dose levels. At 500 mg/kg bw/day one animal aborted and the four remaining animals died. At 1250 and 2500 mg/kg bw/day all animals died.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material:

Identification:	Glyphosate technical
Description:	White powder
Lot/Batch #:	XHJ-64
Purity:	100 %
Stability of test compound:	Not reported

Vehicle:	1 % aqueous Methocel®	
Test Animals:		
Species:	Rabbit	
Strain:	Dutch Belted	
Source:	[redacted]	
Age:	Approx. 6 month	
Sex:	Females	
Weight at first dosing:	2.335 - 3.153 kg	
Acclimation period:	At least 28 days	
Diet/Food:	Purina Rabbit Chow Checkers 5301, *ad libitum*	
Water:	Tap water, *ad libitum*	
Housing:	Individually in suspended wire mesh cages	
Environmental conditions:	Temperature:	controlled but exact values not reported
	Humidity:	controlled but exact values not reported
	Air changes:	Exact values not reported
	Light controlled	

B. STUDY DESIGN

In life dates: 1979-03-02 to 1979-03-30

Animal assignment and treatment:
Thirty female Dutch Belted rabbits were artificially inseminated and randomly assigned to treatment groups of 5 animals. The day of insemination was designated day 0 of gestation. The rabbits received daily doses of 0, 125, 250, 500, 1250 and 2500 mg/kg bw/day test substance by gavage (5 mL/kg bw) from gestation day 6 to 27. Individual doses based on individual body weights determined on gestation day 6.

Dose formulation
Technical glyphosate was suspended in 1% aqueous Methocel® using a tissue homogenizer. The dose solutions were prepared daily.

Clinical observations
A check for mortality or overt changes in appearance and behaviour was made once daily prior to treatment. During the treatment and post-treatment period all rabbits were observed once daily for clinical signs of toxicity and mortality.

Body weight
Individual body weights of dams were recorded on gestation days 0, 6, 12, 18, 24 and 28.

Sacrifice and pathology
Dams
All surviving rabbits were sacrificed at scheduled termination on gestation day 28 by injection of an overdose of sodium pentobarbital. The number and location of viable foetuses, early and late resorptions, the number of total implantations and corpora lutea were recorded. The abdominal and thoracic cavities and organs were examined for gross pathological changes.
Rabbits that died during the study were necropsied to determine the cause of death.

Foetuses

Foetuses were not examined in this pilot study.

II. RESULTS AND DISCUSSION

A. MORTALITY

There was an increased incidence of mortalities from 500 mg/kg bw/day onwards.

Table 5.6.2-61: Glyphosate technical Pilot teratology study in the rabbit ([redacted], 1980): Mortalities

	Dose level in mg/kg bw/day					
	0	**125**	**250**	**500**	**1250**	**2500**
Spontaneous deaths*	0/5	0/5	0/5	4/5	5/5	5/5
Time of death (gestation day)	--	--	--	15-22	10, 11	9,10
% Mortality	0.0	0	0	80	100	100
Sacrificed after abortion	0	0	0	1**	0	0

* deceased animals / total animals in group
** sacrificed on gestation day 26

B. CLINICAL OBSERVATIONS

There were no biologically meaningful differences in appearance or behaviour attributable to treatment with the test item at 125 and 250 mg/kg bw/day when compared to the control. One rabbit at 125 mg/kg bw/day was noted to have a mass in the thoracic area. At necropsy this mass was found to be an abscess. Since similar conditions were not found in any other group, this abscess was considered not test item-related.

One rabbit at 500 mg/kg bw/day group aborted. The four remaining rabbits in this group and all rabbits at 1250 and 2500 mg/kg bw/day died.

C. BODY WEIGHT

There were no biologically meaningful differences in mean maternal body weight gains at 125 and 250 mg/kg bw/day when compared to the control. A severs body weight loss was noted at 500 mg/kg bw/day and higher.

Table 5.6.2-62: Glyphosate technical: Pilot teratology study in the rabbit ([redacted], 1980): Mean body weight development (in g) during gestation

		Dose level in mg/kg bw/day					
		0 (Control)	**125**	**250**	**500**	**1250**	**2500**
Animals per group		5	5	5	5	5	5
Day of gestation	0	2808	2646	2793	2760	2791	2596
	6	2897	2759	2855	2817	2872	2691
	12	2958	2735	2898	2627	--	--
	18	3013	2792	2926	2508	--	--
	24	3036	2886	2987	2413	--	--
	28	3012	2934	2944	--	--	--

D. PATHOLOGY

Necropsy
Yellow foci on the gall bladder were noted in one rabbit at 500 mg/kg bw/day and in one rabbit at 1250 mg/kg bw/day. Erosions of the stomach mucosa were noted in two rabbits at 500 mg/kg bw/day and in one rabbit at 1250 mg/kg bw/day. Erosions of the oesophagus and gall bladder were noted in one rabbit at 1250 mg/kg bw/day. Varying degrees of autolysis were noted in the other animals dying on study. One rabbit at 2500 mg/kg bw/day was found to have a perforated oesophagus at necropsy and death was attributed to an intubation error.

Observations on the ovary and uterus
There were no biologically meaningful differences in the mean number of corpora lutea, total implantations, post-implantation loss or viable foetuses at 125 and 250 mg/kg bw/day. A slight increase in the mean number of early and late resorptions and post-implantation loss was noted at 250 mg/kg bw/day, when compared to the control. No data are available for animals at 500, 1250 and 2500 mg/kg bw/day as in these groups no animals survived until scheduled necropsy.

Table 5.6.2-63: Glyphosate technical: Pilot teratology study in the rabbit ([redacted], 1980): 28–day uterine examination data

	Dose level in mg/kg bw/day					
	0 (Control)	**125**	**250**	**500**	**1250**	**2500**
Surviving dams at caesarean section*	5/5	5/5	5/5	0/5	0/5	0/5
Pregnant rabbits	3/5	3/5	5/5	0/5	0/5	0/5
Non-pregnant rabbits	2/5	2/5	0/5	n.a.	n.a.	n.a.
Abortions	0/5	0/5	0/5	1/5	n.a.	n.a.
Viable foetuses - total	13	24	34	0	0	0
Viable foetuses - mean	4.3	8.0	6.8	0.0	0.0	0.0
Non-viable foetuses total	0	0	0	n.a.	n.a	n.a
Late resorptions -total	0	0	1	n.a.	n.a.	n.a.
Early resorptions - total	1	0	2	n.a.	n.a.	n.a.
Early resorptions - mean	0.3	0.0	0.6	n.a.	n.a.	n.a.
Post-implantation loss - total	1	0	3	n.a.	n.a.	n.a.
Post-implantation loss - mean	0.3	0.0	0.6	n.a.	n.a.	n.a.
Implantations - mean	4.7	8.0	7.4	n.a.	n.a.	n.a.
Corpora lutea - maen	7.0	13.7	12.6	n.a.	n.a.	n.a.

* number of surviving animals / total animals in group
n.a. no animals survived until scheduled caesarean section

III. CONCLUSIONS

The oral administration of glyphosate acid to pregnant rabbits by gavage from gestation day 6-27 did not result in maternal toxicity at 125 and 250 mg/kg bw/day; there were no treatment-related effects on pregnancy or foetuses at these dose levels. At 500 mg/kg bw/day one animal aborted and the four remaining animals died. At 1250 and 2500 mg/kg bw/day all animals died. Accordingly, under the conditions of this study, doses from 500 mg/kg bw/day and higher clearly exceeded the maximum tolerated dose (MTD).

3. Assessment and conclusion

Assessment and conclusion by applicant:

In the pilot dose range-finding study, the oral administration of glyphosate acid to pregnant rabbits by

gavage from gestation gay 6-27 did not result in maternal toxicity at 125 and 250 mg/kg bw/day; there were no treatment-related effects on pregnancy or developmental parameters at these dose levels. At 500 mg/kg bw/day one animal aborted and the four remaining animals died. At 1250 and 2500 mg/kg bw/day all animals died. Accordingly, under the conditions of this study, doses 500 mg/kg bw/day and higher clearly exceeded the maximum tolerated dose (MTD).

Assessment and conclusion by RMS:

1. Information on the study

Data point:	**CA 5.6.2/019**
Report author	[redacted]
Report year	1980
Report title	Technical Glyphosate: Teratology study in rabbits
Report No	401-056
Document No	Not reported
Guidelines followed in study	Not stated. (pre-guideline; satisfies in general the requirements of OECD 414 (1981), but not of OECD 414 (2018))
Deviations from current test guideline (OECD 414, 2018)	Significant mortality at the higest dose tested yielded less litters than necessary to confirm the observed lack of developmental toxicity at 350 mg/kg bw/day
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities[1,2]	No (pre-GLP study)
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Glyphosate acid was administered by gavage to groups of 16 or 17 pregnant Dutch Belted rabbits each, at doses of 75, 175 and 350 mg/kg bw/day from gestation day 6-27 (insemination = day 0). A further group of 16 animals was exposed to the vehicle (0.5 % aqueous Methocel®) to serve as control.

Individual clinical observations and body weights were recorded during the study. The animals were either killed on Day 28 of gestation, or after they had aborted during the study period. All sacrificed animals or animals found dead were subjected to a gross necropsy. The uteri were examined for number of corpora lutea, early and late resorption, and total implantations. Foetuses were weighted, sexed and examined for external malformations, as well as visceral and skeletal anomalies/abnormalities.

At 175 mg/kg bw/day, a slight increase of soft stool and diarrhoea was observed in dams. At 350 mg/kg bw/day, definite signs of maternal toxicity were observed; this toxicity was manifest as signs of soft stool and/or diarrhoea in all high dose animals at least once, nasal discharge, as well as an increase in the number of dams that died. There was no maternal toxicity attributable to the administration of 75 mg/kg bw/day. In addition, there were no signs of developmental effects noted in any dose group.

The oral administration of glyphosate acid to pregnant rabbits by gavage from gestation day 6-27 resulted

in maternal toxicity at 175 and 350 mg/kg bw/day. There were no treatment-related effects on pregnancy or foetuses at any dose level that could not be attributed to maternal toxicity.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material:

Identification:	Glyphosate technical
Description:	White powder
Lot/Batch #:	XHJ-64
Purity:	98.7 %
Stability of test compound:	Not reported
Vehicle:	0.5 % aqueous Methocel®
Test Animals:	
Species:	Rabbit
Strain:	Dutch Belted
Source:	[redacted]
Age:	Approx. 7 month
Sex:	Females
Weight at dosing:	2.533 – 3.234 kg
Acclimation period:	At least 30 days
Diet/Food:	Purina Rabbit Chow Checkers 5301, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in suspended wire mesh cages
Environmental conditions:	Temperature: controlled, but exact values not reported
	Humidity: controlled, but exact values not reported
	Air changes: Exact values not reported
	Light controlled

B. STUDY DESIGN

In life dates: 1979-04-10 to 1979-05-11

Animal assignment and treatment:
Sixty-four female Dutch Belted rabbits were artificially inseminated and randomly assigned to treatment groups of 16 animals. The day of insemination was designated day 0 of gestation. The rabbits received daily doses of 0, 75, 175 or 350 mg/kg bw/day test substance by gavage (1 mL/kg bw) from gestation day 6 to 27. Individual doses based on individual body weights determined on gestation day 6.

Dose formulation
For each dose level an appropriate amount of grounded technical glyphosate was suspended in 0.5 % aqueous Methocel® solution and homogenised. The dose solutions were prepared daily.

Clinical observations
A check for mortality or behavioural changes was made once daily prior to treatment. During the treatment and post-treatment period all rabbits were observed once daily for clinical signs of toxicity, mortality or behavioural changes.

Body weight
Individual body weights of dams were recorded on gestation days 0, 6, 12, 18, 24 and 28. These time points for body weight determination differ from the requirements of the current OECD guideline 414 (2001) (i.e., body weights should be determined on gestation day 0 and at 3–day intervals thereafter). Although the time-intervals were longer than required, the time points for body weight determination are considered to be sufficient to evaluate the body weight development of the pregnant animals.

Sacrifice and pathology
Dams
All surviving rabbits were sacrificed at scheduled termination on day 28. The uterus was excised and weight and the foetuses were removed. The number and location of viable foetuses, early and late resorptions, the number of total implantations and *corpora lutea* were recorded. The abdominal and thoracic cavities and organs were examined for gross pathological changes.
Rabbits that died during the study were necropsied to determine the cause of death.

Foetuses
All foetuses were weighed, sexed and examined for external malformation and variations, as well as for visceral malformations and variations. The carcasses were then fixed in alcohol, macerated in potassium hydroxide and stained with Alizarin Red S for skeletal examination.

Statistics
All statistical analyses compared the treatment groups to the control group with a level of significance at p <0.05. Foetal sex distribution and number of litters with malformations were analysed using the Chi-square test with Yates correction and/or Fisher's exact probability test. Ther number of early and late resorption and post-implantation losses were compared by the Mann-Whitney U-test.
Mean numbers of viable foetuses, total implantations, *corpora lutea* and mean foetal body weights were compared by ANOVA (one-way), Bartlett´s test for homogeneity and appropriate t-test.

II. RESULTS AND DISCUSSION

A. MORTALITY
There was an increased incidence of mortalities in the high dose group (see table below).

Table 5.6.2-64: Glyphosate technical: Teratology study in the rabbit ([redacted], 1980): Mortalities

	Control (0 mg/kg bw/day)	Low (75 mg/kg bw/day)	Intermediate (175 mg/kg bw/day)	High (350 mg/kg bw/day)
Spontaneous deaths*	0/16	1/16	2/16	10/17
Time of death (gestation day)	--	26	22, 25	3 to 21
% mortality	0.0	6.3	12.5	58.8
Sacrificed after abortion	2	0	1	1
Sacrificed on gestation day	22	--	27	23

* dead animals / total animals in group

For five of the rabbits that died spontaneously, the cause of death was attributed to pneumonia, respiratory disease, enteritis or gastroenteritis. For one rabbit of the mid-dose group and the other 7 rabbits of the high dose group, the cause of death could not be determined.
The mortality rates in the intermediate- and especially in the high-dose groups were greater than 10 %, which exceeds the OECD guideline 414 (2001) suggestion of no more than approximately 10 % maternal mortality.

D. CLINICAL OBSERVATIONS
Clinical signs consisting of soft stool and diarrhea were noted in all dose groups during the treatment period. In the 175 mg/kg bw/day dose group, the incidence of this finding was slightly increased when compared with the control group. At 350 mg/kg bw/day, either soft stool, diarrhea or both were observed in each animal at least once during the treatment period. Also in the high dose group, there was an increased incidence of animals with nasal discharge in comparison with the control group.

E. BODY WEIGHT
There were no treatment-related effects on maternal body weights and body weight gain.

Table 5.6.2-65: Glyphosate technical: Teratology study in the rabbit ([redacted], 1980): Mean body weight development (in g) during gestation (mean ± SD)

		Dose level in mg/kg bw/day			
		0 (Control)	75	175	350
Day of gestation	0	2958 ± 146.6	2876 ± 176.3	2983 ± 157.5	2834 ± 196.9
	6	2988 ± 177.5	2937 ± 187.0	3012 ± 206.9	2875 ± 232.1
	12	3039 ± 165.6	2986 ± 191.5	3029 ± 216.1	2732 ± 330.5
	18	3072 ± 166.4	3002 ± 213.4	2959 ± 276.6	2827 ± 317.2
	24	3038 ± 182.3	3005 ± 219.9	2914 ± 321.2	2999 ± 315.1
	28	3030 ± 231.7	3008 ± 142.7	2958 ± 307.5	2948 ± 238.7

F. PATHOLOGY
Necropsy
There were no macroscopic findings in dams that were considered to be related to the administration of glyphosate technical.

Observations on the ovary and uterus
No treatment-related effects were evident in the study.

Table 5.6.2-66: Glyphosate technical: Teratology study in the rabbit ([redacted], 1980): 28–day uterine examination data

	Control (0 mg/kg bw/day)	Low (75 mg/kg bw/day)	Intermediate (175 mg/kg bw/day)	High (350 mg/kg bw/day)
Animals on study	16	16	16	17
Surviving dams at caesarean section*	14/16	15/16	13/16	6/17
Rabbits examined on Day 28	14/16	15/16	13/16	6/17
Pregnant rabbits	12/14	15/15	11/13	6/6
Non-pregnant rabbits	2/14	0/15	2/13	0/6
Abortions	2/16	0/16	1/16	1/17

* number of surviving animals / total animals in group

G. DEVELOPMENTAL PARAMETERS

There were no statistically significant differences in the mean numbers of early or late resorptions, total implantations, corpora lutea, foetal body weights or foetal sex ratio in any of the test substance groups when compared to control. The number of viable foetuses was slightly, but statistically significantly, increased in the low-dose group at 75 mg/kg bw/day. However, this finding was considered incidental and not related to the test substance.

The mean foetal body weights were slightly decreased in the test substance groups as compared to control. However, the mean foetal body weights in all test substance groups were comparable to the historical control data (i.e. 30.9 g) (see table below).

Table 5.6.2-67: Glyphosate technical: Teratology study in the rabbit ([redacted], 1980): 28–day uterine examination data

	Historical control	Control (0 mg/kg bw/day)	Low (75 mg/kg bw/day)	Intermediate (175 mg/kg bw/day)	High (350 mg/kg bw/day)
Pregnant dams[#]	24	12	15	11	6
Viable foetuses/dam	6.7	5.3 ± 2.73	7.6* ± 1.84	5.9 ± 2.77	6.3 ± 2.25
Post implantation loss/dam[##]	0.8	0.7 ± 0.89	0.4 ± 0.63	0.2 ± 0.40	0.8 ± 1.33
Total implantations /dam[##]	7.5	5.9 ± 2.39	8.0 ± 1.81	6.1 ± 2.84	7.2 ± 2.93
Corpora lutea/dam[##]	10.1	9.0 ± 2.13	10.1 ± 1.64	10.5 ± 3.45	8.5 ± 1.87
Foetal sex distribution (males/females)[#]	83/77	28/35	53/61	32/33	17/21
Mean foetal body weight (g)[##]	30.9	33.4 ± 7.27	30.9 ± 4.43	29.9 ± 7.21	29.3 ± 4.82

[#] Total number at caesarean section
[##] Number ± SD; historical control without SD
* Statistically significant difference compared to control ($p \leq 0.05$)

It should be noted that, in all dose groups, the number of pregnant dams were less than the number of pregnant dams required by the current OECD guideline 414 (2001); i.e., 16. Therefore, the evaluation of the developmental parameters may be limited.

Skeletal and visceral examination

The percentages of foetuses with skeletal malformations were 0.0, 2.6, 3.1 and 5.3 in the control, 75, 175 and 350 mg/kg bw/day groups, respectively. Although malformations were observed in the test substance groups, neither the type nor incidence of the malformations provided evidence for an adverse effect of glyphosate acid. There were no visceral malformations observed in any of the dose groups including control. There were no statistically significance differences in the variation observed in the test substance group when compared to the control group (see table below).

Table 5.6.2-68: Glyphosate technical: Teratology study in the rabbit ([redacted], 1980): Summary of foetal malformations and variations

	Hist. contr.	Control (0 mg/kg bw/day)		Low (75 mg/kg bw/day)		Intermediate (175 mg/kg bw/day)		High (350 mg/kg bw/day)	
Number of litters examined		12		15		11		6	
	%	x/y	%	x/y	%	x/y	%	x/y	%
Skeletal malformations		**0/63**	**0.0**	**3/114**	**2.6**	**2/65**	**3.1**	**2/38**	**5.3**
Exencephaly	--	0/63	0.0	0/114	0.0	1/65 (1/11)	1.5	0/38	0.0
Acrania	--	0/63	0.0	0/114	0.0	0/65	0.0	1/38 (1/6)	2.6
Scoliosis with associated rib anomalies	0.6	0/63	0.0	2/114 (2/15)	1.8	0/65	0.0	0/38	0.0

T1 rib absent	--	0/63	0.0	0/114	0.0	1/65 (1/11)	1.5	0/38	0.0
Carpal flexure	0.6	0/63	0.0	0/114	0.0	0/65	0.0	1/38 (1/6)	2.6
Fused cervical vertebral centra	0.6	0/63	0.0	1/114 (1/15)	0.9	0/65	0.0	0/38	0.0
Visceral malformation	--	**0/63**	**0.0**	**0/114**	**0.0**	**0/65**	**0.0**	**0/38**	**0.0**
Total malformations		**0/63**	**0.0**	**3/114**	**2.6**	**2/65**	**3.1**	**2/38**	**5.3**
Variations									
27 presceral vertebrae	8.7	6/63 (5/12)	9.5	7/114 (3/15)	6.1	9/65 (4/11)	13.8	7/38 (5/6)	18.4
13th rudimentary rib(s)	3.7	5/63 (3/12)	7.9	14/114 (6/15)	12.3	3/65 (3/11)	4.6	3/38 (3/6)	7.9
13th full rib(s)	8.1	3/63 (3/12)	4.8	10/114 (4/15)	8.8	5/65 (2/11)	7.7	6/38 (3/6)	15.8
Hyoid arches bent	--	--	--	2/114 (1/15)	1.8	1/65 (1/11)	1.5	--	--
Hyoid body unossified	--	6/63 (2/12)	9.5	2/114 (2/15)	1.8	6/65 (3/11)	9.2	--	--
Parietals reduced in ossification	0.6	1/63 (1/12)	1.6	--		1/65 (1/11)	1.5	--	--
Sternebrae #5 and/or #6 unossified	5.6	6/63 (3/12)	9.5	13/114 (7/15)	11.4	13/65 (5/11)	20.0	4/38 (2/6)	10.5
Pubis unossified	--	4/63 (1/12)	6.3	1/114 (1/15)	0.9	4/65 (1/11)	6.2	--	--
Talus unossified	--	3/63 (1/12)	4.8	--	--	5/65 (3/11)	7.7	--	--
Extra ossification center, cervical area	--	--	--	--	--	1/65 (1/11)	1.5	--	--
Major vessel variations	8.7	11/63 (6/12)	17.5	14/114 (8/15)	12.3	14/65 (5/11)	21.5	6/38 (4/6)	15.8

x/y number of foetuses affected / total number of foetuses examined
(a/b) number of litters affected / total number of litters

III. CONCLUSIONS

The oral administration of glyphosate acid to pregnant rabbits by gavage from gestation gay 6-27 resulted maternal toxicity at ≥175 mg/kg bw/day. There were no treatment-related effects on pregnancy or foetuses at any dose level that could not be attributed to maternal toxicity. Therefore the NOAEL was considered to be 75 mg/kg bw/day for maternal toxicity. The NOAEL for developmental toxicity was considered to be 350 mg/kg bw/day.

I. Assessment and conclusion

<u>Assessment and conclusion by applicant</u>:

The study was performed before GLP and the respective OECD test guidelines were established. Nevertheless, the study is generally in concordance with the current OECD 414 with the restriction that the group sizes are smaller than requested in this guideline. Therefore, the outcome can be reported as valid.

The oral administration of glyphosate acid to time-mated rabbits by gavage from gestation day 6 to 27 resulted in maternal toxicity at ≥175 mg/kg bw/day. There were no treatment-related effects on pregnancy or foetuses at any dose level that could not be attributed to maternal toxicity. Therefore, the

NOAEL was considered to be 75 mg/kg bw/day for maternal toxicity. The NOAEL for developmental toxicity was considered to be 175 mg/kg bw/day because of the high dose offspring NOAEL of 350 mg/kg bw/day could not be relied upon because maternal mortality resulted in only six litters for six surviving dams.

Assessment and conclusion by RMS:

CA 5.7 Neurotoxicity

There was no evidence of neurotoxicity in acute, subchronic or chronic studies in rodents and dogs. An acute neurotoxicity in rats was performed by [redacted] (CA 5.7.1-001) and is considered acceptable in accordance to the conclusion from RAR (2015). A single administration of glyphosate acid produced clinical signs of toxicity (including decreased activity, subdued behaviour, hunched posture, sides pinched in, tip-toe gait and/or hypothermia) at approximately 6 hours after dosing on day 1 in 3/10 females, only, which received 2000 mg/kg bw. One of these females was subsequently found dead on day 2. These clinical signs were considered to reflect general toxicity associated with the administration of high dose levels of glyphosate acid. Quantitative assessment of landing foot splay, sensory perception, muscle weakness and locomotor activity revealed no changes indicative of neurotoxic potential. Histopathological evaluation of the central and peripheral nervous system revealed no treatment-related changes in animals receiving 2000 mg/kg. The no-observed effect level (NOEL) for neurotoxicity, following single oral administration of glyphosate acid was 2000 mg/kg bw.

In addition to the acute study two sub-chronic neurotoxicity studies were also performed ([redacted], 2006, CA 5.7.1/002 and [redacted], 1996, CA 5.7.1/003). The study by [redacted] has not been evaluated at EU level so far and is considered valid. Similarly, the study from [redacted] (1996) is considered acceptable in accordance to the conclusion from RAR (2015). In the [redacted] (2006) study rats were fed glyphosate up to 20000 ppm for 90 consecutive days. Administration of glyphosate produced no unscheduled deaths and no clinically observable signs of systemic toxicity or neurotoxicity. No treatment-related effects were detected in behavioural assessment, functional performance tests, sensory reactivity, or ophthalmoscopy examinations. No adverse effect on bodyweight changed, dietary intake or food efficiency or water consumption were detected. In addition, there were no treatment-related changes in brain weight. No macroscopic changes or neuropathological changes within a comprehensive histopathological evaluation were detected which could be attributed to administration of glyphosate. In conclusion, no evidence of a neurotoxic potential was obtained up to the highest dose of 20000 ppm. Therefore, the no observed adverse effect level (NOAEL) for neurotoxic potential, following dietary administration of glyphosate for at least 90 days, was 20000 ppm (equivalent to 1499 and 1555 mg/kg bw/day for males and females, respectively).

In the study from [redacted] (1996, CA 5.7.1/003) administration of glyphosate acid to rats at up to 20000 ppm for 90 consecutive days produced no clinical signs of toxicity or effects on any of the quantitative functional observation battery tests or on locomotor activity that indicated any neurotoxic potential. In addition, there were no treatment-related changes in brain weight, length or width. Comprehensive histopathological evaluation of the peripheral and central nervous system revealed no evidence of any changes which could be attributed to administration of glyphosate acid. The no observed effect level (NOEL) for neurotoxic potential, following dietary administration of glyphosate acid for at least 90 days, was 20000 ppm (equivalent to 1547/1631 mg/kg bw/day in males and females respectively). There was a treatment-related effect on growth and food utilisation in males receiving 20000 ppm (1547 mg/kg bw/day). In this study the NOEL for systemic toxicity was 8000 ppm (617 mg/kg bw/day) in males and 20000 ppm (1631 mg/kg bw/day) in females.

Glyphosate is often erroneously called an organophosphate pesticide. However, it is important to note that glyphosate is not an organophosphate ester but a phosphonoglycine, a chemical class that does not inhibit cholinesterase activity. Therefore, studies for delayed neurotoxicity are not considered essential. Despite

this, two studies for delayed neurotoxicity in hens ([redacted], 1996 and [redacted], 1987) have been conducted. The study by [redacted] (1987) is found not acceptable by today's standards in accordance with the evaluation in the RAR (2015). Therefore, this study is not used for risk assessment. The study by [redacted] (1996) is considered acceptable in accordance with the evaluation in the RAR (2015). Administration of a single dose of glyphosate acid to hens at a level of 2000 mg/kg bw and an observation for the following 21/22 days revealed no clinical signs of delayed locomotor ataxia. There was no histological evidence of acute delayed neurotoxicity, and there were no significant reductions in neuropathy target esterase levels in the brain and spinal cord. Therefore, the NOAEL for acute delayed neurotoxicity, following single oral administration of glyphosate acid was 2000 mg/kg bw.

In a study performed by [redacted] (1996, see CA 5.8.2) *ex vivo* investigations with isolated rat gastrocnemius muscle were performed. Evaluation of innervated muscle response showed that glyphosate technical, when administered at the maximum solubility concentration in physiological saline (12 mg/mL), did not cause any neuromuscular blocking activity.

Overall, across a wide database of acute and subacute neurotoxicity and repeated dose studies there is no evidence that glyphosate has specific neurotoxic or delayed neurotoxicity potential.

During the literature search the following publications were considered relevant with regards to neurotoxicity:

Martinez et al. (2019) investigated the effect of acute exposure to glyphosate (GPH) on the blood-brain barrier in vitro based on induced pluripotent stem cells (iPSCs) and compared to two chemical analogues: aminomethylphosphonic acid (AMPA) and glycine (GLY), for concentrations ranging from 0.1 µM to 1000 µM. The data from this study demonstrate the relative safety of glyphosate and AMPA with regard to the blood-brain barrier after acute exposure with minimal effects observed at concentrations significantly higher than baseline exposure levels, occupational and non-occupational alike. The presence of an active uptake and passage of glyphosate across the blood-brain barrier suggests the need for extensive brain-centred studies to evaluate the pharmacokinetics and pharmacodynamics of glyphosate on the central nervous system during acute exposure and in individuals exposed to high amounts of such pesticides.

Martinez et al. (2018) examined the effects of glyphosate oral exposure (35, 75, 150 and 800 mg/kg bw, 6 days) on brain region monoamine levels of male Wistar rats. The results demonstrate that glyphosate leads to lower concentrations of 5-HT, DA and NE levels in the CNS. The effects observed in the present study indicates a potential of glyphosate to lower neurotransmitter concentrations in brain regions of Wistar rats. The authors concluded that although no data on humans are available, glyphosate could exert its neurotoxicity, notably on monoamine systems. Further work is needed to investigate glyphosate herbicide and potential links with neurodegenerative diseases. In the study from Martinez no additional investigations related to neurotoxicity were performed on rats during the treatment phase. The study from Martinez (2019) is regarded as an indicator study for neurotoxicity based on the endpoints addressed. Indications of neurotoxicity have not been identified in the available guideline studies focussing on neurotoxic effects (see CA 5.7.1/001 to CA 5.7.2/002).

Chorfa et al. (2013) studied the effects of four pesticides (paraquat, rotenone, maneb and glyphosate) on different molecular events in cell lines which are considered to be related to Parkinson`s disease. Three of the four pesticides triggered molecular events involved in Parkinson`s disease. However, glyphosate was not active in this study.

Table 5.7-1: Studies on neurotoxicity with glyphosate

Annex Point	Study	Study type Species	Substance(s)	Reference list-related category§	Result#
CA 5.7.1/001	[redacted], 1996	Acute neurotoxicity,	Glyphosate acid	Valid, Category	NOEL = 2000

		oral gavage Alpk:AP$_f$sD rats	(Batch: P24 (Y04707/034) Purity:95.6 %)	2a	mg/kg bw
CA 5.7.1/002	[redacted], 2006	90-day neurotoxicity, oral diet Sprague-Dawley rats	Glyphosate (technical) (Batch: H05H016A, Purity: 95.7 %)	Valid, Category 1	NOEL = 20000 ppm (1499 (♂)/ 1555 (♀) mg/kg bw/day
CA 5.7.1/003	[redacted], 1996	90-day neurotoxicity, oral diet Alpk:AP$_f$sD rats	Glyphosate acid (Batch: P24 (Y04707/034) Purity:95.6 %)	Valid, Category 2a	NOEL = 20000 ppm (1547 (♂)/ 1631 (♀) mg/kg bw/day
CA 5.7.2/001	[redacted], 1996	Acute delayed neurotoxicity, oral gavage Domestic chicken	Glyphosate acid (Batch: P24 (Y04707/034/023), Purity: 95.6 %)	Valid, Category 2a	NOAEL = 2000 mg/kg bw
CA 5.7.2/002	[redacted], 1987	28-day delayed neurotoxicity, oral gavage Domestic chicken	Glyphosate (technical) (Batch: not provided, Purity: not provided)	Invalid, Category 3b	No evidence for delayed neurotoxicity

#: Effect level based on neurotoxicity-related observations.
§: The category describes the acceptability/reliability of the study within the AIR 5 submission. This category does not describe hazard classification. (for details please refer to the Doc ID: 110054-B-GRG_Jun_2020)

CA 5.7.1 Neurotoxicity studies in rodents

Acute neurotoxicity

1. Information on the study

Data point:	CA 5.7.1/001
Report author	[redacted]
Report year	1996
Report title	Glyphosate acid: Acute neurotoxicity study in rats
Report No	[redacted]/P/4866
Document No	Not reported
Guidelines followed in study	No guideline stated in the report but in general compliance with OECD 424 (1997)
Deviations from current test guideline	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Groups of 10 animals Alpk:APfSD rats per sex were administered single oral doses of 0, 500, 1000 or 2000 mg/kg bw glyphosate acid. All animals were observed daily for any changes in clinical condition for two weeks post administration. Detailed clinical observations including quantitative assessments of foot landing

splay, sensory perception and muscle weakness were performed at weekly intervals. At scheduled termination 5 rats/sex/group were subjected to full *post mortem* examination. Selected nervous system tissues were examined microscopically.

Clinical signs of toxicity (including decreased activity, subdued behaviour, hunched posture, sides pinched in, tip-toe gait and/or hypothermia) occurred during Day 1 but were limited to 3 females at approximately 6 hours post treatment, in the highest dose group (2000 mg/kg/day). One of these females was subsequently found dead on Day 2.

Slight reductions in food consumption, without any associated effects on body weight, were also observed during Week 1 for both sexes in the highest dose group.

Quantitative assessment of neurotoxic parameters and histopathological evaluation of the central and peripheral nervous system confirmed no neurotoxic potential for glyphosate.

In conclusion, there was no evidence of specific neurotoxicity up to the highest dose tested (2000 mg/kg bw). Therefore, the no observed effect level (NOEL) for neurotoxicity, following single oral administration of glyphosate acid, was 2000 mg/kg bw.

2. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate acid
Description:	White solid
Lot/Batch #:	P24 ([redacted] reference number: Y04707/034)
Purity:	95.6 % w/w
Stability of test compound:	The test substance was shown to be stable for the period of use.

2. Vehicle and/or positive control: Deionised water

3. Test animals:

Species:	Rats
Strain:	Alpk:AP$_f$SD (Wistar-derived)
Source:	[redacted]
Age:	At least 28 days
Sex:	Males and females
Weight at dosing:	♂ 171.4 – 175.0 g; ♀ 144.6 – 148.7 g
Acclimation period:	Approx. 2 weeks
Diet/Food:	CT1 diet (Special Diets Services Limited, Stepfield, Witham, Essex, UK), *ad libitum,* except 24 h prior dosing
Water:	Tap water, *ad libitum*
Housing:	In groups of five, separated by sex, in multiple rats racks.
Environmental conditions:	Temperature: 19 – 23°C Humidity: 40 – 70 % Air changes: 25 – 30/hour I. ours light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: Not reported. The study was conducted during May and June 1995.

Animal assignment and treatment

In an acute neurotoxicity study groups of ten male and ten female Alpk:APfSD (Wistar derived) rats were administered with a single oral dose of 0, 500, 1000 or 2000 mg/kg bw glyphosate acid by gavage.

Dosing Formulation Analysis

Verification of the achieved concentrations was done with samples of each preparation. Homogeneity was determined with samples from the low to high dose levels. The chemical stability of glyphosate acid in water was also determined for all dose formulations over a period of 10 days.

Clinical observations

Clinical observations were made prior to administration and daily thereafter. Any abnormalities together with the observation of no abnormality detected were recorded**.**

Body weight

The body weight of each rat was recorded on Days -7 and -1, immediately before dosing (Day 1), approximately 6 hours after dosing (Day 1) and on Days 8 and 15.

Food consumption

Food consumption for each cage of rats was recorded throughout the study and calculated on a weekly basis**.**

Functional Observational Battery

Prior to the start of treatment (Week -1) and on Day 1, 8 and 15, all animals were observed for signs of functional/behavioural toxicity with quantitative assessment of landing foot splay, sensory perception (tail-flick test) and muscle weakness (fore- and hindlimb grip strength). Detailed clinical assessments and functional performance tests were performed together with an assessment of sensory reactivity to different stimuli. Those included but were not limited to the following list of measures: assessment of autonomic function (e.g. lachrymation, salivation, piloerection, exophthalmus, urination, defecation, pupillary function, ptosis); description, incidence and severity of any convulsions, tremors, abnormal motor function, abnormal behaviour; reactivity to stimuli; changes in level of arousal; sensorimotor responses; alterations in respiration. Locomotor activity was also assessed at these time points. Each observation period was divided into ten scans of five minute duration. Treatment groups were counter balanced across test times and across devices and when the trials were repeated each animal was returned to the same activity monitor at approximately the same time of day. Motor activity was assessed in a separate room to minimise disturbances.

Sacrifice and pathology

At scheduled termination, 5 rats/sex/group designated for neuropathology were sacrificed. Brain weight, brain length and brain width were determined. The following tissues were submitted: brain, spinal cord (cervical and lumbar), Gasserian ganglion, dorsal root ganglia and spinal roots (cervical and lumbar), gastrocnemius muscle, sciatic nerve, sural nerve and tibial nerve.
Submitted tissues were processed as follows: brain (seven levels including the cerebral cortex, the hippocampus, the cerebellum, the pons and medulla), dorsal root ganglia and spinal roots from cervical and

lumbar regions of the cord after decalcification, and gastrocnemius muscle from rats receiving either 0 (control) or 2000 mg/kg bw glyphosate acid were routinely processed, paraffin wax embedded and 5µm thick sections were cut and then stained with haematoxylin and eosin. Sections of brain and cord were in the transverse plane.

The Gasserian ganglion, sciatic nerve, spinal cord (cervical and lumbar portions), sural and tibial nerve from control and high dose group rats were processed and then embedded in Araldite. Semi-thin sections were cut and then stained with toluidine blue. For bilateral tissues only the left was processed. All tissues were sectioned in the transverse plane except the sciatic nerve which was sectioned in both the transverse and the longitudinal plane.

Neuropathological examination was performed on control and highest dose group animals only. All sections were examined by light microscopy.

Statistics

Analyses of variance and covariance were carried out using the GLM procedure in SAS (1989). Least-squares means for each group were calculated using LSMEAN option in SAS PROC GLM. Unbiased estimates of differences from control were provided by the difference between each treatment group least-squares mean and the control group least-squared mean. Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean using a two-sided Student's t-test, based on the error mean square in the analysis.

The levels of probability chosen as significant different from control were $p < 0.01$** and $p < 0.05$* (Student's t-test, two-sided).

II. RESULTS

A. DOSING FORMULATION ANALYSIS

The achieved concentrations of glyphosate acid in water were within 3 % of the nominal levels. The homogeneity was considered acceptable, with a deviation from the overall mean values of approximately ± 8 %. The chemical stability was considered satisfactory.

B. MORTALITY AND CLINICAL OBSERVATIONS

Two females receiving 2000 mg/kg bw glyphosate acid showed subdued behaviour, decreased activity, hunched posture, sides pinched in, tip-toe gait and hypothermia on the day of administration. One of these animals died on the subsequent day. The other one together with an additional female which showed diarrhoea on the day of administration regained full recovery the subsequent day. One female receiving 500 mg/kg bw, was found dead approximately 6 h after administration.
In the absence of any treatment-related clinical signs prior to death, and because no deaths were observed at the intermediate dose level of 1000 mg/kg bw, the death of this animal was considered not to be treatment related.
Distension of the abdomen was recorded for several males from all treated groups on the day of administration. However, in the absence of any dose relationship, this was not considered to be treatment-related.

C. BODY WEIGHT

No treatment-related effects were observed.

D. FOOD CONSUMPTION

During Week 1, mean food consumption was lower in animals receiving 2000 mg/kg bw glyphosate acid compared to controls, although the difference did attain statistical significance only in females (see **Table 5.7.1-1**). There was no evidence of treatment-related effects in animals receiving 500 or 1000 mg/kg bw.

Table 5.7.1-1: Glyphosate acid: Acute neurotoxicity study in rats ([redacted], 1996): Intergroup comparison of food consumption (g/rat/day) during Week 1

Dose level of glyphosate (mg/kg bw)			
0 (control) **Mean ± SD**	**500** **Mean ± SD**	**1000** **Mean ± SD**	**2000** **Mean ± SD**
Males			
29.9 ± 0.7	29.0 ± 0.1	30.1 ± 0.4	28.4 ± 0.2
Females			
22.4 ± 1.0	22.2 ± 0.2	22.8 ± 0.3	20.6* ± 0.3

* Statistically significant difference from the control group mean at the 5 % level (Student's t-test, two-sided)

E. FUNCTIONAL OBSERVATIONAL BATTERY

Examinations of the functional observational battery did not identify any conclusive treatment- and dose-related effects.

Group mean landing foot splay for males receiving 2000 mg/kg bw was statistically significantly lower than that of concurrent controls at approximately 6 hours after dosing on day 1. However, as pre-experimental values for these animals were low, in comparison with control pre-experimental values, the apparent reduction in landing foot splay for high dose group animals on day 1 was considered not to be attributable to administration of glyphosate acid. Group mean landing foot splay for females receiving 500 mg/kg bw was slightly higher than that of concurrent controls on day 15. However, in isolation, this finding was considered to be unrelated to administration of glyphosate acid.

Table 5.7.1-2 Glyphosate acid: Acute neurotoxicity study in rats ([redacted], 1996): Intergroup comparison of landing foot splay (mm)

Dose level of glyphosate (mg/kg bw)				
	0 (control) **Mean ± SD**	**500** **Mean ± SD**	**1000** **Mean ± SD**	**2000** **Mean ± SD**
Males				
Day -7	50.1 ± 7.3	47.7 ± 7.1	51.6 ±6.2	38.9 ± 5.6
Day 1	54.9 ± 7.6	50.0 ± 7.8	56.8 ± 7.8	45.6* ± 6.8
Day8	58.9 ± 8.1	57.3 ± 10.6	58.5 ± 12.1	54.9 ± 3.6
Day 15	62.3 ± 7.6	64.6 ± 6.9	67.9 ± 13.2	59.3 ± 7.3
Females				
Day -7	42.4 ± 7.1	46.6 ± 9.2	38.7 ± 5.1	48.6 ± 8.6
Day 1	47.8 ± 8.1	48.4 ± 7.3	47.5 ± 7.8	51.7 ± 8.4
Day8	51.2 ± 8.0	58.1 ± 9.9	54.7 ± 12.0	56.9 ± 8.9
Day 15	54.1 ± 9.1	64.0* ± 9.8	58.6 ± 10.3	56.8 ± 6.7

* Statistically significant difference from the control group mean at the 5 % level (Student's t-test, two-sided)

Group mean time to tail flick for males receiving 500 mg/kg bw was slightly higher than that of concurrent controls on day 15. However, in isolation, this finding was considered to be unrelated to administration of glyphosate acid.

Table 5.7.1-3 Glyphosate acid: Acute neurotoxicity study in rats ([redacted], 1996): Intergroup comparison of time to tail flick (s) in male rats

Dose level of glyphosate (mg/kg bw)				
	0 (control) Mean ± SD	**500 Mean ± SD**	**1000 Mean ± SD**	**2000 Mean ± SD**
Day -7	8.1 ± 2.9	7.5 ± 2.5	6.0 ± 2.2	7.5 ± 3.7
Day 1	6.3 ± 1.9	6.5 ± 2.6	5.2 ± 2.4	7.5 ± 2.1
Day8	5.3 ± 2.9	5.8 ± 1.9	6.0 ± 2.2	6.1 ± 1.7
Day 15	7.6 ± 3.1	10.9* ± 4.3	6.7 ± 1.9	6.6 ± 2.2

* Statistically significant difference from the control group mean at the 5 % level (Student's t-test, two-sided)

Group mean forelimb grip strength for males receiving 500 mg/kg bw was slightly higher than that of concurrent controls on day 15. However, in isolation, this finding was considered to be unrelated to administration of glyphosate acid.

Table 5.7.1-4 Glyphosate acid: Acute neurotoxicity study in rats ([redacted], 1996): Intergroup comparison of forelimb grip strength (g) in male rats

Dose level of glyphosate (mg/kg bw)				
	0 (control) Mean ± SD	**500 Mean ± SD**	**1000 Mean ± SD**	**2000 Mean ± SD**
Day -7	520 ± 69	538 ± 68	520 ± 89	488 ± 66
Day 1	733 ± 139	705 ± 117	723 ± 99	728 ± 122
Day8	775± 153	798 ±161	850 ± 135	775 ± 129
Day 15	858 ± 175	1015* ± 193	915 ± 147	823 ± 93

* Statistically significant difference from the control group mean at the 5 % level (Student's t-test, two-sided)

Mean overall motor activity values were slightly lower than those of concurrent controls for males and females which received 2000 mg/kg bw on day 1. However, these differences did not attain statistical significance and were considered too small to be of toxicological importance. Mean overall motor activity values were also slightly lower than those of concurrent controls for males which received 2000 mg/kg bw on day 15. However, in the absence of any treatment-related effects for these animals on day 8, this was considered to be incidental and unrelated to administration of glyphosate acid.

Table 5.7.1-5 Glyphosate acid: Acute neurotoxicity study in rats ([redacted], 1996): Intergroup comparison of motor activity (Overall: minutes 1-50)

Dose level of glyphosate (mg/kg bw)				
	0 (control) Mean ± SD	**500 Mean ± SD**	**1000 Mean ± SD**	**2000 Mean ± SD**
Males				
Day -7	208.0 ± 105.2	208.8 ± 92.5	216.7 ± 70.0	238.0 ± 62.1
Day 1	383.1 ± 109.7	356.8 ± 173.5	387.6 ± 177.6	276.5 ± 124.4
Day8	243.9 ± 83.9	307.9 ± 128.4	268.8 ±139.4	279.1 ± 79.9
Day 15	393.6 ± 155.9	402.1 ± 103.1	323.1 ± 89.6	290.7* ± 136.1
Females				
Day -7	291.0 ± 167.9	239.6 ± 127.8	287.4 ± 99.8	288.4 ± 134.0
Day 1	464.1 ± 179.9	396.3 ± 165.0	360.3 ± 115.8	312.3 ± 213.9
Day8	358.1 ± 122.6	382.0 ± 168.4	341.3 ± 135.5	376.9 ± 73.8
Day 15	323.0 ± 170.8	404.8 ± 159.8	379.9 ± 155.7	394.0 ± 136.1

* Statistically significant difference from the control group mean at the 5 % level (Student's t-test, two-sided)

F. PATHOLOGY

Necropsy

No macroscopic findings were detected.

Histopathology

No microscopic findings were considered to be treatment-related.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study is considered acceptable. There was no evidence of specific neurotoxicity up the highest single dose of 2000 mg/kg bw. Based on the study results the NOEL for acute neurotoxicity, following single oral administration of glyphosate acid, is 2000 mg/kg bw.

Assessment and conclusion by RMS:

Subchronic neurotoxicity

1. Information on the study

Data point:	CA 5.7.1/002
Report author	[redacted]
Report year	2006
Report title	Ninety Day Repeated Dose Oral (Dietary) Neurotoxicity Study in the Rat
Report No	2060-0010
Document No	NA
Guidelines followed in study	OECD 424 (1997)
Deviations from current test guideline	None
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

In a subchronic neurotoxicity study, groups of 10 male and 10 female Sprague-Dawley (Crl:CD® (SD) IGS BR) rats were fed diets containing 0, 1000, 5000 or 20000 ppm glyphosate (equivalent to a mean achieved

dosage of 0, 77, 395, or 1499 mg glyphosate /kg bw/day in males and 0, 78, 404, or 1555 mg glyphosate /kg bw/day in females for 90 consecutive days.

Clinical signs, functional observations, bodyweight development and food and water consumption were monitored during the study. Ophthalmoscopic examination was also performed on control group and high dose animals before the start of treatment and during the final week of treatment. Five animals per sex from each dose group were subjected to whole body perfusion with glutaraldehyde: paraformaldehyde, followed by the recording of brain weight. Histopathological examinations of neural tissue were performed on all perfused animals from the high dose and control group animals.

Administration of glyphosate produced no unscheduled deaths and no clinically observable signs of systemic toxicity or neurotoxicity. No treatment-related effects were detected in behavioural assessment, functional performance tests, sensory reactivity, or ophthalmoscopy examinations. No adverse effect on bodyweight changed, dietary intake or food efficiency or water consumption were detected. In addition, there were no treatment-related changes in brain weight. No macroscopic changes or neuropathological changes within a comprehensive histopathological evaluation were detected which could be attributed to administration of glyphosate.

In conclusion, no evidence of a neurotoxic potential was obtained up to the highest dose of 20000 ppm. Therefore, the no observed adverse effect level (NOAEL) for neurotoxic potential, following dietary administration of glyphosate for at least 90 days, was 20000 ppm. (equivalent to 1499 and 1555 mg/kg bw/day for males and females, respectively).

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material:

Identification:	Glyphosate technical
Description:	White crystalline solid
Lot/Batch #:	H05H016A
Purity:	95.7 %
Stability of test compound:	Confirmed for the study period

2. Vehicle and/or positive control: Plain diet

3. Test animals:

Species:	Rats
Strain:	Sprague-Dawley Crl:CD® (SD) IGS BR
Source:	[redacted]
Age:	Approximately 5 - 7 weeks
Sex:	male and female
Weight at dosing:	♂ 115 – 153 g (mean); ♀ 118 – 151 g (mean)
Acclimation period:	Up to 7 days
Diet/Food:	Rodent PMI 5002 (certified) diet (BCM IPS Limited, London, UK) and Rodent Rat and Mouse SQC Ground Diet No. 1 (Special Diets Services Limited, Witham, Essex, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Four per cage per sex in polypropylene grid-floor cages suspended over trays lined with absorbent paper

Environmental conditions:	Temperature:	19-23°C
	Humidity:	40-70 %
	Air changes:	at least 15 / hour
	Photoperiod:	12 hours light/dark cycle

B: STUDY DESIGN AND METHODS

In life dates: August 2005 to February 2006

Animal assignment and treatment

In a subchronic neurotoxicity study, groups of 10 male and 10 female Sprague-Dawley rats were fed diets containing 0, 1000, 5000 or 20000 ppm glyphosate for 90 consecutive days (equivalent to mean achieved dose levels of 0, 77, 395 or 1499 mg/kg bw/day for males, and 0, 78, 404 or 1555 mg/kg bw/day for females).

Dietary admixtures were prepared prior to treatment and then twice during the three month study period (i.e. at approximately monthly intervals). The diet was stored at ambient conditions in labelled, double black plastic bags in labelled, covered plastic bins when not in use.

Prior to the start of treatment, the suitability of the formulation procedure was confirmed by measurement of achieved concentration and homogeneity at concentrations of 20000, 5000 and 500 ppm. Samples were taken of each dietary admixture and were analysed for homogeneity, stability and concentration. The concentration of glyphosate technical in the dietary admixtures was determined by high performance liquid chromatography (HPLC) using an external standard technique.

Mortality/Morbidity

All animals were observed twice daily, early and late during the working day, for morbidity and mortality.

Clinical observations

All animals were examined for overt signs of toxicity, ill-health or behavioural change once daily. All observations were recorded.

Functional observational battery (FOB)

Prior to the start of treatment and during Weeks 3, 5, 9 and 13, all animals were observed for signs of functional/behavioural toxicity. Functional performance tests were also performed together with an assessment of sensory reactivity to different stimuli. Observations were carried out at a similar time on each occasion wherever possible. The minor deviations that occurred were considered not to have affected the purpose or integrity of the results obtained. Detailed individual clinical observations were performed for each animal using a purpose built arena. The following parameters were observed: gait, posture, tremors, twitches, convulsions, bizarre/abnormal/stereotypic behaviour, salivation, pilo-erection, exophthalmia, lachrymation, hyper/hypothermia, skin colour, respiration, palpebral closure, urination, defecation, transfer arousal, tail elevation. Functional performance tests were also performed together with an assessment of sensory reactivity to different stimuli. The examinations included quantitative assessments of muscle weakness (fore- and hind limb grip strength).

Locomotor activity

Locomotor activity was monitored by an automated activity recording apparatus. All animals were tested at weeks 3, 5, 9 and 13. The evaluation period was thirty minutes for each animal (with the exceptions of some females during Week 3, which were assessed for twenty-five minutes). The percentage of time each

animal was active and mobile was recorded for the overall period and also during the final 20 % of the period (considered to be the asymptotic period).

Body weight

Individual bodyweights were recorded on Day 1 (prior the start of treatment) and at weekly intervals thereafter. Bodyweights were also recorded prior to terminal kill.

Food consumption and compound intake

Food consumption was recorded for each cage group at weekly intervals throughout the study. Food utilisation was calculated retrospectively.

Water consumption

Water intake was observed daily, for each cage group, by visual inspection of the water bottles for any overt intergroup differences.

Ophthalmoscopic examination

The eyes of all control and high dose animals were examined pre-treatment and before termination of treatment (during Week 13). Examinations included observation of the anterior structures of the eye, pupillary and corneal blink reflex and, following pupil dilation with a mydriatic, detailed examination of the internal structure of the eye using a direct ophthalmoscope.

Sacrifice and pathology

On completion of the dosing period, all animals were killed by intravenous overdose of sodium pentobarbitone. Five males and five females from each dose group were then perfused with glutaraldehyde: paraformaldehyde.

Following perfusion, all animals (both perfused and non-perfused) were subjected to a gross external and internal necropsy and any macroscopic abnormalities were recorded.

The brain from all perfused animals was weighed prior to immersion in buffered 10 % formalin.

Samples of the following tissues were removed from all perfused animals and were immersed in buffered 10 % formalin for histopathology investigations:
Brain: olfactory bulb, forebrain, centre of cerebrum (including hippocampus), midbrain, cerebellum, pons and medulla oblongata
Dorsal root ganglia: (cervical and lumbar sections)
Dorsal and ventral root fibres: (longitudinal cervical and lumbar sections)
Eyes: (longitudinal sections)
Optic nerve: (longitudinal sections)
Sciatic nerve: proximal (longitudinal and transverse sections)
Tibial nerve: proximal (at the knee) and calf muscle branches - (longitudinal and transverse sections)
Skeletal (calf) muscle: (transverse sections)
Spinal cord: (longitudinal and transverse cervical and lumbar sections)

All tissues from the perfused animals from the high dose and control groups were processed to paraffin wax, sectioned, at a nominal thickness of 5 µm and stained with haematoxylin and eosin for subsequent microscopic examination.

Statistics

Data were processed to give group mean values and standard deviations where appropriate. All data were summarised in tabular form. Where appropriate, quantitative data were analysed by the Provantis™ Tables and Statistics Module. For each variable, the most suitable transformation of the data was found, the use of possible covariates checked and the homogeneity of means assessed using ANOVA or ANCOVA and Bartlett's test. The transformed data were analysed to find the lowest treatment level that showed a significant effect, using the Williams Test for parametric data or the Shirley Test for non-parametric data. If no dose response was found, but the data showed non-homogeneity of means, the data were analysed by a stepwise Dunnett (parametric) or Steel (non-parametric) test to determine significant differences from the control group. Finally, if required, pair-wise tests were performed using the Student t-test (parametric) or the Mann-Whitney U test (non-parametric).

Probability values (p) are presented as follows: $p < 0.01$ (**); $p < 0.05$ (*); $p \geq 0.05$ (not significant).

II. RESULTS

A. DOSING FORMULATION ANALYSIS

The initial dietary admixtures were within 3 % of the nominal concentration and showed the test material to be evenly dispersed with the diet matrix. Stability of the test material in the diet matrix at these concentrations, under the conditions of use during the study, was also confirmed as part of these initial investigations. Achieved concentrations following storage for six weeks were within 6 % of initial concentrations.

Achieved concentrations of dietary admixtures used on the study were measured on three separate occasions and were between 80-102 % of nominal concentration. Homogeneity was also confirmed on these occasions. These results confirmed the continuing accuracy of the formulation procedure.
These results indicate that the mean prepared dietary admixture concentrations were within acceptable limits for the purpose of the study

B. MORTALITY

There were no unscheduled deaths during the treatment period.

C. CLINICAL OBSERVATIONS

There were no neurotoxicologically significant clinical observations detected during the study or at terminal kill.

Incidents of generalised fur loss, staining of the fur and scab formation detected throughout the control and treatment groups, were considered unrelated to treatment. One female treated with 1000 ppm glyphosate displayed a kink in the tail from Day 79 onwards. This was an isolated finding and considered unrelated to treatment.

D. FUNCTIONAL OBSERVATIONS

Functional observational battery (FOB)

No treatment-related effects were detected in the parameters investigated.

All inter and intra group differences in urination, defecation and transfer arousal scores were considered to be a result of normal variation for rats of the strain and age used and were of no toxicological importance.

Sensory Reactivity Assessment

There were no treatment-related changes in the sensory reactivity parameters investigated.

All inter and intra group differences in sensory reactivity scores were considered to be a result of normal variation for rats of the strain and age used and were of no neurotoxicological importance.

Grip Strength Measurements

There were no statistically significant differences detected for mean forelimb and mean hindlimb grip strength test values for treated animals of either sex, in comparison to controls during the study period.

Motor activity

Motor activity assessments did not reveal any obvious signs of neurotoxicity.

Overall activity for females was increased at all treatment levels on Day 57 and Day 85 when compared to control values ($p < 0.01$). No such effects were evident at the previous time-points for females, and in the absence of supporting data to suggest these increases were attributed to treatment, these findings were most probably due to low control values and unrelated to treatment.

Statistically significant increases were also evident for the last 20 % activity for females treated with 5000 and 1000 ppm on Day 57, but this was not apparent at 20000 ppm. No such effects were evident on Day 85 and in the absence of a convincing dose-related response. These findings were considered to have arisen incidentally and were of no toxicological importance.

Effects for males were confined to statistically significant increases in overall activity evident for males treated with 1000 ppm on Day 15 and Day 29 ($p < 0.05$) and males treated with 5000 ppm showed a statistically significant reduction during Day 85 ($p < 0.01$). No effects were evident for males treated with 20000 ppm throughout the treatment periods, and in the absence of a dose-related response, these intergroup differences were considered to be of no toxicological significance.

Table 5.7.1-6: Ninety Day Repeated Dose Oral (Dietary) Neurotoxicity Study in the Rat ([redacted], 2006): Motor activity findings (mean ± SD [s])

		Dietary concentration (ppm)							
		Males				Females			
Day	Assessment	0	1000	5000	20000	0	1000	5000	20000
-1	Overall activity	566.2±174.0	570.2±115.8	542.8±126.7	609 3±207 5	484.8±268.7	492.2±189.7	567.0±128.2	675.0±233.8
	Overall mobile	2.40±2.88	1.20±0 92	2.80±1.4	2.30±1.83	1.70±1.77	1.60±1.43	1.30±1.49	1.20±0.79
	Last 20 % activity	14.60±15.63	12.20±12.20	16.30±15.81	14.40±16.05	25.00±39.69	10 30±10.49	7.90±5.32	72.90±83.98
	Last 20 % mobile	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.10±0.32
15	Overall activity	401.2±119.4	**632.0*±106 93**	543.6±136.6	465.8±243.2	479.0±167.7	444.5±162.3	450.0±239.2	515 2±249.8
	Overall mobile	0.44±0.73	0.78±1.64	0.30±0.48	2.60±3.17	3.20±1.87	2.30±2.21	3.50±4.53	5.60±7.62
	Last 20 % activity	23.8±44.0	9.11±8 31	7.6±10 1	13.20±30.56	55.30±62 58	24 30±35.46	27.10±42.84	82.30±84 54
	Last 20 % mobile	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0 20±0.42	0.10±0.32	0.10±0.32	1.20±1.87
29	Overall activity	431.8±105.3	**594.6*±179 3**	448.5±104.9	423.9±123.8	598.2±178.7	768.7±180.8	648.2±196.0	570 3±197.5
	Overall mobile	0.30±0.67	0.30±0.67	0.10±0 32	0.70±1.06	2.60±2.67	2.60±3.37	3.80±3.68	3.10±3.87
	Last 20 % activity	8.50±7.04	**43.60*±51.88**	8.60±7.76	11.00±8.45	60.20±53 30	7090±63.84	43.60±51.00	75.20±67.80
	Last 20 % mobile	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0 30±0.67	0.20±0.42	0.20±0.63	0.30±0.48
57	Overall activity	496.8±131.5	640.4±195.2	433.2±159.1	524.7±98.2	487.9±170.8	**767.6**±102.2**	**794.9**±84.4**	**528 5**±234.5**
	Overall mobile	0.20±0.42	0.90±1 91	0.30±0.67	0.60±1.07	3 10±4.58	2.30±3.59	4.80±3.05	2.20±2.30
	Last 20 % activity	100.1±293.7	36.50±36.30	24.20±35.99	9.10±10.28	42.90±49.87	**97 80*±48.88**	**104.8*±41.58**	71.40±66.67
	Last 20 % mobile	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.30±0.48	0.80±1.23	0.10±0.32
85	Overall activity	650.9±119.2	508.2±270.0	**344.4**±174.5**	645.2±98.81	363.8±81.61	**655.2**±156.8**	**637.4**±195.3**	**482 2**±161.7**
	Overall mobile	0.00±0.00	0.30±0 95	0.10±0 32	0.30±0.67	1 50±2.12	0.50±0.97	1.20±1.93	0.90±1.37
	Last 20 % activity	41.60±39.07	40.80±42.48	8.10±9 10	43.70±44.38	12.40±15.44	42 30±50.47	81.50±46.39	40.60±53.87
	Last 20 % mobile	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0 10±0.32	0.00±0.00	0.40±0.97	0.10±0.32

* Statistically significant difference from control group mean at the 5 % level
** Statistically significant difference from control group mean at the 1 % level

E. BODY WEIGHT

A slightly inferior bodyweight gain was evident during the first two weeks of treatment for females treated with 20000 ppm ($p < 0.05$) and for males treated with 20000 ppm during the first four weeks of treatment ($p < 0.01$ - $p < 0.05$).

Males treated with 5000 ppm also showed occasional lower bodyweight gain up to Week 9. These changes also showed a dose-related response.

No effects were noted for females treated with 5000 ppm or animals of either sex treated with 1000 ppm throughout the treatment period.

Table 5.7.1-7: Ninety Day Repeated Dose Oral (Dietary) Neurotoxicity Study in the Rat ([redacted], 2006): Group Mean Weekly Bodyweight Gains (mean ± SD [g])

	Dietary concentration (ppm)							
	Males				Females			
Day	0	1000	5000	20000	0	1000	5000	20000
1-8	63.3±6.8	60.8±10.0	60.7±6.5	46.1**±4.3	37.4±8.2	36.9±5.4	41.6±5.7	30.5*±5.3
8-15	55.3±6.9	56.8±5.1	51.3±5.3	41.6**±7.5	27.5±3.5	25.0±6.0	25.3±8.5	20.6*±3.7
15-22	49.5±13.5	49.2±7.3	45.1±9.7	39.4*±7.5	21.1±6.5	18.8±4.8	17.9±7.7	18.3±5.8
22-29	43.8±10.1	45.3±7.3	34.6*±7.2	35.4*±6.6	13.6±6.0	15.9±6.6	13.8±5.9	16.3±5.9
29-36	27.5±5.9	25.3±9.9	25.5±11.8	27.4±4.5	13.5±6.9	11.2±4.7	11.6±6.3	12.1±5.7
36-43	26.5±6.3	33.4±7.5	28.0±5.3	28.1±3.4	13.0±4.3	8.3±4.9	15.2±6.9	9.7±4.9
43-50	20.8±8.3	22.2±6.7	15.4±3.9	20.3±4.6	8.7±4.1	11.9±5.1	10.1±6.8	9.0±6.7
50-57	24.2±5.4	23.4±6.0	18.7±5.4	22.5±6.1	6.4±5.7	7.6±6.2	8.1±7.0	5.9±4.5
57-64	11.1±4.5	13.9±4.2	9.5±4.2	11.3±4.2	7.4±4.5	5.4±5.3	6.2±5.6	9.0±5.2
64-71	22.0±6.7	21.2±5.4	17.3*±4.0	16.1*±3.1	7.1±3.4	7.0±5.5	6.9±7.6	2.7±4.1
71-78	15.4±5.1	15.2±4.8	16.1±4.8	13.6±4.8	7.9±4.2	5.4±5.4	5.9±5.6	7.0±5.9
78-85	19.4±6.7	17.9±5.3	17.4±4.3	18.1±5.9	3.4±6.5	3.0±5.2	5.8±5.2	1.8±5.0
85-91	9.8±4.9	9.6±5.1	11.7±5.5	9.8±3.8	2.2±8.1	6.8±5.1	6.8±5.1	3.2±7.1
Abs. Gain: 1-91	388.6±57.9	394.2±51.6	351.3±36.3	329.7±37.9	169.2±23.5	175.2±22.7	175.2±22.7	146.1±13.1
% Gain: 1-91	283.8±38.4	287.5±37.5	263.0±27.7	247.2±34.7	128.7±17.8	132.4±17.9	132.4±17.9	111.5±12.5

* Statistically significant difference from control group mean at the 5 % level
** Statistically significant difference from control group mean at the 1 % level

F. FOOD/WATER CONSUMPTION AND COMPOUND INTAKE

Dietary intake was slightly lower than the controls during the first two weeks of treatment for males treated with 20000 ppm. Thereafter, recovery of food intake was apparent, although food consumption for males still tended to be marginally lower than controls throughout the treatment period. For females at this level, there was a suggestion of lower intake during the first two weeks of treatment, however, differences from controls were minimal and food intake thereafter was essentially similar to controls. None of these changes achieved statistical significance.

Food consumption for both sexes at 5000 and 1000 ppm were considered to have been unaffected by treatment.

Table 5.7.1-8: Ninety Day Repeated Dose Oral (Dietary) Neurotoxicity Study in the Rat ([redacted], 2006): Group Mean Weekly Food Consumption (g/animal/day)

	Dietary concentration (ppm)

	Males				Females			
Week	**0**	**1000**	**5000**	**20000**	**0**	**1000**	**5000**	**20000**
1-2	25.4	25.5	24.8	21.5	19.3	19.6	20.3	17.6
2-3	27.9	28.0	26.4	23.1	19.0	18.7	20.0	17.5
3-4	25.4	29.0	26.6	26.1	18.6	18.9	19.7	18.1
4-5	25.3	30.0	27.9	25.5	20.0	20.0	20.6	20.4
5-6	30.3	29.4	27.2	25.7	20.4	20.5	21.4	20.2
6-7	29.3	30.9	27.9	26.6	20.7	20.7	21.3	20.4
7-8	29.4	30.7	28.0	27.4	20.8	21.3	21.7	20.2
8-9	29.4	30.8	28.1	26.9	20.6	20.4	21.5	19.9
9-10	29.3	30.5	27.9	25.2	20.4	19.9	20.6	19.8
10-11	29.5	30.4	28.5	26.8	20.4	19.6	19.9	19.4
11-12	32.5	30.8	28.7	27.0	20.4	19.4	20.5	19.7
12-13	26.5	29.8	28.6	27.3	20.0	19.3	20.4	19.0
13-13	29.1	30.0	28.6	27.2	18.9	18.8	20.2	18.4

There was no clear effect on food conversion efficiency at any of the dietary inclusion levels investigated, despite the initial effect observed on bodyweight gain and food consumption at 20000 ppm. This finding suggests that the initial reduced bodyweight gain and food intake at this inclusion level could be attributable to slight unpalatability of the dietary admixture.

The mean doses received for males and females respectively were 77, 395, 1499 and 78, 404, 1555 mg glyphosate /kg bw/day at dose levels of 1000, 5000 and 20000 ppm, respectively

Daily visual inspection of water bottles did not show any overt intergroup differences.

G. OPHTHALMOSCOPY

No treatment-related intergroup differences were detected.

The incidental finding recorded for one control animal (female number 11) at the pre-test observation (diffuse opacity of the right eye) was consistent with a low incidental normal finding in young rats of the strain employed and had resolved by the end of the treatment period.

H. PATHOLOGY

Brain measurements

A slight increase in brain weight, both absolute and relative to terminal bodyweights, was evident for males treated with 20000 ppm ($p < 0.05$). This was most probably a consequence of the slight reduction in bodyweight gain evident during the first weeks of treatment. All values were within the normally expected ranges for these parameters and in the absence of any histopathological correlates, these differences were considered to be of no toxicological importance.
No effect was evident for females treated with 20000 ppm or for animals of either sex treated with 5000 or 1000 ppm.

Table 5.7.1-9: Ninety Day Repeated Dose Oral (Dietary) Neurotoxicity Study in the Rat ([redacted], 2006): Group Mean Brain Weights with Corresponding Relative (% of bodyweight) Brain Weight

		Dietary concentration (ppm)							
		Males				Females			
Week		**0**	**1000**	**5000**	**20000**	**0**	**1000**	**5000**	**20000**
Terminal	Mean [g]	535.4	528.6	486.6	480.6	314.8	295.0	302.6	270.4
Bodyweight[#]	SD	77.4	49.9	47.9	26.9	20.1	17.7	23.4	12.9

	N	5	5	5	5	5	5	5	5
Brain (Including Cerebrum. Cerebellum and Pons	Mean [g]	2.087	2.113	2.075	2.158*	1.939	1.908	1.876	1.940
	SD	0.091	0.082	0.070	0.032	0.023	0.115	0.064	0.055
	N	5	5	5	5	5	5	5	5
	Mean [%]	0.395	0.402	0.429	0.450*	0.619	0.647	0.623	0.719
	SD	0.048	0.038	0.039	0.022	0.049	0.024	0.055	0.048
	N	5	5	5	5	5	5	5	5

[#]: numbers may slightly differ compared to the study report due to rounding.

Necropsy

No macroscopic abnormalities were detected in control or treatment group animals at terminal kill.

Histopathology

Histopathological examination of the selected tissues from animals of either sex treated with 20000 ppm did not show any treatment-related pathological changes.

Study conclusion:
Dietary administration of the test material, glyphosate technical, to rats for a period of ninety consecutive days at dietary concentrations of up to 20000 ppm did not result in any neurotoxic effects. The "No Observed Adverse Effect Level" (NOAEL) for neurotoxicity was, therefore, considered to be 20000 ppm.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study is considered acceptable. No evidence of a neurotoxic potential was observed up to the highest dose of 20000 ppm. The no observed effect level (NOEL) for neurotoxic potential, following dietary administration of glyphosate acid for at least 90 days, was 20000 ppm, corresponding to 1499 / 1555 mg/kg bw/day for males and females, respectively.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.7.1/003
Report author	[redacted]
Report year	1996
Report title	Glyphosate Acid: Subchronic Neurotoxicity Study In Rats
Report No	[redacted]/P/4867
Document No	NA
Guidelines followed in study	Study was pre-guideline, but satisfies in general the requirements of OECD 424 (1997)
Deviations from current test guideline	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised	Yes

testing facilities	
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

In a subchronic neurotoxicity study, groups of 12 male and 12 female Alpk:APfSD (Wistar-derived) rats were fed diets containing 0, 2000, 8000 or 20000 ppm glyphosate acid for 13 weeks.

All animals were observed prior to the study start and daily throughout the study for any changes in clinical condition. In addition, detailed clinical observations, including quantitative assessments of landing foot splay, sensory perception and muscle weakness, were performed at intervals. Locomotor activity was also monitored at intervals. At the end of the study, 6 rats/sex/group were killed and subjected to a full post mortem examination. Selected nervous system tissues were removed, processed and examined microscopically.

Administration of glyphosate acid produced treatment-related effects on growth and food utilisation in males receiving 20000 ppm, with no associated effects on food consumption. There were no treatment-related effects on bodyweight, food consumption or food utilisation for males receiving 2000 or 8000 ppm, or for females from all treated groups.

There were no clinical signs of toxicity or effects on any of the quantitative functional observation battery tests or on locomotor activity that indicated any neurotoxic potential. In addition, there were no treatment-related changes in brain weight, length or width. Comprehensive histopathological evaluation of the peripheral and central nervous system showed no evidence of any changes which could be attributed to administration of glyphosate acid.

In conclusion, no evidence of a neurotoxic potential was obtained up to the highest dose of 20000 ppm. Therefore, the no observed effect level (NOEL) for neurotoxic potential, following dietary administration of glyphosate acid for at least 90 days, was 20000 ppm (equivalent to 1546.5 and 1630.6 mg/kg bw/day for males and females respectively).

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material:

Identification:	Glyphosate acid (technical)
Description:	White solid
Lot/Batch #:	P24 ([redacted] reference number: Y04707/034)
Purity:	95.6 %
Stability of test compound:	Confirmed for the study period
2. Vehicle and/ or positive control:	Plain diet

3. Test animals:

Species:	Rats
Strain:	Alpk:APfSD
Source:	[redacted],

Age:	At least 6 weeks
Sex:	male and female
Weight at dosing:	♂ 215.0 – 218.6 g (mean); ♀ 173.5 – 178.8 g (mean)
Acclimation period:	Approximately 2 weeks
Diet/Food:	CT1 diet (Special Diet Services Limited, Witham, Essex, UK), *ad libitum* (except up to 24 hours prior to dosing)
Water:	Tap water, *ad libitum*
Housing:	Four per cage per sex in stainless steel cages (26.5 x 50.0 x 20.7cm)
Environmental conditions:	Temperature: 19-23°C Humidity: 40-70 % Air changes: 25-30/hour Photoperiod: 12 hours light/dark cycle

B: STUDY DESIGN AND METHODS

In life dates: 1995-05-09 to August 1995

Animal assignment and treatment

In a subchronic neurotoxicity study, groups of 12 male and 12 female Alpk:APfSD (Wistar-derived) rats were fed diets containing 0, 2000, 8000 or 20000 ppm glyphosate acid for 13 weeks (equivalent to mean achieved dose levels of 0, 155.5, 617.1 and 1546.5 mg/kg bw/day for males, and 0, 166.3, 672.1 and 1630.6 mg/kg bw/day for females) glyphosate technical.

All diets were based on CT1 diet supplied by Special Diets Services Limited, Stepfield, Witham, Essex, UK. The experimental diets were prepared in 30 kg batches by direct addition of the test substance to 30 kg of CT1 diet and mixing thoroughly. The diets were stored at room temperature until required for use.

Samples from all dietary levels (including controls) were taken at intervals throughout the study and analysed quantitatively for glyphosate acid. The homogeneity of glyphosate acid in CT1 diet was determined by analysing samples from the low and high dose levels. The chemical stability of glyphosate acid in diet, under the conditions of storage used on this study, was determined for 2000 ppm and 20000 ppm diets prepared for use on a concurrent 1 year feeding study in the rat in the same laboratory.

Clinical observations

A check for clinical signs of toxicity, ill health and behavioural changes was made once daily on all animals. All observations were recorded. A detailed physical examination was performed on each rat prior to start of treatment, and at weekly intervals thereafter.

Functional observational battery (FOB)

Prior to the start of treatment and during Weeks -1, 5, 9 and 14, all animals were observed for signs of functional/behavioural toxicity. The assessment involved observations in the home cage and/or while the rat was moving freely in a standard arena followed by manipulative/in hand tests Functional performance tests were also performed together with an assessment of sensory reactivity to different stimuli. The examinations included quantitative assessments of landing foot splay, sensory perception (tail-flick test) and muscle weakness (fore- and hind limb grip strength). The clinical observations included, but were not limited to, the following list: assessment of autonomic function (e.g. lachrymation, salivation, piloerection,

exophthalmus, urination, defecation, pupillary function, ptosis); description, incidence and severity of any convulsions, tremors, abnormal motor function, abnormal behaviour; reactivity to stimuli; changes in level of arousal; sensorimotor responses; alterations in respiration.

Locomotor activity

Locomotor activity was monitored by an automated activity recording apparatus. All animals were tested at weeks -1, 5, 9 and 14. Each observation period was divided into ten scans of five minute duration. Treatment groups were counter balanced across test times and across devices and when the trials were repeated each animal was returned to the same activity monitor at approximately the same time of day. Motor activity was assessed in a separate room to minimise disturbances.

Body weight

Individual body weights were recorded in week -1, immediately prior to treatment, at weekly intervals thereafter, and at necropsy.

Food consumption and compound intake

Food consumption was recorded as required for each cage group throughout the study and calculated on a weekly basis. Food utilisation and compound intake were calculated.

Water consumption

Not reported.

Ophthalmoscopic examination

Not performed. However, ophthalmological data are available from other repeated dose studies.

Sacrifice and pathology

At the scheduled termination, all main study animals not required for neuropathology, were killed by overexposure to rising concentrations of carbon dioxide gas and were discarded without examination.

At termination, the six rats/sex/group designated for neuropathology were deeply anaesthetised with intraperitoneal sodium pentobarbitone and killed by whole body perfusion fixation with modified Karnovsky's solution. The following tissues were submitted: brain, spinal cord (cervical and lumbar), Gasserian ganglion, dorsal root ganglia and spinal roots (cervical and lumbar), gastrocnemius muscle, sciatic nerve, sural nerve and tibial nerve.

Brain weight, brain length and brain width were determined.

Submitted tissues were processed as follows: brain (seven levels including the cerebral cortex, the hippocampus, the cerebellum, the pons and medulla), dorsal root ganglia and spinal roots from cervical and lumbar regions of the cord after decalcification, and gastrocnemius muscle from rats receiving either control diet or diet containing 20000 ppm glyphosate acid were routinely processed, paraffin wax embedded and 5µm thick sections were cut and then stained with haematoxylin and eosin. Sections of brain and cord were in the transverse plane.

The Gasserian ganglion, sciatic nerve, spinal cord (cervical and lumbar portions), sural and tibial nerve from control and high dose group rats were processed and then embedded in Araldite. Semi-thin sections were cut and then stained with toluidine blue. For bilateral tissues only the left was processed. All tissues were sectioned in the transverse plane except the sciatic nerve which was sectioned in both the transverse

and the longitudinal plane.

Neuropathological examination was performed on control and highest dose group animals only. All sections were examined by light microscopy.

Statistics

All data were evaluated using analysis of variance and/or analysis of covariance for each specified parameter using the GLM procedure in SAS (1989)[32]. Least-squares means for each group were calculated using LSMEAN option in SAS PROC GLM. Unbiased estimates of differences from control were provided by the difference between each treatment group least-squares mean and the control group least-squared mean. Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean using a two-sided Student's t-test, based on the error mean square in the analysis.

The levels of probability chosen as significant different from control were $p < 0.01$** and $p < 0.05$* (Student's t-test, two-sided).

II. RESULTS

A. DOSING FORMULATION ANALYSIS

The achieved mean concentrations of glyphosate acid in diet were within 4 % of the nominal levels, with individual values being within 15 % of nominal. There were considered acceptable. The homogeneity of the low- and high-dose diets was considered acceptable, with a deviation from the overall mean values of ± 4 %. The chemical stability was considered satisfactory.

B. MORTALITY

No deaths occurred during the study.

C. CLINICAL OBSERVATIONS

There were no treatment-related clinical signs of toxicity.

D. FUNCTIONAL OBSERVATIONS

Functional observational battery (FOB)

There were no clinical signs that could be attributed to administration of glyphosate acid.

There was an apparent increase in the incidence of miosis and decreased pupil response to light in males receiving 20000 ppm. However, as these signs were seen for several of these males pre-experimentally and were also present at a similar incidence in females with no obvious relationship to treatment, this was considered to be incidental and unrelated to administration of glyphosate acid.

Landing Foot Splay Measurements

There was no evidence of any treatment-related effect on landing foot splay.

Time to Tail-Flick

[32] SAS Institute Inc. SAS/STAT User's Guide, Version 6, Fourth Edition, Volume 2. Cary, NC: SAS Institute Inc., 1989

There was no evidence of any treatment-related effect on time to tail-flick.

Grip Strength Measurements

There was no evidence of any treatment-related effect on forelimb or hind limb grip strength.

Motor activity

There was no evidence of any treatment-related effect on locomotor activity.

During week 5, slightly reduced locomotor activity was recorded on occasions for females receiving 20000 ppm. However, in the absence of any treatment-related effects on motor activity for these animals at other time points during the study, this is considered to be incidental and unrelated to administration of glyphosate acid.

Table 5.7.1-10: Glyphosate Acid: Subchronic Neurotoxicity Study In Rats ([redacted], 1996): Selected motor activity findings

		Dietary concentration (ppm)							
		Males				Females			
Week	Assessment period (min)	0	2000	8000	20000	0	2000	8000	20000
5	1-50	388.7	472.1	335.6	384.4	441.2	379.3	457.8	359.3
9	1-50	304.7	413.4*	298.4	327.3	512.3	488.9	555.1	557.0
14	1-50	299.4	395.1	292.2	372.8	553.0	512.7	569.3	514.7

* Statistically significant difference from control group mean at the 5 % level (Student's t-test, 2-sided)
** Statistically significant difference from control group mean at the 1 % level (Student's t-test, 2-sided)

E. BODY WEIGHT

Group mean bodyweight for males receiving 20000 ppm was statistically significantly lower than that of controls throughout the study. At week 14, group mean bodyweight for these animals was 92.8 % that of controls, equating to a reduction in bodyweight gain of approximately 12 %.

Group mean bodyweight for males receiving 8000 ppm was also marginally lower than that of controls from weeks 6 to 14. However, these differences did not attain statistical significance and were considered too small to be of biological importance.

For males receiving 2000 ppm, and for females at all dose levels, mean bodyweight was essentially similar to that of concurrent controls throughout the study.

Table 5.7.1-11: Glyphosate Acid: Subchronic Neurotoxicity Study In Rats ([redacted], 1996): Intergroup comparison of bodyweights (g)

	Dietary concentration (ppm)							
	Males				Females			
Week	0	2000	8000	20000	0	2000	8000	20000
1	216.0	217.0	218.6	215.0	173.5	178.8	175.6	175.3
2	263.5	264.7	264.9	254.6**	192.7	200.6	196.1	194.3
4	338.2	340.7	339.6	323.7*	214.3	228.3**	224.9**	219.2
8	440.7	440.1	429.1	405.8**	253.6	262.1	260.4	255.4
14	534.7	532.8	526.5	496.1**	285.1	291.5	287.9	281.0

* Statistically significant difference from control group mean at the 5 % level (Student's t-test, 2-sided)
** Statistically significant difference from control group mean at the 1 % level (Student's t-test, 2-sided)

F. FOOD CONSUMPTION AND COMPOUND INTAKE

There were no effects on food consumption. The efficiency of food utilisation for males receiving 20000 ppm was statistically significantly lower than that of concurrent controls during weeks 1 to 8. There were no changes in the efficiency of food utilisation for males receiving 2000 or 8000 ppm or for females from all treated groups.

Table 5.7.1-12: Glyphosate Acid: Subchronic Neurotoxicity Study In Rats ([redacted], 1996): Intergroup comparison of food utilisation (g growth/100 g food)

	Dietary concentration (ppm)							
	Males				Females			
Week	0	2000	8000	20000	0	2000	8000	20000
1-4	18.13	17.16	16.94	16.28*	9.42	9.73	9.36	9.61
5-8	11.52	10.69	10.35	9.93*	5.99	5.55	5.39	5.70
1-13	12.00	11.45	11.38	10.87**	6.08	6.03	6.06	5.96

* Statistically significant difference from control group mean at the 5 % level (Student's t-test, 2-sided)
** Statistically significant difference from control group mean at the 1 % level (Student's t-test, 2-sided)

The mean doses received for males and females respectively were 155.5, 617.1, 1546.5 and 166.3, 672.1, 1630.6 mg glyphosate acid/kg/day at dose levels of 2000, 8000 and 20000 ppm, respectively

G. PATHOLOGY

Brain measurements

There was no evidence of any effects on brain weight, length or width.

Necropsy

There were no macroscopic findings that were considered to be attributable to treatment.

Histopathology

There were no microscopic findings in the peripheral or central nervous system that were considered to be attributable to treatment.

Study conclusion

Dietary administration of glyphosate acid to rats for a period of ninety consecutive days at dietary concentrations of up to 20000 ppm produced evidence of toxicity in the form of reduced growth and reductions in food utilisation for males. Comprehensive histopathological evaluation of the nervous system showed no evidence of any changes in the peripheral or central nervous system which could be attributed to administration of glyphosate acid.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study is considered acceptable. No evidence of a neurotoxic potential was obtained up to the highest

dose of 20000 ppm. The no observed effect level (NOEL) for neurotoxic potential, following dietary administration of glyphosate acid for at least 90 days, was 20000 ppm, corresponding to 1546.5 / 1630.6 mg/kg bw/day for males and females, respectively.

Assessment and conclusion by RMS:

CA 5.7.2 Delayed polyneuropathy studies

1. Information on the study

Data point:	CA 5.7.2/001
Report author	[redacted]
Report year	1996
Report title	Glyphosate acid: Acute delayed neurotoxicity study in domestic hen
Report No	[redacted]/C/3122
Document No	NA
Guidelines followed in study	No guideline stated in the report but in general compliance with OECD 418 (1995)
Deviations from current test guideline	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Twenty birds were given a single dose of glyphosate acid at a level of 2000 mg/kg bw and observed for the following 21/22 days. Twelve negative control (vehicle, distilled water) and twelve positive control (tri-ortho-cresyl phosphate, TOCP, 1000 mg/kg bw) were also dosed. All birds were observed at least twice daily for any changes in clinical condition. Mortality, bird health and clinical signs were recorded at each observation. Following treatment, the birds were examined daily for signs of delayed ataxia. Bodyweight was measured at weekly intervals. Forty eight hours after dosing, pre-determined birds (three from each group) were examined for brain acetylcholinesterase and brain and spinal cord neuropathy target esterase (NTE) activities. At the end of the 21-day post-treatment observation period six birds from each group were examined histopathologically.

No clinical signs of delayed locomotor ataxia were observed in any birds treated with glyphosate acid. Five birds in the positive control group developed clinical ataxia. Brain acetylcholinesterase activity was slightly depressed in birds treated with glyphosate acid and TOCP. This was considered to be of no toxicological significance. Brain and spinal cord neuropathy target esterase (NTE) activities in birds treated with glyphosate acid were similar to negative controls. In the positive control group NTE activity was significantly reduced. Over the 21-day post-treatment period body weight gains were observed in the negative control and in the birds dosed with glyphosate acid. Most birds dosed with TOCP lost weight over

the same period. At necropsy, no changes attributable to treatment with glyphosate acid were detected. Histopathological examination revealed no evidence of acute delayed neurotoxicity or any other treatment-related changes in glyphosate acid-treated hens.

In conclusion, oral administration of a single dose of glyphosate acid at dose level of 2000 mg/kg bw did not produce any clinical signs of delayed neurotoxicity when assessed in terms of clinical ataxia. There was no histological evidence of acute delayed neurotoxicity, and there were no significant reductions in neuropathy target esterase levels in the brain and spinal cord. Therefore, the NOAEL for acute delayed neurotoxicity, following single oral administration of glyphosate acid was 2000 mg/kg bw.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material:

Identification:	Glyphosate acid
Description:	White powder
Lot/Batch #:	P24 (Y04707/034/023)
Purity:	95.6 % w/w
Stability of test compound:	The test substance was shown to be stable for the period of use.
2. Vehicle and positive control:	Vehicle control: Distilled water Positive control: tri-ortho-cresylphosphate (TOCP, purity: 99.0 %, vehicle: corn oil)
3. Test animals:	
Species:	Domestic chicken (Gallus gallus domesticus) female
Strain:	Lohmann Brown (a hybrid brown laying strain)
Source:	[redacted]
Age:	Approximately 12 months
Sex:	Females
Weight at dosing:	1927 – 2215 g
Acclimation period:	Approx. 2 weeks
Diet/Food:	HRC layer ration in pellet form (Parker Bros. Ltd., Lark Mills, Suffolk, UK, *ad libitum* (except overnight starvation prior dosing)
Water:	Tap water, *ad libitum*
Housing:	Floor pens (galvanised steel, concrete floor) measuring 1.8 x 1.4 m. Number of hens per pen not specified.
Environmental conditions:	Temperature: 15 – 17°C Humidity: 79 % Air changes: not given in the report but ventilation considered “adequate” by study author 12 hours light/dark cycle

B: STUDY DESIGN AND METHODS

In life dates: 1996-01-09 to 1996-02-14.

Animal assignment and treatment

Twenty hens were administered glyphosate acid as a single dose of 2000 mg/kg bw by oral gavage. Twelve birds were employed as positive controls and received a single dose of 1000 mg TOCP/kg bw. The negative control group consisted also of 12 hens and received once distilled water also by gavage. The same volume of 10 mL/kg bw was applied to all hens. Treatment was followed by an observation period of 21 or 22 days.

Dosing Formulation Analysis

Verification of the achieved concentrations was done with samples of each preparation. Homogeneity and the chemical stability of glyphosate acid in water was also determined over a period of 2 hours.

Clinical observations

A check for mortality, clinical signs of toxicity, ill health and behavioural changes was made twice daily on all birds.

Body weight

Individual body weights were recorded weekly.

Food consumption

Food consumption was not recorded

Ataxia assessment

Following treatment, hens were examined daily for signs of (delayed) ataxia.

Sacrifice and pathology

Three pre-determined chicken from each group were sacrificed 48 hours after dosing to determine brain cholinesterase, brain neuropathy target esterase and lumbar spinal cord neuropathy target esterase (NTE) activities.

At the scheduled termination, 6 hens from each group were selected for necropsy and histopathological examinations. Whereas in the negative control and glyphosate-treated groups the first six birds in numerical order (because of the absence of clinical signs) were employed, care was taken in the TOCP-treated group to include all animals that had shown clinical ataxia. The remaining hens from all three groups were killed and discarded.

At termination, after perfusion through the heart with fixative, head and spinal column (with brain and spinal cord exposed but left in place) and dissected sciatic nerves (including tibial branches) from the six hens/group designated for neuropathology were taken and stored. The following tissues were used to take samples for histological examination: brain (forebrain, mid and hindbrain), spinal cord (upper and lower cervical, mid-thoracic and lumbo-sacral parts), sciatic nerve (proximal and distal, above knee), tibial nerve. One transverse and two longitudinal sections were performed at each level.

Statistics

No statistical analysis was necessary since the results were quite clear and number of animals limited.

II. RESULTS

A. DOSING FORMULATION ANALYSIS

The achieved concentrations of glyphosate acid in water were within 4 % of the nominal levels. The results confirm that the formulations were homogenous and stable during ambient temperature storage for 2 hours, a period representing the maximum time from preparation to completion of dosing.

B. MORTALITY AND CLINICAL OBSERVATIONS

There were two unscheduled deaths during the study. In the test group receiving glyphosate acid, one bird was found dead on day 10 after dosing. This hen had not exhibited any signs of toxicity prior to death. The cause of death was apparently not elucidated but in the absence of clinical signs and isolated occurrence it was concluded that this mortality was unrelated to treatment. In the positive (TOCP) control group, one bird had been severely pecked by other hens and was sacrificed on humane grounds, during the acclimation period.

In the glyphosate-treated and negative control groups, there were no uncommon clinical signs observed. In the positive control group one more hen was pecked and had to be treated by applying Stockholm tar to the wounds.

C. BODY WEIGHT

Group mean body weight increased in the glyphosate-treated and negative control groups but weight loss was observed in the positive controls receiving TOCP.

D. FOOD CONSUMPTION

food consumption was not recorded.

E. ATAXIA ASSESSMENT

Ataxia was confined to the positive control group receiving 1000 mg TOCP/kg bw. Five of 11 hens were affected. Signs occurred for the first time between post-observation days 11 and 21. The severity of ataxia ranged from 1 – 5 (No ataxia to continuous staggering gait) in a scale of 0-8.

F. PATHOLOGY

Clinical chemistry

In line with ataxia observations, NTE levels in brain and spinal cord were clearly reduced in the positive control group (by 84 % for brain and by 78 % for spinal cord as compared to negative control group) but no effect was seen in the group receiving glyphosate. A very low reduction of brain cholinesterase (mean 6 % less than in negative control) was seen in the hens that had that received glyphosate. In the positive control group, the mean decrease in brain cholinesterase activity was 19 %. No statistical analysis was possible in the study due to the low number (3) of birds investigated. Taking into account the very low difference to negative control birds and the fact that glyphosate is known not to inhibit cholinesterases, a treatment-related effect is unlikely.

Table 5.7.2-1: Glyphosate acid: Acute delayed neurotoxicity study in domestic hen ([redacted], 1996): Clinical chemistry – group mean values

		Group/treatment		
		Negative control **Mean ± SD**	**Glyphosate acid 2000 mg/kg bw** **Mean ± SD**	**Positive control 1000 mg/kg bw TOCP** **Mean ± SD**
Brain	AChE	12.75	11.95 (-6 %)	10.37 (-19 %)

[µmol/g/min]			
Brain NTE [nmol/g/min]	2232	2436 (+9 %)	360** (-84 %)
Spinal cord NTE [nmol/g/min]	544	547 (+1 %)	118** (-78 %)

Number in parentheses refer to percentage reduction in relation to negative control
** $p<0.01$

Necropsy

There were no macroscopic findings that were attributable to treatment.

Histopathology

The evaluation of histological findings is complicated by the fact that axonal degeneration in the spinal cord and peripheral nerves were observed in all three groups in nearly all birds suggesting high background incidence. In the TOCP-treated group, the cerebellum was also affected in five out of six animals (as compared to only one bird in the glyphosate group). Furthermore, axonal degeneration in general was more severe in the positive control group.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study is considered acceptable. Hens receiving glyphosate showed no occurrence of ataxia nor changes in NTE activity. Thus, no delayed neuropathy was observed for glyphosate. Observations of a positive control group receiving TOCP (1000 mg/kg) confirmed the sensitivity of the test. In conclusion, the NOAEL for acute delayed neurotoxicity, following single oral administration of glyphosate acid was 2000 mg/kg bw.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.7.2/002
Report author	[redacted]
Report year	1987
Report title	A 21 Day Oral Neurotoxicity in Domestic Hen of Glyphosate (technical) of Excel Industry Limited
Report No	NA
Document No	NA
Guidelines followed in study	No guideline followed
Deviations from current test guideline	Major deviations from currently adopted OECD 419 (only 21 days treated and no post treatment observation performed). It should be noted that at the time this study was run OECD 419 recommended 90 days exposure but no post exposure observation period.
Previous evaluation	Not accepted in RAR (2015)

GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities (GLP was not compulsory at the time the study was performed)
Acceptability/Reliability:	Invalid
Category study in AIR 5 dossier (L docs)	Category 3b

2. Full summary

Groups of three birds per dose were orally administered daily doses of 0, 250, 500, or 1000 mg/kg bw/day glyphosate in corn oil for 21 consecutive days. The hens were examined at least once daily for signs of overt toxicity. Body weights and egg weights were recorded daily. Food consumption was recorded on days -7 and -3 before the study and on days 1, 4, 8, 11, 15, 18 and 21 during the treatment period. Blood samples were collected from alar vein and haematological and biochemical parameters were monitored prior to treatment, on the 11th day of the study and at termination. Spinal cord and sciatic nerve were histologically analysed using Holmes silver stain.

All hens survived the treatment period and did not exhibit any signs of neurotoxicity except one high dose hen showing slight ataxia on day 18. All hens of the highest dose group appeared hunched and lethargic from day 5 to 11. Red liquid and matting of feathers in anogenital region were noticed from day 16 to the end of the study. In the other groups, hens did not exhibit any clinical symptoms. An overall reduction in body weight of about 20 % as well as a decrease in food consumption occurred in the highest dose group. A slight reduction in haematological parameters (haemoglobin, packed cell volume and red blood cell count) was also found in this group. Blood chemistry, gross pathology and histology did not provide indications of adverse effects. Egg weight were unaffected in all hens.

In conclusion, oral administration of glyphosate for 21 consecutive days up to 1000 mg/kg bw/day did not produce neurotoxicity. Systemic toxicity was observed in the 1000 mg/kg bw/day group.

I. MATERIALS AND METHODS

A: MATERIALS

1. Test material:

Identification:	Glyphosate (technical)
Description:	Not provided
Lot/Batch #:	Not provided
Purity:	Not provided
Stability of test compound:	Not provided

2. Vehicle and positive control: Vehicle control: corn oil

3. Test animals:

Species:	Chicken
Strain:	Gallus domesticus
Source:	[redacted]
Age:	8 - 10 months
Sex:	Females
Weight at dosing:	2110 – 2830 g
Acclimation period:	7 days

Diet/Food:	Poultry feed by Maidc LTD, Bombay, *ad libitum*	
Water:	water, *ad libitum*	
Housing:	Single in wire mesh battery cages	
Environmental conditions:	Temperature:	not provided
	Humidity:	not provided
	Air changes:	not provided
	Light/dark cycle: not provided	

B: STUDY DESIGN AND METHODS

In life dates: Not provided in the study report.

Animal assignment and treatment

Twelve hens were allocated to four dose groups: control (0 mg/kg bw/day), low (250 mg/kg bw/day), mid (500 mg/kg bw/day, and high (1000 mg/kg bw/day). Glyphosate in corn oil was administered by oral intubation into the crop once daily for 21 days.

Dosing Formulation Analysis

No information provided.

Clinical observations

The hens were examined at least once daily for signs of ill health or overt toxicity.

Body weight

Individual body weights were recorded daily from day -7 of the pre-dose period and throughout the treatment period.

Egg weight

The eggs were collected daily and their weights were recorded.

Food consumption

Group food consumption was recorded on days -7 and -3 before the start of the treatment and on days 1, 4, 8, 11, 15, 18, and 21 during the treatment period.

Ataxia assessment

The hens were examined daily and the findings were recorded for each hen. The following score system was used:

No ataxia	0
Doubtful or minor signs	1 (leg weakness)
Positive paralytic signs	2 (lack of leg coordination, loss of balance, tendency to fall back)
Advanced paralytic signs	3 (inability to walk, ataxia, hyperextension, complete prostration and morbidity)
Death	4

Haematology

Blood samples were collected from alar vein and the haematological parameters were examined prior to treatment, on the 11th day of treatment and at termination of the study. The following parameters were examined: haemoglobin (Hb), packed cell volume (PCV), red blood cell count (RBC), white blood cell count (WBC), neutrophils (N), lymphocytes (L), eosinophils (E), basophils (B).

Clinical chemistry

Blood samples were collected from alar vein and the biochemical parameters were examined prior to treatment, on the 11th day of treatment and at termination of the study. The following parameters were examined: serum glutamic pyruvate transaminase (SGPT), serum alkaline phosphatase (SAP), total serum protein, blood urea nitrogen (BUN), cholinesterase (in plasma) (ChE).

Sacrifice and pathology

The hens were killed intravenous injection of pentobarbitone sodium and exsanguinated by cutting cervical blood vessels.
The brain was removed intact and was fixed in 10 % formalin. The entire spinal cord along with the vertebral column was excised and fixed in 10 % formalin containing formic acid. Both sciatic nerves, together with the proximal part of the pereneal and tibial nerves of each leg were removed along with some muscle and fixed in 10 % formalin.
The following tissue from all hens were preserved: brain (intact), spinal cord (entire cord in the vertebral column), sciatic nerves (disted endes, together with the proximal part of the pereneal and tibial nerves with some muscle attached from leg). Transverse and longitudinal sections were made both sciatic nerves and proximal ends of the pereneal and tibial nerves. The spinal cord and sciatic nerve from all the hens were processed to paraffin wax blocks, sections cut at nominal thickness of 10 µm and stained with Holmes silver stain.

Statistics

No information provided.

II. RESULTS

A. DOSING FORMULATION ANALYSIS

Not applicable.

B. MORTALITY AND CLINICAL OBSERVATIONS

All hens survived the treatment period. Hens of the control, low and mid dose groups did not exhibit any clinical symptoms. All hens of the high dose group appeared hunched and lethargic from day 5 to day 11. Red liquid and matting of feathers in anogenital region was seen in all the hens of the high dose group on day 16 onwards.

C. BODY WEIGHT

Overall reduction in body weight of about 20 % occurred in the hens of the high dose group only. The body weights of the low and mid dose groups were found to be comparable with those of hens of the control group.

Table 5.7.2-2: A 21 Day Oral Neurotoxicity in Domestic Hen of Glyphosate (technical) of Excel Industry Limited ([redacted], 1987): Intergroup comparison of body weight (kg)

Dose level of glyphosate (mg/kg bw/day)				
	0 (control) Mean ± SD	250 Mean ± SD	500 Mean ± SD	1000 Mean ± SD
Day 1	2.31 ± 0.14	2.42 ± 0.22	2.47 ± 0.12	2.37 ± 0.09
Day 5	2.34 ± 0.13	2.40 ± 0.25	2.42 ± 0.08	2.36 ± 0.10
Day 10	2.29 ± 0.14	2.43 ± 0.23	2.41 ± 0.10	2.36 ± 0.13
Day 15	2.31 ± 0.13	2.42 ± 0.24	2.38 ± 0.10	2.27 ± 0.11
Day 20	2.29 ± 0.16	2.38 ± 0.22	2.35 ± 0.12	2.02 ± 0.13
Day 22	2.32 ± 0.12	2.41 ± 0.22	2.38 ± 0.14	1.91 ± 0.11

Egg weights: There was no significant difference in the number of egg laid in control, low and mid dose groups. Slight reduction in the number of eggs laid in the high dose group was observed.

D. FOOD CONSUMPTION

The food consumption was unaffected in control hens and hens of low and mid dose group. Food consumption was progressively reduced in the high dose group from day 8 compared to the control group.

Table 5.7.2-3: A 21 Day Oral Neurotoxicity in Domestic Hen of Glyphosate (technical) of Excel Industry Limited ([redacted], 1987):Intergroup comparison of food consumption (g)

Dose level of glyphosate (mg/kg bw/day)				
	0 (control)	250	500	1000
Day -7 to -1	1880	1740	1590	1830
Day 1 to 7	1760	1680	1810	1770
Day 8 to 14	1690	1870	1750	1310
Day 14 to 21	1830	1940	1710	830

E. ATAXIA ASSESSMENT

No hens exhibited any signs of neurotoxicity during the study except one hen of the high dose group which showed slight ataxia on day 18.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Slight reduction in Hb, PCV and RBC was noted in hens of the high dose group at the end of treatment as compared to those in control hens. No changes in haematological parameters were found in the low and mid dose group.

Table 5.7.2-4 A 21 Day Oral Neurotoxicity in Domestic Hen of Glyphosate (technical) of Excel Industry Limited ([redacted], 1987): Haematology – group mean values

Dose level of glyphosate (mg/kg bw/day)				
	0 (control) Mean ± SD	250 Mean ± SD	500 Mean ± SD	1000 Mean ± SD
	Pre-treatment			
Hb [g %]	9.23 ± 0.45	9.57 ± 0.77	9.53 ± 0.45	9.9 ± 0.67
PCV [%]	29 ± 1.6	31 ± 2.12	31 ± 0.12	31.67 ± 1.8
RBC [x 10^6/mm³]	3.03 ± 0.12	3.3 ± 0.24	3.1 ± 0.06	3.4 ± 0.21
WBC – total [x 10^3/mm³]	9.17 ± 0.48	12.3 ± 1.21	10.0 ± 1.03	11.77 ± 0.73

Table 5.7.2-4 A 21 Day Oral Neurotoxicity in Domestic Hen of Glyphosate (technical) of Excel Industry Limited ([redacted], 1987): Haematology – group mean values

	Dose level of glyphosate (mg/kg bw/day)			
	0 (control) Mean ± SD	**250 Mean ± SD**	**500 Mean ± SD**	**1000 Mean ± SD**
N [%]	25.67 ± 4.41	27.33 ± 6.17	30.67 ± 5.34	25 ± 6.63
L [%]	68.67 ± 2.45	64.67 ± 5.89	63.33 ± 7.94	64.33 ± 5.47
M [%]	5.57 ± 2.23	7.67 ± 0.9	8.67 ± 2.45	10.67 ± 1.8
E [%]	0.66 ± 0.68	0.33 ± 0.34	0.66 ± 0.34	0 ± 0
	After 10 days			
Hb [g %]	9.37 ± 0.37	9.87 ± 0.8	9.77 ± 0.63	10.03 ± 0.27
PCV [%]	29.67 ± 0.68	31.67 ± 1.8	31 ± 1.56	31.33 ± 0.09
RBC [x 10^6/mm³]	3.07 ± 0.18	3.3 ± 0.16	3.23 ± 0.09	3.33 ± 0.18
WBC – total [x 10^3/mm³]	10.43 ± 0.44	11.67 ± 0.63	11.37 ± 0.85	11.97 ± 0.92
N [%]	22.67 ± 3.74	32 ± 3.28	29 ± 5.8	32.33 ± 2.41
L [%]	70.33 ± 3.78	58.67 ± 4.42	62.3 ± 4.42	57.3 ± 5.24
M [%]	6.33 ± 1.22	8.67 ± 1.48	8.33 ± 2.07	9 ± 2.56
E [%]	0.67 ± 0.68	0.67 ± 0.34	0.33 ± 0.34	1.33 ± 0.90
	After 21 days			
Hb [g %]	9.43 ± 0.5	9.83 ± 0.74	10.03 ± 0.6	8.6 ± 0.21
PCV [%]	30.67 ± 1.22	32 ± 1.56	32.33 ± 1.89	28.33 ± 0.68
RBC [x 10^6/mm³]	3.17 ± 0.09	3.33 ± 0.17	3.33 ± 0.27	2.87 ± 0.07
WBC – total [x 10^3/mm³]	9.9 ± 0.56	10.46 ± 0.5	10.63 ± 1.3	12.77 ± 0.65
N [%]	31.33 ± 2.65	24.67 ± 4.34	29.67 ± 5.34	26.67 ± 4.75
L [%]	59 ± 4.24	66 ± 2.56	63.67 ± 6.61	65.67 ± 4.75
M [%]	9 ± 1.56	9 ± 3.28	6 ± 1.18	7.33 ± 1.22
E [%]	0.66 ± 0.34	0.33 ± 0.34	0.66 ± 0.68	0.33 ± 0.34

The levels of SGPT, SAP, BUN, total serum protein and blood sugar were comparable in treated hens compared to the control hens.

H. PATHOLOGY

Necropsy

There were no macroscopic findings that were attributable to treatment.

Histopathology

There were no evidence of neurological changes in the spinal cord and peripheral nerves of treated hens and control hens.

3. Assessment and conclusion

Assessment and conclusion by applicant:

To evaluate delayed neurotoxicity this study is considered to be not valid due to major deviations from the current guideline protocol. However, it can be regarded as supportive, that glyphosate does not elicit delayed neurotoxicity in hens up to a dose of 1000 mg/kg bw/day orally for 21 days.

Assessment and conclusion by RMS:

Publications on neurotoxicity

A literature search for the active substance glyphosate was performed in accordance to the provisions of the EFSA Guidance "Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) 1107/2009" and updated Appendix to this Guidance document. The following publications were found relevant and reliable for this section and the summaries are thus presented below and are part of the general discussion at the beginning of the section.

Table 5.7- 1: Overview on literature found relevant for evaluation of glyphosate in section 5.7

Annex Point	Study	Study type	Substance(s)	Reliability & restriction comments	Result
CA 5.7/001	Martinez *et al*., 2019	Acute neurotoxicity, *in vitro* Induced pluripotent stem cells	Glyphosate (Sigma Aldrich, Purity: analytical grade)	Valid, Category 2a Glyphosate and AMPA not adequately characterized No positive controls to verify assays	High levels of glyphosate (100 µM) may result in neurological damage
CA 5.7/002	Martinez *et al*, 2018	Acute neurotoxicity, oral gavage Wistar rats	Glyphosate (Purity: ≥ 98 %)	Valid, Category 2a No positive or negative historical controls No concurrent positive historical control No dose verificaiton	loss of 5-HT, DA and NE levels in CNS
CA 5.7/003	Chorfa *et al*, 2013	Acute neurotoxicity, *in vitro* human neuroblastoma (SH-SY5Y) and melanoma (SK-MEL-2) cell lines	Glyphosate (Sigma Aldrich, Purity: 99.5 %)	Valid, Category 2a No positive control Only two dose groups	No effects observed

12 Information on the study

Data point:	CA 5.7/001
Report author	Martinez, A. *et al.*
Report year	2019
Report title	Effects of glyphosate and aminomethylphosphonic acid on an

	isogeneic model of the human blood-brain barrier.
Document No	doi.org/10.1016/j.toxlet.2018.12.013 E-ISSN: 1879-3169
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	None
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary of the study according to OECD format

In this study, the effect of acute exposure to glyphosate (GPH) on the blood-brain barrier in vitro was investigated based on induced pluripotent stem cells (iPSCs). Two chemical analogues: aminomethylphosphonic acid (AMPA) and glycie (GLY) were used as comparators, Concentrations tested ranged from 0.1 µM to 1000 µM..

I. MATERIALS AND METHODS

Chemicals - Glyphosate (EPA 547 1,000 µg/mL solution), aminomethylphosphonic acid (AMPA) and glycine (GLY) were purchased as analytical grade reagents from Sigma-Aldrich, St. Louis, USA. The purity of the test chemicals was not reported.

Cell culture - Induced pluripotent stem cell line IMR90-c4 iPSC (RRID: CVCL_C437) was purchased from WiCell cell repository (WiCell, Madison, WI). iPSC colonies were maintained on hPSC-grade growth factor reduced Matrigel (C-Matrigel, Corning, Corning, MA) in the presence of Essential 8 medium (E8, ThermoFisher, Waltham, MA).

iPSC differentiation - iPSCs were differentiated into brain microvascular endothelial cells (BMEC). iPSCs were seeded as single cells on T-Matrigel at a cell density of 20,000 cells/cm^2 in E8 supplemented with 10 µM Y-27632. 24 hours after seeding, cells were maintained in E8 for 5 days prior to differentiation. Cells were maintained for 6 days in unconditioned medium (UM: DMEM/F12 with 15 mM HEPES, 20 % knockout serum replacement, 1 % non-essential amino acids, 0.5 % Glutamax and 0.1 mM β-mercaptoethanol). After 6 days, cells were incubated for 2 days in the presence of EC+/+ (EC medium supplemented with 1 % platelet-poor derived serum, 20 ng/mL human recombinant basic fibroblast growth factor (bFGF) and 10 µM retinoic acid). After such maturation process, cells were dissociated by Accutase® treatment and seeded as single cells on tissue-culture plastic surface (TCPS) coated with a solution of collagen from human placenta and bovine plasma fibronectin at 80 µg/cm^2 and 20 µg/cm^2, respectively. Twenty-four hours after seeding, cells were incubated in presence of EC-/- (EC medium supplemented with 1 % platelet poor derived serum (PDS)). Barrier phenotype experiments were performed 48 hours after seeding. Differentiation of iPSCs into neurons was done using an adherent 3-step differentiation method. Co-culture experiments were performed by seeding iPSC-derived BMECs at day 8 of differentiation on inserts juxtaposed over 16-days iPSC-derived neurons. BMECs were maintained in EC medium, whereas neurons were maintained in neuron maturation medium (NMM).

Glyphosate, AMPA and glycine treatment - Dilutions of glyphosate, AMPA and glycine were made immediately before the experiments and maintained in cell medium for 24 or 48 hours. In co-culture experiments, the test compounds were added in the apical chamber at a concentration of 100 µM and incubated for 6 hours. iPSC-derived neuron monocultures exposed to similar concentrations served as controls.

Cell metabolic activity - Following treatment, CellTiter Aqueous® MTS reagent ([3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt) was added to each sample following recommendations by the manufacturer. Cells were maintained for 60 minutes at 37 °C followed by a measurement of absorbance at 490 nm using an ELISA plate reader.
Absorbance obtained from the test samples were subtracted from background absorbance and normalized against controls (untreated cells).

iPSC-derived BMECs barrier function - iPSC-derived BMECs were seeded at a seeding density of 10^6 cells/cm^2 on Transwells (polyester, 0.4 µm pore size, Corning) and coated as previously described. Barrier function was assessed 48 hours after seeding of iPSC-derived BMECs monolayers. Barrier tightness was measured by assessing both the transendothelial electrical resistance (TEER) and paracellular diffusion. TEER was measured using an EVOHM STX2 chopstick electrode. For each experiment, three measurements were performed for each insert and the average resistance obtained was used for the determination of the barrier function.

Fluorescein, glyphosate and mannitol permeability assay - To assess changes in paracellular permeability, sodium fluorescein was added in the apical (top) chamber at a final concentration of 10 µM. 100 µL aliquots were sampled from the basolateral (donor) chamber every 15 minutes for up to 60 minutes. Each aliquot sample was replaced with 100 µL of cell medium. Fluorescein content in the samples was assessed using a fluorimeter ELISA plate reader. Glyphosate permeability was assessed by incubating cells in the presence of 100 µM glyphosate dissolved in EC-/- in the apical chamber. Sampling in the basolateral chamber occurred as previously described. For the determination of glyphosate the samples taken were alkalinized with 17 µL of borax solution followed by the addition of 17 µL of 9-fluorenylmethoxycarbonyl chloride (FMOC-Cl) solution for the derivatisation of glyphosate. Samples were allowed to incubate in the dark under gentle shaking for 2 hours. The derivatisation process was terminated by adding 137 µL dichloromethane. The sample was homogenized and centrifuged at 2000 rpms for 5 minutes to separate the organic phase and analysis by spectrophotometry at 265 nm. Blank EC-/- medium was used as the blank, whereas glyphosate dissolved in EC-/- at concentrations ranging from 10 nM to 10 µM was used to establish a standard curve. For the measurement of mannitol permeability, [^{14}C] D-mannitol was added in the apical chamber with sampling in the basolateral chamber as described previously. Radioactivity was assessed by adding 100 µL sample to 5 mL liquid scintillation cocktail and counted using a Beckman-Coulter LS6500 liquid scintillation counter. The permeability across BMECs monolayers was obtained by calculating the clearance slope from both samples and blank inserts and by the calculation of the Pe value.

Immunocytochemistry - Cells were stained on tissue culture polystyrene (TCPS) plates and fixed with 4 % paraformaldehyde. Cells were blocked for 1 hour at room temperature in PBS supplemented with 10 % normal goat serum (PBS-G) with 0.2 % Triton-X100 and were then incubated overnight in the presence of claudin-5, occludin, GLUT1 or βIII tubulin. Cells were washed with PBS containing 1 % bovine serum album, and incubated in the presence of Alexa Fluor®-488 conjugated secondary antibodies for 1 hour at room temperature. Thereafter the cells were counterstained with DAPI and observed on a Leica inverted epifluorescence microscope. Micrograph pictures were acquired using Leica Acquisition Suite X and processed using ImageJ. Semi-quantitative analysis was done by measuring the average fluorescence intensity of each micrograph picture using the built-in measure tool in ImageJ. Average fluorescence values from negative controls were subtracted from the fluorescence values obtained in the test samples.

Flow cytometry - iPSC-derived BMECs at Day 10 of differentiation were treated with 100 µM glyphosate, AMPA or glycine for 24 hours. Cells were harvested by enzymatic dissociation using Accutase® and fixed with 4 % paraformaldehyde. Cells were blocked in PBS-G supplemented with 0.2 % Triton-X100 dissolved in PBS for 30 minutes, following by an overnight incubation at 4 °C in primary antibody solution (GLUT-1, SPM498 dissolved in PBS-G). Cells were then washed with PBS containing 1 % BSA and incubated in the presence of Alexa Fluor® 555-conjugated antibody. As isotype control, cells were exposed to mouse IgG as primary antibody and analysed using a BD FACSVerse®, with a fluorescence photomultiplier tube (PMT) adjusted to IgG isotype control. Fluorescence intensity for each sample was obtained from a count of 10,000 cellular events. Median fluorescence intensity (MFI, geometric mean) was

determined for each sample and corrected against IgG isotype.

Glucose and doxorubicin uptake assay - Glucose uptake assays were performed by incubating cells grown on TCPS in presence of cell medium supplemented with [^{14}C] D-glucose. Cells were incubated for 60 minutes at 37 °C. Afterwards the cells were washed with ice-cold PBS and homogenized with PBS + 0.2 % Triton-X100 for 10 minutes. Radioactivity was assessed by adding 100 µL sample to 5 mL liquid scintillation cocktail and counted using a Beckman-Coulter LS6500 liquid scintillation counter. The doxorubicin uptake assay was performed by pre-incubating iPSC-derived BMECs in the presence of glyphosate, AMPA or glycine at 100 µM for 2 hours. Doxorubicin was added to obtain 5 µM as a final concentration and allowed to incubate for 1 hour. Cells were homogenized as previously described and total fluorescence assessed by fluorimetry. Total protein content obtained from cell homogenates was determined using a BCA protein assay.

Statistical analysis - Cells were randomly assigned treatment conditions prior each experiment. Data are represented as mean ± S.D. from three or more independent experiments. One-way analysis of the variance (ANOVA) coupled with Dunnett (or Kruskal-Wallis) tests analysis were performed using Prism 7.0 built-in package (GraphPad Software). A p-value < 0.05 was considered as statistically significant.

II. RESULTS

BMEC cell viability – For the assessment of the effect of glyphosate on the viability of BMECs cell monolayers a range of concentrations was used partially overlapping the levels found in patients reported as asymptomatic, minor and moderate (17, 241, and 428 µM, respectively). Treatment with glyphosate, AMPA or glycine for 24 hours at concentrations ranging from 10 to 1000 µM resulted in no changes in cell metabolic activity. This indicates that glyphosate and AMPA unlikely have toxicity towards the blood-brain barrier.

Fluorescein permeability in BMECs monolayers - Changes in the barrier function in BMECs monolayers were measured using TEER and fluorescein permeability. In addition to the previous concentrations used, 2 concentrations (0.1 and 1 µM) were included to reflect average plasma concentrations reported in occupational exposure. No changes in TEER were noted for any of the concentrations tested of glyphosate, AMPA or glycine. However, a biphasic response was noticed in fluorescein permeability. At 0.1 µM, a slight but not statistically significant decrease in fluorescein permeability was found for glyphosate, AMPA and glycine followed by a statistically significant increase for both glyphosate and AMPA at 1 and 10 µM but not at 100 and 1,000 µM. To confirm the increase in the paracellular profile of glyphosate, changes in paracellular permeability were investigated using [^{14}C]-mannitol, an alternative paracellular flux marker at 1 and 10 µM. A modest but statistically significant increase in mannitol permeability was observed at 10 µM glyphosate. No significant increase was noted following AMPA or glycine treatment.

Tight junction complexes integrity - To better understand the effect of glyphosate and AMPA on the barrier function, changes in tight junction complexes were investigated, in particular changes in claudin-5 and occludin, by immunocytochemistry. No changes in claudin-5 immunolocalisation were observed. However, a dose-dependent decrease in claudin-5 relative expression was noted in all groups as quantified by fluorescence intensity. Glyphosate decreased claudin-5 fluorescence intensity at 100 and 1000 µM, but treatment with AMPA already decreased significantly claudin-5 fluorescence intensity at 10 µM. No changes in occludin localization occurred following treatment although a significant decrease in occludin protein levels was noted in all treatment groups with the exception of 10 µM glyphosate. Unlike claudin-5, this effect appeared to be dose-independent.

Diffusion across the BBB - The ability of glyphosate to cross the blood brain barrier (BBB) was investigated following a single exposure at 100 µM in the apical chamber for 2 hours and measurement of the amount of glyphosate present in the basolateral chamber. After 2 hours of diffusion, the amount of glyphosate capable of crossing BMEC monolayers was about 1.67 ± 0.31 % of the applied dose. It was found that the permeability of glyphosate was significantly greater than fluorescein (18.67 ± 3.55 × 10^{-6} cm/min versus

10.59 × 10^{-6} cm/min) or mannitol (13.10 ± 2.03 × 10^{-6} cm/min). Also the effect of glyphosate and AMPA on drug efflux transporters was investigated using doxorubicin as a drug efflux substrate. With the exception of AMPA that showed a 2-fold increase over control, exposure to 100 µM glyphosate or glycine for 24 hours showed no differences compared to controls.

Modulation of glucose uptake in BMECs - As previous studies reported changes in glucose levels in certain vertebrates following exposure to glyphosate and AMPA changes in GLUT1 (the main glucose transporter at the BBB) localization and expression in BMECs following treatment by immunocytochemistry were studied. Following exposure at 100 µM glyphosate, but not at 1,000 µM, an apparent increase in GLUT1 immunoreactivity was noted. Similar results were obtained with AMPA although less pronounced. A flow cytometry analysis was performed where changes in mean fluorescence indexes were compared following exposure at 100 µM for 24 hours. Exposure to glyphosate yielded an increase in GLUT1 expression levels compared to control. Although AMPA showed no differences in GLUT1 expression, glycine exposure resulted in a significant decrease compared to control.

Barrier function of neurons co-cultured with BMECs - As glyphosate showed the ability to cross the BBB and produced changes in GLUT1 expression and glucose uptake in BMECs monolayers, the effects of glyphosate on neurovascular coupling using a BMEC/neurons co-culture model was investigated. First, the ability of such co-cultures to yield barrier function was assessed by measuring differences in TEER between BMECs monocultures and BMECs co-cultured with iPSC-derived neurons. A 3-fold increase in TEER in BMECs co-cultured with neurons compared to BMECs maintained in monocultures was observed. These co-cultures were then exposed to 100 µM glyphosate, AMPA or glycine for 24 hours. TEER measurements indicated that there was no statistically significant difference in barrier tightness when compared to controls. A mild increase in fluorescein permeability was noted with glyphosate when compared to control, but not with AMPA or glycine.

Neurovascular coupling - The effect of glyphosate on neurovascular coupling was investigated by measuring changes in neural cell metabolic activity using MTS following exposure to 100 µM for 6 hours. A significant decrease in cell metabolic activity was observed in co-cultured neurons when compared to monocultures. When co-cultured neurons were exposed to glyphosate and AMPA the metabolic activity was statistically significantly increased when compared to controls. Glyphosate and AMPA produced no statistically significant changes in metabolic activity of mono-cultured neurons. When the effect of glyphosate, AMPA and glycine was investigated in iPSC-derived neuron colonies by immunocytochemistry against βIII-tubulin, no changes were observed both in monocultures and co-cultures, suggesting that changes in cell metabolic activity is unlikely due to cell death.

Neuron progenitor cells (NPC) - The effect of glyphosate on differentiating and differentiated neurons was investigated by exposing cells to a concentration considered representative of the amount crossing the BBB i.e. 0.1–1 µM. First, the effect of an exposure of 24 hours on the cellular metabolic activity of undifferentiated NPCs was investigated using the MTS assay. A significant decrease in cell metabolic activity was seen at concentrations of glyphosate and glycine of 1 µM although immunofluorescence analysis of these NPCs showed no major alterations in the relative cell density and nestin (a cellular marker of neural stem cells/progenitor cells) immunoreactivity. Then, differentiating NPCs were treated continuously for 16 days (by replacing cell medium every 48 hours) in the presence of 0.1 µM glyphosate, AMPA or glycine. The concentration tested is representative of plasma concentrations reported in occupational workers and is 20 times higher than values reported in non-occupational population. No significant changes in cell metabolic activity were observed between the different groups compared to controls. After 16 days of exposure to glyphosate or AMPA no changes in gross morphology of iPSC-derived neuron colonies were evident.

Neurites density - The exposure of iPSC-derived neurons seeded at low density (50,000 cells/cm^2) to glyphosate or AMPA for 24 hours at concentrations ranging from 1 µM to 1000 µM decreased statistically significantly cell metabolic activity at 10 µM and beyond. AMPA showed similar results, albeit not statistically significant. Treatment with glycine showed only small effects on cell metabolic activity.

Changes in cell density and neurites formation by immunocytochemistry were also investigated and, with the exception of glycine, no depletion in neurites was observed with glyphosate and AMPA. Upon quantification of cell nuclei and neurites per surface area a progressive decrease in neuron density was noted for both glyphosate and AMPA, with a significant decrease at 1000 µM. However, no differences in neurite density were noted with glyphosate and AMPA, with the exception of glycine at 100 and 1000 µM.

Discussion
The toxicity of acute glyphosate poisoning on blood-brain barrier integrity was investigated by assessing its activity on the different cell types of the neurovascular unit. Cells were exposed to glyphosate and AMPA at concentrations ranging from 10 to 1000 µM. This is the concentration range of glyphosate plasma values in patients with self-inflicted poisoning. An increase in fluorescein permeability was noted for glyphosate and AMPA at 1 µM and 10 µM. A similar outcome was noted for mannitol in cells exposed to 1 µM glyphosate. This suggests a possible detrimental effect of glyphosate and AMPA on blood-brain barrier function. Although no major changes in tight junction complexes localization were observed, a decrease was found in both claudin-5 and occludin protein levels after exposure to glyphosate, AMPA and glycine, suggesting that glyphosate and AMPA may interfere with tight junction complexes integrity. Yet, the interference of such compounds on tight junction proteins remains unclear. Although not statistically significant, a 50 % increase in paracellular permeability was noted with glycine at 100 and 1000 µM. The data from this study suggest that high levels of glycine may increase the permeability of the BBB and disrupt tight junction complexes. In addition to changes in barrier function, glyphosate permeability in BMEC monolayers was assessed. It is estimated that about 1 % of the applied dose (100 µM) diffused across BMECs monolayers. However, the permeability for glyphosate was significantly higher than that for fluorescein despite its high hydrophilicity (xLogP = -4.63). This indicates that glyphosate crosses the BBB via carrier-mediated diffusion. Amongst the different cell types, neurons displayed the most important changes in metabolic activity following exposure to glyphosate and AMPA. Significant changes in neuronal cell metabolic activity were observed following exposure to glyphosate, AMPA or glycine whereas such changes we not observed in BMECs. Such decrease in cell metabolic activity was unlikely to be considered as neurotoxicity since these effects didn't translate in changes in neuronal cell density and neurites formation. The change in cell metabolic activity observed may be due to changes in glucose metabolism, as changes in glucose uptake in BMECs, as well as some changes in GLUT1 expression levels were noted.

III. CONCLUSION

The data from this study demonstrate the relative safety of glyphosate and AMPA with regard to the blood-brain barrier after acute exposure with minimal effects observed at concentrations significantly higher than baseline exposure levels, occupational and non-occupational alike. The presence of an active uptake and diffusion of glyphosate across the blood-brain barrier suggests the need of extensive brain-centered studies to evaluate the pharmacokinetics and pharmacodynamics of glyphosate on the central nervous system during acute exposure and in individuals exposed to high amounts of such pesticides.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The effect of glyphosate, AMPA and glycine was investigated on the integrity of the blood-brain barrier *in vitro* using an induced pluripotent stem cell line differentiated into brain microvascular endothelial cells (BMEC) and neurons. The endpoints investigated were BMEC cell viability, fluorescein permeability in BMEC cell monolayers, tight junction complexes integrity, diffusion across the blood-brain barrier, modulation of glucose uptake in BMECs, barrier function of neurons co-cultured with BMECs, neurovascular coupling, differentiation of neuron progenitor cells and neurites density. The results of this study indicate that glyphosate or AMPA are unlikely to present toxicity towards the blood-brain barrier. Minimal effects on single parameters were observed with glyphosate or AMPA, but were comparable with effects of the amino acid glycine.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate used was not sufficiently characterized and no positive controls were used in any of the assays conducted.

Assessment and conclusion by RMS:

Reliability criteria for *in vitro* toxicology studies

Publication: Martinez *et al.,* 2019	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity of glyphosate and AMPA not reported. Source: Sigma-Aldrich, St. Louis, USA.
Only glyphosate acid or one of its salts is the tested substance	N	Also glycine and AMPA tested.
AMPA is the tested substance	Y	
Study		
Test system clearly and completely described	Y	
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	NA	
Test concentrations in physiologically acceptable range (<1 mM)	Y	Concentration range *in vitro* from 0.1 to 1000 µM for some tests.
Cytotoxicity tests reported	Y	
Biochemical methods described	Y	Some could be better documented.
Analytical method described	Y	The method for the analysis of glyphosate.
Positive and negative controls	N	No positive controls were used.
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	Y	For some tests
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Reliability not assignable		
Not reliable		

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate used was not sufficiently characterized and no positive controls were used in any of the assays conducted.

1. Information on the study

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2. Full summary of the study according to OECD format

The effects of glyphosate oral exposure on brain region monoamine levels in male Wistar rats were examined. Glyphosate-treated rats (35, 75, 150 and 800 mg/kg bw, 6 days), had no visible injury, i.e., no clinical signs of dysfunction were observed. After the last dose of glyphosate, the levels of serotonin (5-HT), dopamine (DA) and norepinephrine (NE) and its metabolites were determined in the brain regions striatum, hippocampus, prefrontal, cortex, hypothalamus and midbrain, by HPLC. Glyphosate caused statistically significant changes in the 5-HT and its metabolite 5-hydroxy-3-indolacetic acid (5-HIAA), DA and its metabolites 3,4-hydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), and NE and its metabolite 3-metoxy-4-hydroxyphenylethyleneglycol (MHPG) levels in a brain regional- and dose-related manner. Moreover, glyphosate, dose-dependently, evoked a statistically significant increase in 5-HT turnover in striatum and hypothalamus and in DA turnover in prefrontal cortex and hippocampus, and a statistically significant decrease in NE turnover in prefrontal cortex and hypothalamus. The present findings indicate that glyphosate significantly altered central nervous system (CNS) monoaminergic neurotransmitters in a brain regional- and dose-related manner.

I. MATERIALS AND METHODS

Chemicals; Glyphosate [N-(phosphonomethyl) glycine], molecular formula $C_3H_8NO_5P$ CAS RN 107-83-6, purity ≥ 98 %, serotonin (5-HT) and its metabolite [5-hydroxy-3-indolacetic acid (5-HIAA)], dopamine (DA) and its metabolites [3,4-hydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA)] and norepinephrine (NE) and its metabolite [3-metoxy-4-hydroxyphenylethyleneglycol (MHPG)] were purchased from Sigma-Aldrich, St Louis, MO, 63103 USA. All other chemicals were of the highest quality grade and obtained from commercial sources.

Animals and experimental design; All experiments using live animals were undertaken in accordance with the ethics requirements and authorized (protocol number 086) by the official ethical committee of our university. Male Wistar rats of 60 days old each weighing 200–210 g (Charles River Inc., Margate, Kent, UK) were used. The animals were individually housed in polycarbonate cages with sawdust bedding and maintained in environmentally controlled rooms (22 ± 2°C and 50 ± 10 % relative humidity) with a 12 h

light/dark cycle (light from 08.00 to 20.00 h). Food (A04 rodent diet, Scientific Animal Food & Engineering, SAFE, Augy, France) and water were available *ad libitum*. Thirty male rats were assigned randomly to five groups of 6 animals each, a control group and four glyphosate treated groups. Animal treated groups received glyphosate orally at the dose of 35, 75, 150 and 800 mg/kg bw [equivalent to 1/160, 1/75, 1/37 and 1/7 of the acute oral rat LD50 ≈ 5.6 g/kg bw] for 6 consecutive days. The doses were chosen taking into account the LD50 oral value as well as the NOAEL (no observed adverse effect level) described in the literature. The glyphosate treated group rats were deprived of food for 6 h before the oral administration of glyphosate, but were allowed water ad libitum. Glyphosate was dissolved in water and was administered orally by gavage in a maximum volume of 2 mL/rat. Control animals received the vehicle (water) on the same schedules. The animal body weights were measured during the study and food and water consumption of each animal was also assessed. The animals received the treatment at the same time each day, specifically between 10:00 and 11.00 a.m. Three hours after the last dose, the animals were sacrificed by decapitation. The brain was removed quickly and hypothalamus, midbrain, hippocampus, striatum and prefrontal cortex tissues rapidly dissected out at 4 °C. Tissues were rapidly weighed and stored at -80 °C until analysis.

Determination of monoamine levels; The five brain regions analysed in the present study were hypothalamus, midbrain, hippocampus, striatum and prefrontal cortex. Following sample collections, 300–800 µL of 0.4 M $HClO_4$ containing 0.1 % (w/v) $Na_2S_2O_5$ was added to the tissues, and the mixture was homogenized (1 min) by sonication (Labsonic U-Braun). The homogenates were centrifuged (RC5C, Sorvall Instruments) at 12,000g for 20 min at 4 °C and aliquots of supernatants were taken for analysis of 5-HT and its metabolite 5-HIAA, DA and its metabolites DOPAC and HVA and NE, using a high performance liquid chromatography (HPLC) technique with electrochemical detection. Also, aliquots of supernatants were taken for analysis of the norepinephrine metabolite MHPG by HPLC with fluorimetric detection. An acid-catalysed procedure was used to hydrolyse MHPG-sulphate in homogenates of brain region tissues. Volumes of 200–300 µL of the supernatants (in 0.4 M $HClO_4$) were treated for 3 min at 100 °C in a water bath. The samples were then cooled and 30 – 45 µL of 2 M NaOH were added (final pH: ca. 1.5) and aliquots were injected into a reverse phase HPLC system. For the analysis of catecholamines NE, DA, DOPAC and HVA, the mobile phase consisted of 0.1 M $Na_2HPO_4 \cdot 2H_2O$, 0.1 M citric acid (pH 3.5), 1.6 mM octane sulphonic acid, 0.9 mM EDTA and 10 % (v/v) methanol. For the analysis of the indolalkylamines 5-HT and 5-HIAA, the mobile phase consisted of 0.1 M $Na_2HPO_4 \cdot 2H_2O$, 0.1 M citric acid (pH 3.5) and 10 % (v/v) methanol. Elution was performed at a flow rate of 1 mL/min and the working electrode potential was set at 0.8 V for catecholamines and 0.7 V for indolalkylamines. The HPLC system consisted of a Shimadzu liquid chromatograph, model LC-9A equipped with a 5 µm particle size C18-Nucleosil reversed phase column (4 mm i.d. × 125 mm) proceeded by a C18 pre-column, an electrochemical detector (Shimadzu, model L-ECD-6A), a sample injector (20 µL valve) and an integrator (Shimadzu, model C-R6A Chromatopac). For the analysis of the norepinephrine metabolite (MHPG), the mobile phase consisted of 0.06 M $Na_2HPO_4 \cdot 2H_2O$, 0.03 M citric acid and 6 % (v/v) methanol. Elution was performed at a flow rate of 1.5 mL/min. The HPLC system consisted of a Shimadzu liquid chromatograph, model LC-10AS, a 25 µm particle size Tracer Extrasil ODS reversed phase column (4 mm i.d. × 125 mm), a fluorescence detector (Shimadzu, model RF-551), a sample injector (20 µL valve) and an integrator (Shimadzu, model C-R6A Chromatopac). Excitation and emission wavelengths of the detector were 275 and 315 nm, respectively. Peak areas from the sample chromatograms were used to quantify the analytes by external standard technique using solutions of catecholamines (NE, DA, DOPAC y HVA), indolalkylamines (5-HT and 5-HIAA) and norepinephrine metabolite (MPHG) reference standards (Sigma Chemical Co., St Louis, MO, USA). For tissue specimens as determined by use of a linear least squares regression procedure, a linear relationship existed in the calibration curve of catecholamines (NE, DA, DOPAC, HVA), indolalkylamines (5-HT, 5-HIAA) and norepinephrine metabolite (MPHG) over the range of 0.002–100 µg/g, which always yielded a correlation coefficient exceeding 0.9998. Overall mean recovery of catecholamines (NE, DA, DOPAC and HVA), indolalkylamines (5-HT and 5-HIAA) and norepinephrine metabolite (MPHG) from tissues was 100 % for every analyte. Within- and between-day variation was < 4 %. Quantification limit (LOQ) was 2 ng/g for NE, DA, DOPAC, 5-HT and 5-HIAA and 10 ng/g for HVA and MPHG in the different tissue matrices. NE, DA and 5-HT turnover were calculated as ratios of metabolites to neurotransmitter.

Data analysis; Statistical analysis of data was performed using GraphPad Prism 6 for Windows. Results

are presented as mean ± S.D. of 6 animals per group. Results significantly different from controls are also presented as percentage change over control. One-way ANOVA was carried out to determine significant dose-dependent effect of glyphosate on 5-HT, DA, NE and metabolite levels and the corresponding turnover values in the brain regions studied, followed by Tukey's post hoc test. Statistical significance was set at $P < 0.05$. ANOVA's F values are presented in the Tables. F distribution was calculated with numerator degrees (DFn) and denominator degrees of freedom (DFd).

II. RESULTS

The glyphosate-treated rats at oral doses of 35, 75, 150 and 800 mg/kg bw/day for 6 days had no visible injury. These doses were selected based on preliminary experiments where the doses and route of administration did not show any adverse effects, abnormal clinical signs as well as changes in body weight, food and water consumption in the animals (see table below).

Table 5.7- 2: Effect of glyphosate on body weight gain and food and water consumption in male rats

Parameter	Animal groups				
	Control	Glyphosate (35 mg/kg bw, 6 days)	Glyphosate (75 mg/kg bw, 6 days)	Glyphosate (150 mg/kg bw, 6 days)	Glyphosate (800 mg/kg bw, 6 days)
Body weight gain (g)	29.17 ± 2.64	27.67 ± 4.23	29.33 ± 3.08	30.00 ± 4.00	27.33 ± 4.50
Food consumption (g)	96.33 ± 7.45	98.00 ± 7.27	95.66 ± 7.39	93.67 ± 9.24	95.50 ± 9.05
Water consumption (mL)	133.83 ± 20.92	131.50 ± 20.12	129.5 ± 21.50	140.50 ± 24.77	151.00 ± 9.19

Results are presented as means ± SD for six rats.
Results are not significantly different from control group.
Continuous probability distribution (F) for all parameters were lower than 1.140 (DFn = 4, DFd = 25).

All the rat groups exposed to glyphosate by oral route did not show statistically difference on weight of tissues (brain regions) or the ratio weight tissue/body weight (%) compared to control group (data not shown). Glyphosate at a dose of 35 mg/kg bw did not affect the 5-HT, DA, NE and metabolite levels in the brain regions studied. In this study, 35 mg/kg bw might be identified as the NOAEL based on neurotransmitter changes in CNS. Glyphosate at doses of 75, 150 and 800 mg/kg bw produced in a dose-dependent manner a significant decrease of 5-HT content respect to control in striatum. Moreover, glyphosate at doses of 150 and 800 mg/kg bw produced a significant decrease of 5-HT content respect to control in hippocampus and prefrontal cortex and only at a dose of 800 mg/kg bw in hypothalamus and midbrain. In addition, the highest dose (800 mg/kg bw) of glyphosate resulted in a significant decrease in the 5-HIAA levels in hippocampus compared to control group. Also, glyphosate at doses of 150 and 800 mg/kg bw significantly increased the turnover (5-HIAA/5-HT) in striatum and hypothalamus compared to control groups (see table below).

Table 5.7- 3: Effect of glyphosate on 5-HT and 5-HIAA levels and turnover (5-HIAA/5-HT) in brain regions of male rats

Parameter	Animal groups: oral dose of glyphosate during 6 consecutive days	Brain regions				
		Striatum	Hippocampus	Prefrontal cortex	Hypothalamus	Midbrain
5-HT (ng/g)	Control	696.28 ± 14.11	321.60 ± 12.99	710.24 ± 39.86	1698.90 ± 15.31	1551.79 ± 70.09
	35 mg/kg bw	650.05 ± 38.04	319.00 ± 8.17	675.01 ± 49.65	1642.00 ± 74.65	1541.00 ± 62.59
	75 mg/kg bw	557.50 ± 112.42** (−20)[a]	315.16 ± 6.98	603.64 ± 133.99	1575.00 ± 147.39	1515.42 ± 51.99
	150 mg/kg bw	445.57 ± 85.33*** (−36)[a]	293.97 ± 6.23* (−9)[a]	507.20 ± 42.11*** (−29)[a]	1360.75 ± 452.82	1495.33 ± 64.02
	800 mg/kg bw	355.11 ± 16.48*** (−49)[a]	291.06 ± 27.67* (−9)[a]	482.80 ± 69.26*** (−32)[a]	1103.07 ± 111.29*** (−35)[a]	1210.46 ± 134.13*** (−22)[a]
		27.45[b]	5.793[b]	10.51[b]	7.330[b]	18.18[b]
5-HIAA (ng/g)	Control	365.50 ± 75.88	390.31 ± 99.55	299.85 ± 48.05	743.32 ± 78.12	865.30 ± 123.9
	35 mg/kg bw	366.30 ± 55.29	383.04 ± 6.35	297.00 ± 48.98	755.00 ± 33.45	846.00 ± 95.25
	75 mg/kg bw	366.49 ± 68.09	351.54 ± 8.99	295.10 ± 64.32	736.09 ± 55.73	820.32 ± 120.66
	150 mg/kg bw	368.70 ± 31.54	307.93 ± 91.06	270.69 ± 58.58	733.13 ± 116.03	805.00 ± 66.93
	800 mg/kg bw	352.41 ± 60.91	269.81 ± 55.41* (−31)[a]	259.39 ± 37.20	726.33 ± 40.54	708.82 ± 151.74
		0.06677[b]	3.669[b]	0.7277[b]	0.175[b]	1.661[b]
5-HIAA/5-HT	Control	0.53 ± 0.12	1.22 ± 0.30	0.42 ± 0.06	0.45 ± 0.04	0.56 ± 0.09
	35 mg/kg bw	0.56 ± 0.06	1.20 ± 0.04	0.45 ± 0.11	0.47 ± 0.20	0.55 ± 0.08
	75 mg/kg bw	0.66 ± 0.08	1.12 ± 0.04	0.5 ± 0.15	0.52 ± 0.19	0.54 ± 0.09
	150 mg/kg bw	0.86 ± 0.26*** (63)[a]	1.06 ± 0.35	0.54 ± 0.14	0.59 ± 0.18 (30)[a]	0.54 ± 0.04
	800 mg/kg bw	0.99 ± 0.14*** (88)[a]	0.93 ± 0.21	0.54 ± 0.09	0.67 ± 0.15** (43)[a]	0.59 ± 0.13
		20.8[b]	1.591[b]	1.355[b]	13.31[b]	0.3139[b]

** Results are presented are means ± SD from six animals in each group and are significantly different from the control value at $P < 0.01$.
*** Results are presented are means ± SD from six animals in each group and are significantly different from the control value at $P < 0.001$.
* Results are presented are means ± SD from six animals in each group and are significantly different from the control value at $P < 0.05$.
[a] Percentage change over control in parenthesis.
[b] F (continuous probability distribution; DFn = 4, DFd = 25).

With respect to DA, DOPAC and HVA levels and turnover in brain regions (see table below), glyphosate at doses of 75, 150 and 800 mg/kg bw produced, in a dose-dependent manner, a statistically significant decrease of DA levels in prefrontal cortex and midbrain compared to control groups. Likewise, glyphosate at the highest dose (800 mg/kg bw) decreased significantly the DA levels in hypothalamus, striatum and hippocampus compared to control group. Moreover, glyphosate at doses of 150 and 800 mg/kg bw decreased significantly the DOPAC metabolite levels in hypothalamus and glyphosate at highest dose only produced a significant decrease of DOPAC levels in hippocampus respect to control group. In addition, the HVA levels significantly decreased after doses of glyphosate 75, 150 and 800 mg/kg bw in hypothalamus, after doses of glyphosate 150 and 800 mg/kg bw in midbrain; and after dose of 800 mg/kg bw in prefrontal cortex respect to control groups. Glyphosate (75, 150 and 800 mg/kg bw) produced a significant increase of the turnover (DOPAC+HVA/DA) in prefrontal cortex respect to control groups. Glyphosate at highest dose only produced a significant increase of the turnover (DOPAC+HVA/DA) in hippocampus (see table below).

Table 5.7- 4: Effect of glyphosate on DA, DOPAC and HVA levels and turnover (DOPAC+HVA/DA) in brain regions of male rats

Parameter	Animal groups: oral dose of glyphosate during 6 consecutive days	Brain regions				
		Striatum	Hippocampus	Prefrontal cortex	Hypothalamus	Midbrain
DA (ng/g)	Control	6406.35 ± 502.83	248.48 ± 59.36	209.00 ± 45.43	600.20 ± 35.36	776.95 ± 34.09
	35 mg/kg bw	6332.00 ± 296.00	231.00 ± 34.60	186.00 ± 21.33	581.00 ± 51.76	732.00 ± 20.86
	75 mg/kg bw	5614.83 ± 1040.66	213.55 ± 29.46	109.14 ± 16.61*** (−48)[a]	555.48 ± 102.85	676.48 ± 28.33*** (−13)[a]
	150 mg/kg bw	5563.85 ± 223.28	177.84 ± 39.32	97.28 ± 10.78*** (−53)[a]	528.52 ± 39.21	666.07 ± 29.05*** (−14)[a]
	800 mg/kg bw	5009.86 ± 468.29** (−22)[a]	73.07 ± 67.49*** (−71)[a]	34.99 ± 33.65*** (−83)[a]	480.02 ± 23.93* (−20)[a]	642.72 ± 22.98*** (−17)[a]
		6.044[b]	12.49[b]	36.86[b]	4.001[b]	23.71[b]
DOPAC (ng/g)	Control	691.04 ± 64.66	9.51 ± 1.77	21.43 ± 2.68	93.22 ± 21.18	48.75 ± 13.78
	35 mg/kg bw	689.00 ± 74.87	9.44 ± 1.29	21.00 ± 2.23	85.00 ± 18.34	47.00 ± 8.38
	75 mg/kg bw	688.17 ± 221.53	9.00 ± 1.22	19.19 ± 1.23	68.19 ± 21.13	45.78 ± 9.33
	150 mg/kg bw	689.62 ± 37.04	7.66 ± 1.26	18.46 ± 1.01	57.55 ± 6.11** (−38)[a]	44.81 ± 4.09
	800 mg/kg bw	683.38 ± 72.08	6.66 ± 1.95* (−30)[a]	20.04 ± 0.84	57.26 ± 4.70** (−39)[a]	43.78 ± 1.93
		0.00389[b]	4.005[b]	2.958[b]	6.156[b]	0.3051[b]
HVA (ng/g)	Control	907.87 ± 258.95	18.29 ± 1.95	50.75 ± 8.78	68.91 ± 14.12	71.46 ± 3.82
	35 mg/kg bw	906.00 ± 132.32	18.00 ± 3.48	50.60 ± 4.97	64.00 ± 8.34	68.00 ± 5.32
	75 mg/kg bw	901.87 ± 260.40	17.45 ± 4.11	50.33 ± 6.29	44.34 ± 17.40** (−36)[a]	65.10 ± 4.41
	150 mg/kg bw	846.17 ± 48.38	16.17 ± 1.26	46.38 ± 4.27	34.77 ± 5.77*** (−50)[a]	52.91 ± 6.04*** (−26)[a]
	800 mg/kg bw	839.75 ± 32.86	16.40 ± 2.59	30.84 ± 11.55** (−39)[a]	36.82 ± 6.41*** (−47)[a]	38.09 ± 11.10*** (−47)[a]
		0.226[b]	0.6519[b]	7.480[b]	11.50[b]	25.26[b]
(DOPAC+HVA)/DA	Control	0.25 ± 0.05	0.12 ± 0.02	0.36 ± 0.09	0.28 ± 0.25	0.15 ± 0.02
	35 mg/kg bw	0.25 ± 0.04	0.12 ± 0.01	0.39 ± 0.02	0.27 ± 0.06	0.16 ± 0.02
	75 mg/kg bw	0.3 ± 0.11	0.13 ± 0.03	0.64 ± 0.07** (78)[a]	0.22 ± 0.07	0.16 ± 0.02
	150 mg/kg bw	0.28 ± 0.02	0.14 ± 0.03	0.67 ± 0.07** (86)[a]	0.18 ± 0.02	0.15 ± 0.01
	800 mg/kg bw	0.31 ± 0.04	0.98 ± 0.91*** (150)[a]	2.88 ± 2.62*** (153)[a]	0.20 ± 0.02	0.13 ± 0.02
		1.269[b]	5.254[b]	39.28[b]	2.009[b]	2.647[b]

*** Results are presented are means ± SD from six animals in each group and are significantly different from the control value at $P < 0.001$.
** Results are presented are means ± SD from six animals in each group and are significantly different from the control value at $P < 0.01$.
* Results are presented are means ± SD from six animals in each group and are significantly different from the control value at $P < 0.05$.
[a] Percentage change over control in parenthesis.
[b] F (continuous probability distribution; DFn = 4, DFd = 25).

In relation to NE and MHPG levels and turnover, glyphosate at doses of 75, 150 and 800 mg/kg bw produced a significant decrease of the NE levels in striatum and midbrain compared to control. Moreover, glyphosate at doses of 150 and 800 mg/kg bw produced a significant decrease of NE levels in hippocampus

and only at a dose of 800 mg/kg bw in prefrontal cortex respect to control groups. Moreover, MHPG levels significantly decreased after doses of glyphosate 150 and 800 mg/kg bw in hippocampus and after dose of glyphosate 800 mg/kg bw in striatum, prefrontal cortex, hypothalamus and midbrain. Glyphosate at dose of 800 mg/kg bw produced a significant decrease of the turnover (MHPG/NE) in prefrontal cortex and hypothalamus compared to control group (see table below).

Table 5.7- 5: Effect of glyphosate on NE and MHPG levels and turnover (MHPG/NE) in brain regions of male rats

Parameter	Animal groups: oral dose of glyphosate during 6 consecutive days	Brain regions				
		Striatum	Hippocampus	Prefrontal cortex	Hypothalamus	Midbrain
NE (ng/g)	Control	210.64 ± 79.72	190.74 ± 13.01	145.81 ± 3.08	1006.77 ± 195.09	452.04 ± 76.01
	35 mg/kg bw	202.00 ± 37.93	183.00 ± 11.02	145.00 ± 9.34	1005.00 ± 74.85	422.00 ± 39.44
	75 mg/kg bw	121.07 ± 42.09* (−43)[a]	159.24 ± 20.21	138.54 ± 11.18	1000.88 ± 76.64	357.03 ± 55.11* (−21)[a]
	150 mg/kg bw	118.13 ± 24.65* (−44)[a]	150.02 ± 30.98* (−21)[a]	137.29 ± 6.11	901.07 ± 72.69	358.61 ± 31.77* (−21)[a]
	800 mg/kg bw	102.74 ± 27.10** (−51)[a]	137.34 ± 15.13** (−28)[a]	127.64 ± 12.35* (−12)[a]	878.22 ± 71.54	358.92 ± 17.12* (−21)[a]
		7.194[b]	7.981[b]	3.908[b]	2.007[b]	5.083[b]
MHPG (ng/g)	Control	161.88 ± 21.25	67.07 ± 8.13	109.60 ± 28.56	50.29 ± 8.27	71.69 ± 15.56
	35 mg/kg bw	159.00 ± 35.00	61.00 ± 5.85	100.00 ± 12.67	46.00 ± 11.27	70.00 ± 9.32
	75 mg/kg bw	155.46 ± 36.00	54.90 ± 6.67	84.67 ± 12.91	43.40 ± 7.21	69.11 ± 8.54
	150 mg/kg bw	132.94 ± 31.34	51.22 ± 9.28* (−24)[a]	84.46 ± 12.31	43.03 ± 21.58	67.72 ± 10.57
	800 mg/kg bw	108.95 ± 10* (−33)[a]	50.47 ± 9.85* (−25)[a]	71.11 ± 10.32** (−35)[a]	20.22 ± 9.25*** (−60)[a]	29.01 ± 4.66*** (−60)[a]
		3.748[b]	4.451[b]	4.810[b]	5.198[b]	18.61[b]
MHPG / NE	Control	0.85 ± 0.29	0.35 ± 0.06	0.77 ± 0.18	0.05 ± 0.01	0.16 ± 0.03
	35 mg/kg bw	0.84 ± 0.33	0.34 ± 0.05	0.69 ± 0.09	0.05 ± 0.02	0.17 ± 0.02
	75 mg/kg bw	1.42 ± 0.59	0.35 ± 0.08	0.61 ± 0.08	0.04 ± 0.01	0.20 ± 0.04
	150 mg/kg bw	1.14 ± 0.23	0.35 ± 0.08	0.62 ± 0.10	0.05 ± 0.03	0.19 ± 0.03
	800 mg/kg bw	1.10 ± 0.19	0.37 ± 0.07	0.56 ± 0.05* (−23)[a]	0.02 ± 0.01** (−55)[a]	0.18 ± 0.01
		2.733[b]	0.1513[b]	2.792[b]	5.423[b]	1.923[b]

* Results are presented are means ± SD from six animals in each group and are significantly different from the control value at $P < 0.05$.
** Results are presented are means ± SD from six animals in each group and are significantly different from the control value at $P < 0.01$.
*** Results are presented are means ± SD from six animals in each group and are significantly different from the control value at $P < 0.001$.
[a] Percentage change over control in parenthesis.
[b] F (continuous probability distribution; DFn = 4, DFd = 25).

Discussion

Pesticides are widely used in agricultural and other settings, resulting in continuing human exposure. The nervous system represents a prime target for both the acute and chronic effects of pesticides. Among them are the organochlorines, pyrethroids, organophosphates, neonicotinoids, herbicides and some novel agents. Acute symptoms can include headache, nausea, dizziness, and sensory paraesthesia. Toxicity often involves neuronal hyper-excitability, and disorders of cognition. Toxicological in vitro and in vivo studies have demonstrated specific neurodegenerative effects from exposure to certain pesticides, and human case reports have suggested a causal relationship between certain pesticide exposures and Parkinson's disease (PD) typically associated with degeneration of the dopaminergic neurons. Evidence is also now accumulating that organophosphate pesticides target serotonin and noradrenergic systems contributing to adverse outcomes related to emotional and social behaviours. Ingestion of the herbicide glyphosate may cause significant toxicity including nausea, vomiting, diarrhea, oral and abdominal pain, renal and hepatic impairment, and pulmonary oedema. Impaired consciousness and seizures have also been reported as sequelae but there are limited data of glyphosate on central nervous system (CNS) toxicity. This study was designed to investigate the effects of glyphosate on CNS monoaminergic neurotransmitter contents (5-HT, DA and NE) in male Wistar rats in order to generate more data on the glyphosate neurotoxicity. The current study showed that exposure to glyphosate, in a region and dose-dependent manner, was accompanied by a significant decrease in the 5-HT, DA and NE contents in the brain regions studied (striatum, hippocampus, prefrontal cortex, hypothalamus, hypothalamus and midbrain), which indicated that glyphosate transfer across the blood-brain barrier, enters the brain, probably accumulates in significant quantity, and exerts neurotoxicity altering the serotonergic, dopaminergic and noradrenergic systems. Researchers have reported similar changes of these brain neurotransmitters after exposure to the insecticide pyrethroid cyfluthrin. It should be noted that the rats treated with glyphosate at dose of 35 mg/kg bw per day did not exhibit any effects on the 5-HT, DA and NE contents in the brain regions studied. In our study, this NOAEL

observed (35 mg/kg bw per day) was lowest to that identified on the maternal and developmental toxicity studies (NOAEL of 50 mg/kg bw per day) and used to establish the ADI. For current regulatory evaluation of risks associated with glyphosate exposure, a NOAEL of 35 mg/kg bw per day could be used instead of a NOAEL of 50 mg/kg bw per day. In this regard, taking into account that glyphosate probably accumulates in the CNS and considering that in the present study the glyphosate exposure was only during only 6 days, further research with a longer period of exposure should be necessary to corroborate the proposed NOAEL of 35 mg/kg bw per day. In the present study, quantitative analysis of 5-HT, DA and NE contents showed that the loss of these neurotransmitters was mainly observed in the striatum [5-HT and NE contents decreased significantly (-49 % and -51 %, respectively) after the highest dose of 800 mg glyphosate/kg bw] and in prefrontal cortex and hippocampus [DA contents decreased significantly (-83 % and -71 %, respectively) after the highest dose of 800 mg glyphosate/kg bw]. Previously, Researchers also showed in rats that glyphosate decreased DA but not 5-HT levels in striatum as well as reduced the locomotor activity suggesting that the decrease in striatal DA levels could also explain a behavioural hypoactivity. Moreover, in our study, glyphosate after the highest dose of 800 mg/kg bw produced a significant increase of the (5-HIAA/5-HT) turnover in striatum (88 %) and of the (DOPAC+HVA/DA) turnover in prefrontal (153 %) and hippocampus (150 %), but a significant decrease of the (NE/MHPG) turnover in prefrontal cortex (-23 %) and hypothalamus (-55 %), critical brain regions that regulate cognitive functions. The cooperation of the hippocampus and the prefrontal cortex is vital in spatial working memory performance and decision making. Disconnection or damage to either of the two brain regions induces impaired cognitive behaviours. Because cognitive functions are quite complex, more details are required for the complete understanding of glyphosate-induced neurotoxicity. It would be of interest to investigate the developmental changes of the hippocampus and prefrontal cortex in prenatal glyphosate.

III. CONCLUSION

In conclusion, the results demonstrate that glyphosate leads to loss of 5-HT, DA and NE levels in the CNS. The neurochemical effects observed in the present study are an important public health concern. Although we have no data on humans, glyphosate could exert its neurotoxicity, notably on monoamine systems, by inducing DNA damage, neuronal inflammation and oxidative stress mechanisms. Further investigation is needed to involve the glyphosate herbicide with neurodegenerative diseases.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Although the study concludes "loss of 5-HT, DA and NE levels in the CNS", no historical controls are available to assess and compare the changes in the treatment-groups to ascertain if the effects are within background or if they are biologically relevant.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because there were no negative or positive historical control data to establish whether changes in the levels of neurotransmitters were biologically meaningful. No concurrent positive control was included to demonstrate assay viability. Also no analytical verification of dose levels are available.

Assessment and conclusion by RMS:

Reliability criteria for *in vivo* toxicology studies

Publication: Martínez, M. *et al.,* 2018	**Criteria met? Y/N/?**	**Comments**

Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	Non-guideline
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Technical glyphosate purity of ≥98 %. Source: Sigma-Aldrich. No information on storage,
Only glyphosate acid or one of its salts is the tested substance	Y	Yes
AMPA is the tested substance	N	
Study		
Test species clearly and completely described	Y	Wistar rat (male)
Test conditions clearly and completely described	Y	Yes
Route and mode of administration described	Y	Oral by gavage.
Dose levels reported	Y	35, 75, 150, 800 mg/kg bw/day for 6 days
Positive control	N	-
Number of animals used per dose level reported	Y	6/dose group.
Method of analysis described for analysis test media	N	-
Validation of the analytical method	N	-
Analytical verifications of test media	N	-
Complete reporting of effects observed	N	
Statistical methods described	Y	
Historical control data of the laboratory reported	N	
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	No positive or negative historical control data and no concurrent positive control data included, no analytical verification of dose or stability.
Reliability not assignable		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because there was no historical control to determine if changes in the levels of neurotransmitters were within historical controls. No positive control was included. Also no analytical verification of dose levels are available.		

1. Information on the study

Data point:	CA 5.7/003
Report author	Chorfa, A. *et al*.
Report year	2013

Report title	Specific pesticide-dependent increases in α-synuclein levels in human neuroblastoma (SH-SY5Y) and melanoma (SK-MEL-2) cell lines.
Document No	doi:10.1093/toxsci/kft076 E-ISSN: 1096-0929.
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	Yes, evaluated in RAR (2015)
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary of the study according to OECD format

The objective was to precisely assess changes in α-syn levels in human neuroblastoma (SH-SY5Y) and melanoma (SK-MEL-2) cell lines following acute exposure to glyphosate using Western blot and flow cytometry. The study was conducted using an in vitro test system. Glyphosate did not have any impact on the endpoints measured in this study.

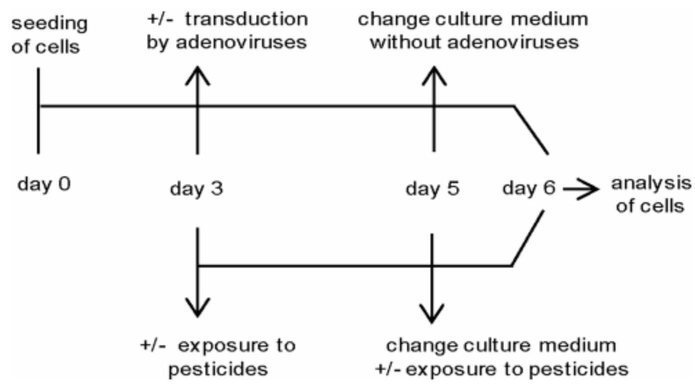
I. MATERIALS AND METHODS

Cell culture: SH-SY5Y (a human dopaminergic neuroblastoma cell line) and SK-MEL-2 (a human cutaneous melanoma cell line) obtained from American Type Culture Collection (ATCC, Rockville, MD) were maintained in Dulbecco's modified Eagle's (DMEM-F12-GlutamaxI) medium containing 10 % fetal bovine serum (Invitrogen), 100 U/mL penicillin, and 100 µg/mL streptomycin in an incubator at 37 °C and 5 % CO2.

Recombinant AdV-mediated overexpression of α-syn. Cell transduction was performed as previously described (see study report). Briefly, a recombinant adenoviral genome containing the full-length complementary DNA encoding human WT and mutant A53T α-syn in frame with a C-terminal myc-His epitope tag was generated by homologous recombination. Cells were infected with α-syn AdV or GFP AdV. On day 2 of infection, the medium was replaced with AdV-free DMEM.

Pesticides exposure: Cells at 70 % confluence were exposed to glyphosate (N-(phosphonomethyl)-glycine) (Sigma-Aldrich). Purity was 99.5 %. Glyphosate was dissolved in ultrapure water. Glyphosate concentrations for cell exposures (at 75 and 50 % viability) were chosen based on the evaluation of toxicity following exposure of the SH-SY5Y cell line (0.005-800 µM) using the 3-(3,4-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The protocol used for cell exposure and / or transduction with recombinant AdVs is shown in the figure below.

Figure 5.7-1: Experimental protocol of exposure to pesticides and recombinant AdV transduction of SH-SY5Y neuroblastoma and SK-MEL-2 melanoma cell lines.



At approximately 70 % cell confluence (day 3), cells were transduced with recombinant AdVs for protein overexpression (WT α-syn, A53T α-syn, GFP) and/or exposed to pesticides (rotenone, paraquat, maneb, and glyphosate) at concentrations corresponding to the IC50 determined on the SH-SY5Y cell line. For certain experiments, these two steps (pesticides exposure/adenoviral transduction) were combined. After 48 h (day 5), two protocols were followed. When the cells were only transduced with AdV, the culture medium was replaced by fresh AdV-free culture medium. When the cells were exposed to pesticides, the culture medium was replaced by fresh AdV-free culture medium supplemented with pesticides. Experiments were ended 24 h later (day 6), and adherent cells were collected for analyses by flow cytometry or Western blot.

Cell death and viability assays: The MTT assay was performed with the Celltiter96 nonradioactive kit (Promega, France). MTT is metabolically converted into formazan by mitochondrial dehydrogenases of healthy, living cells. Briefly, 5 × 10^4 cells per well were seeded into 96-well plates in duplicate and then treated with different concentrations of glyphosate. Cell death was assessed after 72 h of pesticide exposure. Then, 15 µL of "dye solution" was added to each well, and the plates were incubated at 37 °C, 5 % CO_2, for 4 h. Finally, 100 µL of "solubilization/stop solution" was added. After incubation for 1 h at 37 °C, the optical density of the dissolved formazan grains within the cells was measured spectrophotometrically at 560 nm (BioTek ELx808, France). Results were expressed as percentage of the control. The IC_{50}, half-maximal (50 %) inhibitory concentration, was determined for each pesticide from the graph of cell viability.

Protein extraction and Western blotting: After pesticide exposure, the cells were harvested and lysed in Laemmli buffer and then heated for 10 min at 100 °C. The cell extract was then centrifuged (15,000 × g, 30 min at 4 °C) before being loaded on to 12 % gel for SDS-PAGE. Western blots were performed as previously described (see study report). Blots were hybridized with monoclonal antibodies against β-actin (Abcam, dilution 1:1000) and against α-syn (clone 42, BD Biosciences, dilution 1:2000) overnight at 4 °C. The membranes were then washed and further hybridized with goat anti-mouse immunoglobulin G (IgG) horseradish peroxidase–conjugated antibody (Pierce, dilution 1:1000) for 30 min at room temperature. Protein bands were detected with chemiluminescent reagents (SuperSignal West Dura Extended Duration Substrate Kit, Pierce), then exposed to autoradiographic films or to a CCD camera (Versadoc system 5000, Bio-Rad), and quantified by Quantity One soft- ware (Bio-rad).

Flow cytometry: The cells were harvested by centrifugation at 1000 × g for 5 min at 4 °C. The cell pellets were resuspended in a blocking solution (2 % BSA-PBS) at 4 °C. The cells were simultaneously permeabilized and fixed for 20 min at 4 °C with BD Cytofix/Cytoperm Kit (BD Biosciences). All steps were carried out with permwash solution (BD Permwash Kit). After another centrifugation step, the cell pellets were incubated with α-syn clone 42 antibody in permwash solution for 30 min at 4 °C. They were then rinsed with permwash solution and incubated with goat anti-mouse IgG R-phycoerythrin conjugate (Invitrogen) at 4 °C for 30 min. The specific fluorescence intensities were measured with a BD FACS LSRII analyzer (BD Biosciences). Data were acquired using Diva software (BD Biosciences) and analyzed with FlowJo software (v7.6.5-TreeStar, Ashland, Oregon).

Statistical analysis: The results represent means ± SEM from at least 3 and up to 15 independent

experiments. The effects of glyphosate on cell survival, following exposures of the four cell types (SH-SY5Y ± WT α-syn AdV and ± A53T α-syn AdV and ± GFP AdV) to different concentrations, were determined from an analysis of covariance. The results from Western blot studies were subjected to a Wilcoxon's test. For the flow cytometry studies of AdV-transduced cells, the mean fluorescence intensities of cells exposed or not exposed to glyphosate were compared using Student's test. Then, we realized a Levene's analysis in order to verify the hypothesis of homogeneity of variances. If the hypothesis was not verified, the Welch's analysis was used.

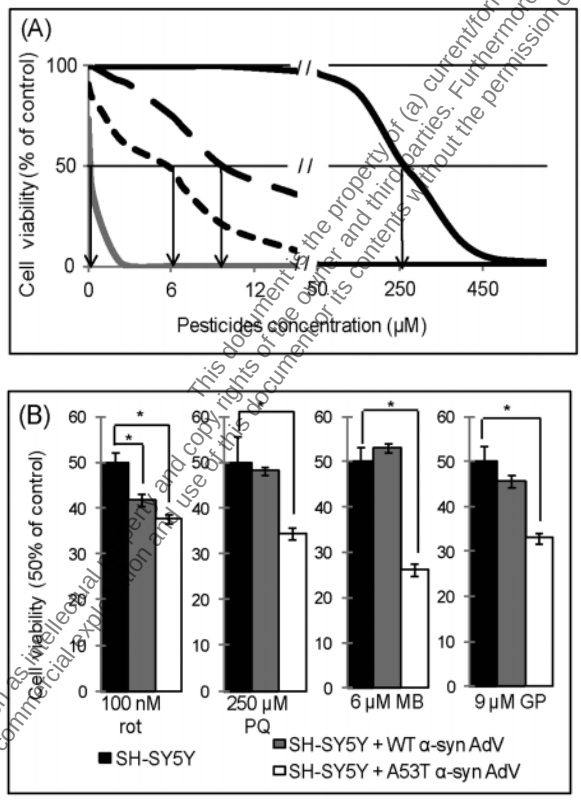
II. RESULTS

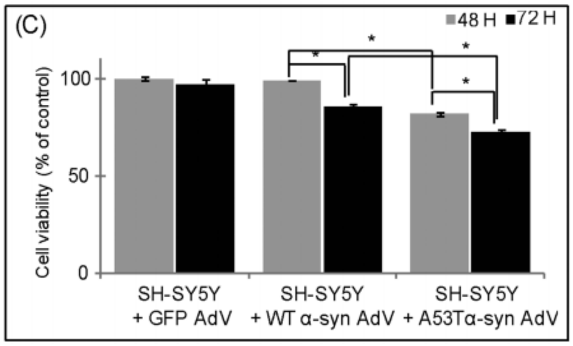
The impact of in vitro exposure to glyphosate on α-syn levels was assessed in two human cell lines of neuronal (SH-SY5Y) or melanocytic (SK-MEL-2) origin. The levels of endogenous α-syn or of recombinant α-syn levels after transduction with recombinant AdVs (WT and A53T α-syn AdV) were analyzed by Western blot and flow cytometry.

Cytotoxicity Associated With Pesticide Exposure and/or Adenoviral Transduction

The SH-SY5Y neuronal cell line was first exposed to various concentrations of glyphosate (0.005-800 µM) for 72 h. The respective cytotoxicity of glyphosate (relative amounts of living and metabolically active cells) was estimated by MTT assay (see figure below). This showed that glyphosate had a distinct effect on the survival of SH-SY5Y cells after 72 h of exposure. The half-maximal (50 %) inhibitory concentrations (IC50) for glyphosate was 9 µM. It was 11 µM for glyphosate for the SK-MEL-2 cell line. The IC50 value, determined after exposure of the SH-SY5Y cell line for 72 h, was chosen for further investigations of the effects on α-syn levels. The same concentration was used in experiments with the SK-MEL-2 melanoma cell line.

Figure 5.7- 2: Effects of pesticides and adenoviral transduction on viability of SH-SY5Y neuroblastoma cells.





(A) Cell viability was assessed using the MTT assay. Data represent mean ± SEM numbers of viable treated cells/numbers of viable untreated cells from three separate experiments (*$p < 0.05$). The p values were determined by Wald's test. The cell viability percentage after 72-h treatment with increasing concentrations (0.005-800 µM) of pesticide was measured: rotenone (grey curve), maneb (dashed black curve), glyphosate (black curve em dash), or paraquat (black solid curve).

(B) Part of the concentration range has been removed to facilitate comparison of the results. We then used the IC_{50} previously determined on SH-SY5Y cells (100 nM and 6, 9, and 250 µM) to quantify the viability of cells transduced with WT or A53T α-syn AdV after pesticide exposure for 72 h in comparison to untransduced SH-SY5Y cells.

(C) The cytotoxicities associated with the recombinant Advs transduction alone, with either the GFP protein, WT or A53T α-syn AdV, were measured at 48 h (grey bars) and 72 h (black bars).

Glyphosate cytotoxicity, following exposure for 72 h of SH-SY5Y cells transduced with recombinant AdVs, was then analyzed using the protocol summarized in Figure 5.7-1. The MTT assay did not reveal a significant decrease in the viability of WT α- syn AdV-transduced cells after exposure to glyphosate [-2 % [$p = 0.6$]) at the previously determined IC50 (see Figure 5.7-2 (B)). Viability was significantly decreased (-17 %) with glyphosate ($p < 0.01$) when the cells were transduced with A53T α-syn AdV. Thus, glyphosate induced a greater reduction of viability in cells transduced with A53T α-syn AdV than in nontransduced SH-SY5Y cells.

It should be noted, however, that viability was also consistently decreased in the absence of pesticide exposure, after transduction with WT α-syn AdV (-14 %)($p < 0.05$) and even more so with A53T α-syn AdV (-38 %)($p < 0.05$) at 72 h, as shown in Figure 5.7-2 (C) representing three different experiments. Viability was already decreased 48 h after transduction with A53T α-syn AdV (-18 %) ($p < 0.05$). Cytotoxicity seemed to be related to α-syn overexpression, as viability was unchanged 48 or 72 h after transduction with the GFP AdV used as a control in these experiments.

Specific Increase of Endogenous α-syn in Human Neuroblastoma and Melanoma Cells Exposed to Pesticides

Both SH-SY5Y neuroblastoma and SK-MEL-2 melanoma cell lines express α-syn. We therefore assessed endogenous α-syn expression using Western blot and flow cytometry and examined the ability of glyphosate exposure to modulate changes in α-syn levels. The Western blot experiments indicated that endogenous α-syn levels (**Error! Reference source not found.** A) were significantly increased by 100 nM rotenone (~1.81×) ($p < 0.001$), whereas no change was observed in the closely related, but nonamyloidogenic, β-synuclein protein **Error! Reference source not found.** C). The impacts of glyphosate on the level of endogenous α-syn in SH-SY5Y neuroblastoma cells was measured in comparison (**Error! Reference source not found.** A). A significant increase in α-syn levels was not observed with glyphosate up to 9 µM ($p = 0.6553$).

Quantification of Recombinant α-Syn Levels by Flow Cytometry Following Pesticide Exposure

We then attempted to quantify the changes in α-syn levels following transduction with AdV designed to overexpress α- syn. Transduction of SH-SY5Y cells with WT or A53T α-syn AdV resulted in similar levels of α-syn expression as measured by flow cytometry. In comparison to the isotype control, a 5.1 % increase

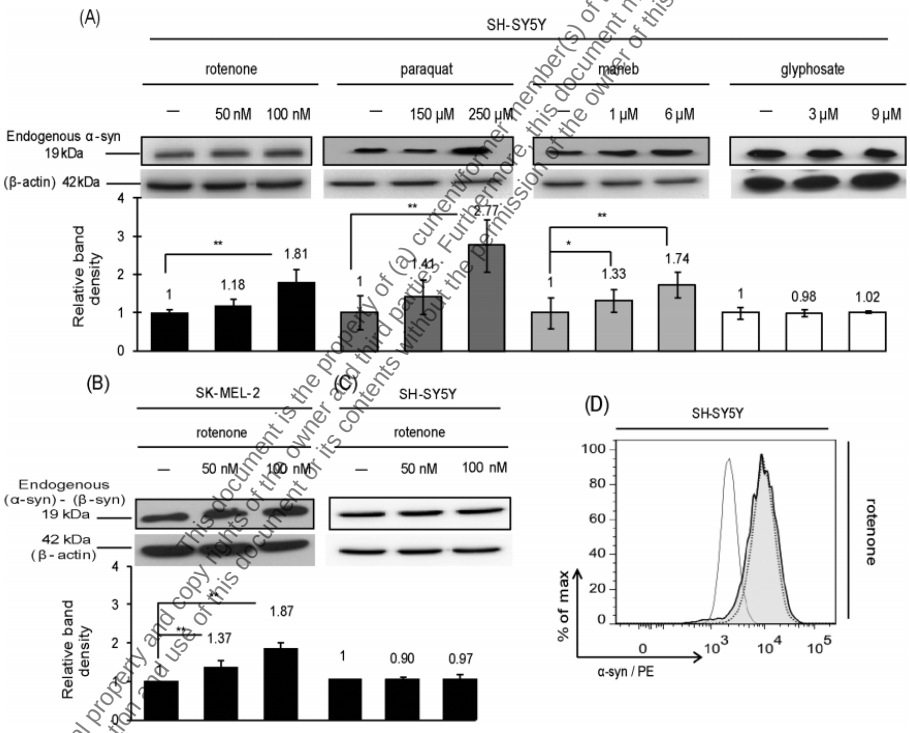
in fluorescence intensity was observed compared with 1.7 % for the endogenous protein. Similar levels of α-syn were produced in α-syn AdV-transduced SK-MEL-2 cells (data not shown). The Western blot revealed that transduction with recombinant α-syn AdV was associated with a predominant band at 22.5 kDa, resulting from the presence of the myc-His tag epitope, in addition to the 19 kDa band representing the endogenous α-syn.

We then confirmed the effects of pesticides on AdV-associated α-syn levels by Western blot. In AdV-transduced SH-SY5Y cells, 1.38- and 1.70-fold increases were observed with WT (left panel) and A53T (right panel) α-syn, respectively, following exposure to 100 nM rotenone ($p < 0.001$).

Pesticide impact was then assessed by flow cytometry after 72-h exposure of SH-SY5Y cells transduced with WT or A53T α-syn AdV to the IC_{50} (determined on the SH-SY5Y cell line) in comparison to a GFP AdV control. The observed increases in α-syn levels were specific, as no change in GFP fluorescence was apparent after exposure to glyphosate (data not shown) of cells transduced with a GFP AdV control.
No increase in α-syn levels was detected after exposure of SH-SY5Y cells transduced with WT or A53T α-syn AdV to glyphosate.

No significant increase in α-syn levels was found after the exposure to glyphosate of SK-MEL-2 cells transduced with WT or A53T α-syn AdV (compared with those transduced with GFP AdV).

Figure 5.7- 3: Characterization of the impact of pesticides on endogenous α-syn levels in SH-SY5Y and SK-MEL-2 cells by Western blot and flow cytometry.



(A) The levels of endogenous α-syn were estimated by Western blot after exposure of SH-SY5Y cells to different pesticides at concentrations corresponding to 25 and 50 % of the IC_{50} (50 and 100 nM rotenone; 150 and 250 µM paraquat; 1 and 6 µM maneb; 3 and 9 µM glyphosate).

(B) Similarly, the levels of endogenous α-syn in SK-MEL-2 cells exposed to 50 and 100 nM rotenone were also assessed.

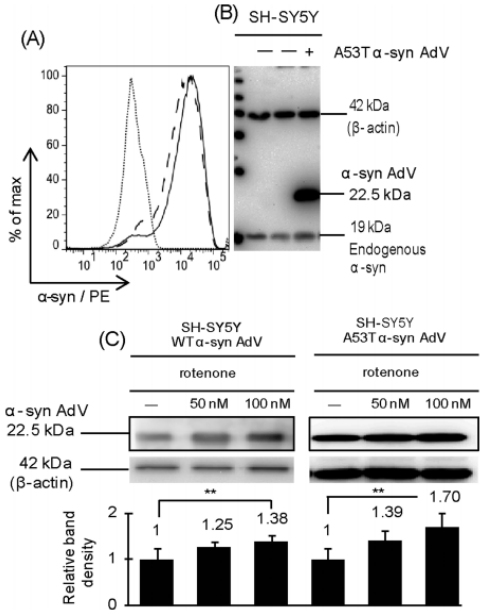
(C) In parallel, we estimated the amounts of β-synuclein in SH-SY5Y cells after rotenone exposure.

(D) β-actin was used as a loading control. Data represent the means ± SEM from four to six independent experiments obtained by Wilcoxon's test (**$p < 0.001$; *$p < 0.05$). The fold increase is indicated above the histogram for each experimental condition. Finally, the fluorescence curves corresponding to the levels of endogenous α-syn (dotted curve) in SH-SY5Y cells observed by

flow cytometry after exposure to 100 nM rotenone (black curve, shaded area) are compared with SH-SY5Y cells immunostained with an isotype control (grey curve)(D).

Data represent the means ± SEM from three independent experiments.

Figure 5.7- 4: Characterization of the impact of pesticides on recombinant α-syn levels in SH-SY5Y cells by Western blot and flow cytometry.



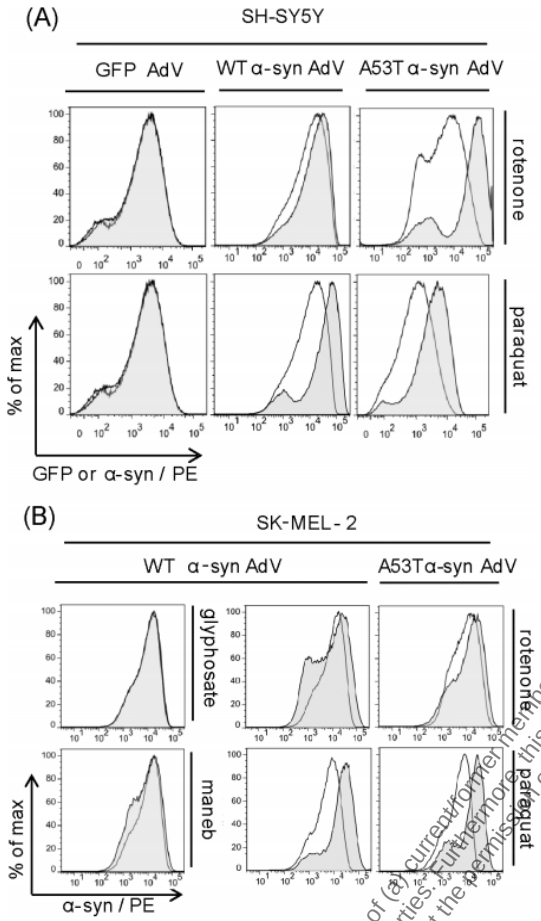
(A)Three days after adenoviral transductions, the cells were permeabilized and immunostained with clone 42 antibody raised against α-syn. The flow cytometry histogram shows the levels of recombinant α-syn in SH-SY5Y cells overexpressing either WT (dashed line) or A53T (solid line) α-syn AdV, in comparison to endogenous α-syn (grey line).

(B) Western blot detection of recombinant α-syn in comparison to endogenous α-syn is shown.

(C) Finally, the levels of recombinant α-syn were quantified in SH-SY5Y cells transduced with either WT or A53T α-syn AdV following rotenone exposure at 50 and 100nM.

Data represent the mean ± SEM α-syn/β-actin ratios from four independent experiments (**$p < 0.001$ obtained by Wilcoxon's test).

Figure 5.7- 5: Characterization of the impact of pesticides on α-syn levels in AdV-transduced SH-SY5Y and SK-MEL-2 cells by flow cytometry.



(A) Fluorescence curves correspond to the α-syn levels following AdV transduction of SH-SY5Y cells by GFP AdV (left column, solid curve not shaded), WT α-syn AdV (center column, solid curve not shaded), and A53T α-syn AdV (right column, solid curve not shaded) alone or following exposure to either 100nM rotenone (upper panel, solid curve, grey shaded) or 250µM paraquat (lower panel, solid curve, grey shaded).

(B) Fluorescence curves correspond to the α-syn levels in SK-MEL-2 after transduction with WT α-syn AdV (left and center column, solid curve not shaded) or A53T α-syn AdV (right column, solid curve not shaded) alone or following exposure to 9µM glyphosate (solid curve, grey shaded), 6µM maneb (solid curve, grey shaded), 100nM rotenone (upper panel, solid curve, grey shaded), or 250µM paraquat (lower panel, solid curve, grey shaded).

Data represent the means ± SEM of 6 to 12 separate experiments for each of the different pesticide exposures.

III. DISCUSSION & CONCLUSION

Overall, the specific effects of the pesticides were quite consistent for both endogenous and AdV-produced α-syn. The mechanisms involved in the pesticide-induced increases were not examined but could reflect a decreased efficiency of the cellular mechanisms involved in the degradation of misfolded proteins such as the proteasome pathway, and/or, for the endogenous protein, increased synthesis of the protein. The changes in protein levels were specific to α-syn, as no changes were observed for a GFP protein encoded by recombinant AdVs or for the endogenous β-synuclein expressed in the SH-SY5Y cell line. β-Synuclein shares a high homology sequence with α-syn but is less prone to aggregation in relation with the absence of 11 amino acids in the central region of the protein.

No effect of the pesticide glyphosate on α-syn levels was detected. Although a case of parkinsonian

syndrome was reported following acute poisoning with glyphosate, it was concluded in a new study that there is little evidence to suggest a causal relationship between glyphosate exposure and noncancer diseases, including PD.

It was found that paraquat, and to a lesser extent rotenone, but not maneb or glyphosate, also increased the α-syn levels in SK-MEL-2 cells, whether produced endogenously or after adenoviral transduction. In summary, we provide an approach based on commonly used methods, which allow the quantification of cellular α-syn levels, and we show here that these levels are greatly increased following exposure to certain pesticides, which have been specifically associated with PD. This experimental strategy could provide useful and readily available information specific to each pesticide so that the neurotoxic potential of the chemical can be assessed prior to large scale use in the field. On this basis, further studies are now focusing on the development of analytical methods in microplate format, which would be more convenient for large scale screening. To what extent such in vitro observations reflect specific events involved in the molecular pathogenesis of human α-syn associated diseases remains to be determined.

1. Assessment and conclusion

Assessment and conclusion by applicant:

The objective was to precisely assess changes in α-syn levels in human neuroblastoma (SH-SY5Y) and melanoma (SK-MEL-2) cell lines following acute exposure to glyphosate using Western blot and flow cytometry. The study was conducted using an *in vitro* test system. Glyphosate did not have any impact on the endpoints measured in this study. This is not a guideline study, nor did this study evaluate an endpoint used in risk assessment. Therefore, this study in not usable for quantitative human health risk assessment or hazard assessment.

This publication is considered reliable with restrictions (no positive control was included and only 2 test concentrations were used) but is not relevant for the risk assessment of glyphosate.

Assessment and conclusion by RMS:

Reliability criteria for *in vitro* toxicology studies

Publication: Chorfa *et al.*, 2013	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity of 99.5 %. Source: Sigma-Aldrich.
Only glyphosate acid or one of its salts is the tested substance	N	Also other pesticides tested (rotenone, paraquat, maneb).
AMPA is the tested substance	N	
Study		

Test system clearly and completely described	Y	Human neuroblastoma (SH-SY5Y) and melanoma (SK-MEL-2) cell lines.
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	NA	
Test concentrations in physiologically acceptable range (< 1 mM)	Y	0, 3, and 9 µM.
Cytotoxicity tests reported	Y	
Positive and negative controls	N	No positive controls used.
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	X	
Reliability not assignable		
Not reliable		
This publication is considered not relevant for the risk assessment of glyphosate because it is not a guideline study, nor did this study evaluate an endpoint used in risk assessment. It is reliable with restrictions because no positive control was included and only 2 test concentrations were used.		

CA 5.8 Toxicity studies of metabolites

CA 5.8.1 Toxicity studies of metabolites

Studies with AMPA

The metabolite aminomethyl phosphonic acid (AMPA) was investigated for metabolism/excretion, acute and subchronic toxicity, mutagenicity and teratogenicity. Overall the present studies show that AMPA has a lower toxicity than the parent compound glyphosate and is devoid of any mutagenic or teratogenic potential.

Absorption, Distribution, Metabolism and Excretion
A metabolism and excretion study in rats shows that AMPA is moderately absorbed from the gastrointestinal tract and excretion is rapidly and nearly complete. Main excretion route is *via* faeces (74 %). Approximately 20 % of the material is absorbed and most of that amount is rapidly excreted unchanged in the urine (13 % within 12h). Of the material absorbed, very small amounts are catabolized as evidenced by the facts that less than 0.1 % of the dose is expired in air and tissue residues are less than 10 ppb (Colvin, Moran & Miller, 1973, CA 5.8.1/001).

Acute toxicity
A total of five acute oral toxicity studies and two acute dermal toxicity studies are available. From the acute oral studies an oral LD_{50} of > 5000 mg/kg bw both in rats and mice was derived ([redacted] 1996, CA 5.8.1/002; [redacted], 1993, CA 5.8.1/003; [redacted], 1991, CA 5.8.1/004, [redacted] 1988, CA 5.8.1/005). Diarrhoea and general clinical signs of toxicity were observed at dose levels of 5000 mg/kg bw. An even higher LD50 was obtained one study in rats (LD50 of > 8300 mg/kg bw) based on mortality occurring in 1/5 rats at 6310 mg/kg bw, 2/5 at 7940 mg/kg bw and 4/5 at 10000 mg/kg bw ([redacted], 1973; CA 5.8.1/006). However, this study is considered to provide only supportive information as the reporting was very brief.

The dermal LD_{50} was found to be >2000 mg/kg bw for rats ([redacted], 2002, CA 5.8.1/007; [redacted], 1993, CA 5.8.1/008).

Studies assessing the skin and eye irritation of AMPA are available ([redacted], 1973, CA 5.8.1/009 and 010). No skin irritation potential was found, but a slight irritating effect in the eye comprising slight to moderate erythema was observed. However, it can be concluded that the test substance is not corrosive to the eye as all observations were reversible within 120 h. Since experimental procedures are described very briefly and no individual animal data are given, the study is considered as supportive only.

In two Magnusson and Kligman Maximisation Tests, no sensitising effects of AMPA were observed ([redacted], 2002, CA 5.8.1/011; [redacted], 1993, CA 5.8.1/012).

Short-term toxicity
The short-term toxicity of AMPA was investigated in rats and dogs.
In rats, a NOAEL of 400 mg/kg bw/day was derived from a 90-day study ([redacted], 1979, CA 5.8.1/017) based on decreased body weight gain/food consumption, gastrointestinal clinical signs and urogenital lesions (epithelial hyperplasia of bladder and renal pelvis) and concomitant increase in lactic dehydrogenase activity in blood at 1200 mg/kg bw/day. However, in a 13-week study in rats, no adverse effects were observed up to a dose of 1000 mg/kg bw/day, although lactic dehydrogenase activity was not determined in this study ([redacted], 1993, CA 5.8.1/016). In other rat studies of shorter duration, except for slight to moderate decreases in body weight gain in male rats when compared with control and a very slight decrease in food consumption at 4000 mg/kg bw in a 14-day dose range finding study (no histopathology findings) and a slight increase in kidney weights in male rats at 350 and 1000 mg/kg bw/day in a 4-week study no further adverse effects were detected ([redacted], 1978, CA 5.8.1/013; [redacted], 1993, CA 5.8.1/014).

In dogs, the most sensitive NOAEL was obtained from a 1-month study. Based on haemotology findings indicative for mild to moderate anaemia, a NOAEL of 300 mg/kg bw/day in males and 100 mg/kg bw/day in females was obtained ([redacted] 1991, CA 5.8.1/015). No further adverse effects were observed. In a 90-day study in dogs, no treatment related effects were observed up to a nominal highest dose of 300 mg/kg bw/day (263 mg/kg bw/day after adjustment for purity) neither in males nor females ([redacted], 1991, CA 5.8.1/018).

Genotoxicity
AMPA was tested negative for bacterial mutagenicity in four Ames tests and for mammalian cells mutagenicity in one Mouse lymphoma assay ([redacted], 1996, CA 5.8.1/019; [redacted] 1993, CA 5.8.1/020; [redacted], 1988, CA 5.8.1/021 and [redacted], 1980, CA 5.8.1/022; [redacted] 1993, CA 5.8.1/023). Further, in two *in vitro* UDS assays, AMPA did not induce unscheduled DNA synthesis in primary rat hepatocytes ([redacted], 2002, CA 5.8.1/024; [redacted], 1991, CA 5.8.1/025). However, both tests were considered to provide only supportive information, as the UDS assay is no longer a standard method described by current guidelines for this endpoint.

The clastogenic/aneugenic activity of AMPA was investigated in two *in vivo* micronucleus tests in mice. Both tests were negative for micronuclei after single administration of 5000 mg/kg bw or at doses of up to 1000 mg/kg bw, respectively ([redacted], 1993, CA 5.8.1/026; [redacted], 1993, CA 5.8.1/027).
In order to comply with the current OECD testing guidelines a set of 3 *in vitro* genotoxicity studies conducted according to the most recent guidance document requirements is currently being performed with AMPA (Ames test, HPRT test, micronucleus test in human lymphocytes).

However, the overall weight of evidence based on available data are that metabolites of glyphosate are non-genotoxic.

Developmental toxicity

Three developmental toxicity studies in rats are available.
The most sensitive NOAEL for maternal toxicity was 150 mg/kg bw/day based on clinical signs, decrease of bw gain and food consumption at 400 and 1000 mg/kg bw/day. Further, the NOAEL for developmental toxicity was 400 mg/kg bw/day based on decreased fetal weight ([redacted], 1991, CA 5.8.1/030). In the two other developmental studies, there was no evidence of toxicity in either the dam or embryo-fetal development at any of the dose levels applied (up to 1000 mg/kg bw/day) ([redacted], 1992, CA 5.8.1/028; [redacted], 1991, CA 5.8.1/029). No teratogenic effects were observed in any of the three studies.

Literature evaluation

In addition to studies commissioned by representatives of the Glyphosate Renewal Group, several publications identified from the open literature have been submitted.
One publication is available addressing the effects of AMPA (and further glyphosate, it´s metabolite methylphosphonic acid and some impurities) on apoptosis induction in human peripheral blood mononuclear cells (PBMCs) (Kwiatkowska *et al*, 2020, CA 5.8.1/031). All substances changed PBMCs membrane permeability, activated caspase-8, -9, -3 and caused chromatin condensation, which showed that they were capable of inducing apoptosis both via extrinsic and particularly intrinsic pathway at high concentrations only. Generally the study demonstrated that there were no differences between apoptotic changes induced by glyphosate, its metabolites or impurities, and observed changes were provoked by high concentrations of investigated compounds. Since clear changes were only seen at high concentrations, a low apoptotic potential of these compounds was concluded.

This publication was considered relevant for the risk assessment of AMPA but reliable with restrictions because no proper cytotoxicity tests were performed, no positive controls were used and the concentration range at which most of the effects were observed is beyond the acceptable physiological range (> 1 mM).

Mañas F. *et al.* (2020, CA 5.4/012) reported that AMPA did not have an effect on oxidative stress parameters in liver, kidney, lung and heart in a Comet assay in Balb C mice after 14-day exposure to AMPA via drinking water. A statistically significant increase in DNA damage parameters was observed for AMPA in blood and liver. Nevertheless, the increase of tail intensity was rather low (1.23- and 1.65-fold in blood and liver). No positive or historical control data were provided for further evaluation. In addition, no information on animals (clinical signs, systemic toxicity) was provided. No histopathology of investigated tissues was performed and therefore no final conclusion on cytotoxicity in the respective tissues is possible, which may have an impact on and explain the outcome of the comet assay.

No induction of double strand breaks was observed in the experiments from Suárez-Larios *et al.* (2017, CA 5.4/009).

Roustan *et al.* (2014) reported that AMPA did result in increased ROS level in CHO-K1 cells. Nevertheless, due the reporting of the results (only one of four tested concentrations of AMPA reported; no standard deviation provided) and the lack of statistical evaluation a final conclusion on the ROS induction by AMPA is not possible. With regards to micronucleus induction a statistical significant increase of cells with micronuclei was observed after treatment with AMPA at concentrations as low as 0.01 ~~m~~µg/mL (equivalent to a high physiological dose of about 60 µM). However, when tested in combination with other pesticides including glyphosate no induction of cells with micronuclei was observed at concentrations from 5 to 100 µg/mL. Taking into account the available in vivo micronuclei tests yielding negative results, a clastogenic potential of AMPA is unlikely.

Nota bene: In autumn of 2019 NGOs and media published accusations against the German based contract research organization "[redacted]" on animal welfare and GLP. In the frame of this glyphosate renewal dossier GRG is submitting 24 studies performed during the years 1995 – 2010 at [redacted]. All these studies were conducted during the validity periods of GLP certificates routinely renewed and re-confirming the GLP-standard every three years. Studies performed at this laboratory are clearly indicated in the overview table (Table 5.8.1-1) as well as in the respective study header. Due to the large number of corroborative studies available within the Glyphosate Renewal Group,

the overall endpoint conclusion of glyphosate is not reliant on the results of the studies performed at [redacted].

Table 5.8.1-1: Studies on toxicity with metabolite AMPA

Annex Point	Study	Study type	Substance(s)	Reference list- related category§	Exposure conditions, dose levels	Result
Absorption, Distribution, Metabolism and Excretion						
CA 5.8.1/001	[redacted], 1973	Metabolism study, Wistar rat, ♂	AMPA (Purity: not available)	Valid, Category 3a	Single oral dose of 6.7 mg [^{14}C]-AMPA/kg bw *via* gavage	Moderate absorption; rapid excretion: 74 % *via* faeces, 20 % *via* urine and < 0.1 % exhaled air; 0.06 of the total dose recovered from carcass; liver, kidney, and muscle exhibited residues of 6, 6 and 3 ppb
Acute oral toxicity						
CA 5.8.1/002	[redacted], 1996	Acute oral toxicity, CD mice, ♀/♂	AMPA (Purity: 99.33 %)	Valid, Category 2a	Limit test	LD_{50} oral > 5000 mg/kg bw (males and females), no evidence of toxicity
CA 5.8.1/003	[redacted] 1993	Acute oral toxicity, SD rats, ♀/♂	AMPA (Purity: 99.2 %)	Valid, Category 2a	Limit test	LD_{50} oral > 5000 mg/kg bw (males and females); some general and gastrointestinal clinical signs observed
CA 5.8.1/004	[redacted], 1991	Acute oral toxicity, Wistar rats, ♀/♂	AMPA (Purity: 97.3 %)	Valid, Category 2a	Limit test	LD_{50} oral > 5000 mg/kg bw (males and females); some general and gastrointestinal clinical signs observed
CA 5.8.1/005	[redacted], 1988	Acute oral toxicity, Wiastar rat, ♀/♂	AMPA (Purity: 100 %)	Valid, Category 2a	Limit test	LD_{50} oral > 5000 mg/kg bw (males and females); decreased bw, some general clinical signs and diarrhoea
CA 5.8.1/006	[redacted], 1973	Acute oral toxicity, rat,	AMPA (Purity: not	Supportive, Category 3a	5010 mg/kg bw, 6310 mg/kg bw,	LD_{50} oral > 8300 mg/kg bw

Table 5.8.1-1: Studies on toxicity with metabolite AMPA

Annex Point	Study	Study type	Substance(s)	Reference list- related category§	Exposure conditions, dose levels	Result
		♀/♂	available)		7940 mg/kg bw and 10000 mg/kg bw, single oral gavage	(males and females); some general clinical signs and increased mortality
Acute dermal toxicity						
CA 5.8.1/007	[redacted], 2002	Acute dermal toxicity (Limit test), CD rats, ♀/♂	AMPA (Purity: 98 %)	Valid#, Category 2a	Limit test	LD_{50} dermal > 2000 mg/kg bw (males and females); no evidence of toxicity
CA 5.8.1/008	[redacted], 1993	Acute dermal toxicity (Limit test), SD rats, ♀/♂	AMPA (Purity: 99.2 %)	Valid, Category 2a	Limit test	LD_{50} dermal > 2000 mg/kg bw (males and females); no evidence of toxicity
Skin and Eye irritation						
CA 5.8.1/009	[redacted], 1973	Skin irritation study, rat, ♀/♂	AMPA (Purity: not available)	Supportive, Category 3a	Moistened test material applied on skin	No skin irritation
CA 5.8.1/010	[redacted], 1973	Eye irritation study, rabbit, ♀/♂	AMPA (Purity: not available)	Supportive, Category 3a	Instillation of unchanged test material	Slightly irritating (slight to moderate erythema), no corrosion
Skin sensitisation						
CA 5.8.1/011	[redacted], 2002	Magnusson and Kligman Maximisation Test, Dunkin-Hartley guinea pigs, ♂	AMPA (Purity: 98 %)	Valid#, Category 2a	Maximisation test	Not sensitising
CA 5.8.1/012	[redacted], 1993	Magnusson and Kligman Maximisation Test, Dunkin-Hartley guinea pigs, ♀	AMPA (Purity: 99.2 %)	Valid, Category 2a	Maximisation test	Not sensitising
Short-term toxicity						
CA 5.8.1/013	[redacted], 1978	14-day oral toxicity, CD rats, ♀/♂	AMPA (Purity: 99.9 %)	Supportive, Category 3a	1000, 2000 and 4000 mg/kg bw/day *via* diet	No NOAEL derived ↓ bw gain and food consumption at 4000 mg/kg bw/day
CA	[redacted]	4-week oral	AMPA	Valid,	10, 100, 350 and	NOAEL =

Table 5.8.1-1: Studies on toxicity with metabolite AMPA

Annex Point	Study	Study type	Substance(s)	Reference list- related category§	Exposure conditions, dose levels	Result
5.8.1/014	1993	toxicity, SD rat, ♀/♂	(Purity: 99.2 %)	Category 2a	1000 mg/kg bw/day *via* gavage	100 mg/kg bw/day (males) and 1000 mg/kg bw/day (females) based on ↑ kidney weight in males
CA 5.8.1/015	[redacted], 1991	1 month oral toxicity, Beagle dog	AMPA (Purity: 87.8 %)	Supportive, Category 3a	10, 30, 100, 300 and 1000 mg/kg bw/day *via* capsule treatment	NOAEL = 300 mg/kg bw/day (males) and 100 mg/kg bw/day (females), based on haemotology findings indicative for mild to moderate anaemia
CA 5.8.1/016	[redacted], 1993	13-week oral toxicity, SD rat, ♀/♂	AMPA (Purity: 99.2 %)	Valid, Category 2a	10, 100 and 1000 mg/kg bw/day *via* gavage	NOAEL = 1000 mg/kg bw/day
CA 5.8.1/017	[redacted], 1979	90-day oral toxicity, CD rats, ♀/♂	AMPA (Purity: 99.96 %)	Valid, Category 2a	400, 1200 and 4800 mg/kg bw/day *via* diet	NOAEL = 400 mg/kg bw/day based on bw gain↓, urothelial hyper-plasia (bladder) and gastro-intestinal clinical signs; at top dose level in addition mortality following blood collection (f), food consumption↓, clinical chemistry findings (LDH activity↑) and hyper-plasia of renal pelvis epithelium
CA 5.8.1/018	[redacted], 1991	90-day oral toxicity, Beagle dog	AMPA (Purity: 87.8 %)	Valid, Category 2a	10, 30, 100 and 300 mg/kg bw/day (adjusted for purity: 8.8, 26.3, 87.8 and 263 mg/kg bw/day) *via* capsule	NOAEL = 300 (263 adjusted for purity) mg/kg bw/day

Table 5.8.1-1: Studies on toxicity with metabolite AMPA

Annex Point	Study	Study type	Substance(s)	Reference list- related category§	Exposure conditions, dose levels	Result
Genotoxicity - *in vitro*						
CA 5.8.1/019	[redacted], 1996	Genotoxicity in bacteria (Ames); *S. typhimurium* TA 98, TA 100, TA 1535 and TA 1537, and *E. coli* WP2 uvrA	AMPA (Purity: 99.33 %)	Supportive, Category 2a	313 - 5000 µg/plate, ± S9, pre-incubation method	Negative
CA 5.8.1/020	[redacted] 1993	Genotoxicity in bacteria (Ames); *S. typhimurium* TA 98, TA 100, TA 1535 and TA 1537	AMPA (Purity: 99.2 %)	Supportive, Category 2a	310 - 5000 µg/plate, ± S9, plate incorporation and pre-incubation method	Negative
CA 5.8.1/021	[redacted], 1988	Genotoxicity in bacteria (Ames); *S. typhimurium* TA 98, TA 100, TA 1535, TA 1537 and TA 1538, and *E. coli* WP2 uvrA	AMPA (Purity: > 99 %)	Valid, Category 2a	1.8 - 5000 µg/plate, ± S9, plate incorporation method	Negative
CA 5.8.1/022	[redacted] 1980	Genotoxicity in bacteria (Ames); *S. typhimurium* TA 98, TA 100, TA 1535, TA1537 and TA 1538, and *E. coli* WP2 hcr	AMPA (Purity: 99 %)	Invalid, Category 3b	10 - 5000 µg/plate, ± S9, plate incorporation method	Negative
CA 5.8.1/023	[redacted] 1993	Genotoxicity in mammalian cells, Mouse lymphoma (L5178Y) cells	AMPA (Purity: 99.2 %)	Valid, Category 2a	310 – 5000 µg/mL, ± S9	Negative
CA 5.8.1/024	[redacted] 2002	*In vitro* unscheduled DNA synthesis (UDS) assay, primary rat hepatocytes	AMPA (Purity: 99.9 %)	Supportive, Category 2a	0.625 - 10 mM	Negative
CA 5.8.1/025	[redacted], 1991	*In vitro* unscheduled DNA synthesis (UDS) assay, primary rat hepatocytes	AMPA (Purity: 94.38 %)	Supportive, Category 2a	5 - 5000 µg/mL	Negative up to 2500 µg/mL, meaningful evaluation of higher concentrations not possible due to cytotoxicity
Genotoxicity - *in vivo*						

Table 5.8.1-1: Studies on toxicity with metabolite AMPA

Annex Point	Study	Study type	Substance(s)	Reference list- related category§	Exposure conditions, dose levels	Result
CA 5.8.1/026	[redacted] 1993	*In vivo* Micronucleus test, NRMI mice, ♀/♂	AMPA (Purity: 99.2 %)	Valid, Category 2a	Single oral dose of 5000 mg/kg bw *via* gavage	Negative
CA 5.8.1/027	[redacted], 1993	*In vivo* Micronucleus test, CD-1 mice, ♀/♂	AMPA (Purity: 94.38 %)	Valid, Category 2a	Single doses of 100, 500 and 1000 mg/kg bw *via i.p.* injection	Negative
Developmental Toxicity						
CA 5.8.1/028	[redacted], 1992	Develop-mental toxicity, SD rat	AMPA (Purity: 99.2 %)	Valid, Category 2a	100, 350 and 1000 mg/kg bw/day *via* gavage on gestation days 6 - 16	NOAEL = 1000 mg/kg bw/day (maternal and developmental)
CA 5.8.1/029	[redacted], 1991	Range finder developmental toxicity study, SD rat	AMPA (Purity: 94.38 %)	Supportive, Category 3a	125, 250, 500, 750 and 1000 mg/kg bw/day *via* gavage on gestation days 6 - 15	NOAEL = 1000 mg/kg bw/day (maternal and developmental)
CA 5.8.1/030	[redacted], 1991	Developmental toxicity study, SD rat	AMPA (Purity: 94.38 %)	Valid, Category 3a	150, 400 and 1000 mg/kg bw/day *via* gavage on gestation days 6 - 15	NOAEL (maternal) = 150 mg/kg bw/day, based on clinical signs, ↓bw gain/ food consumption NOAEL (developmental) = 400 mg/kg bw/day, based on ↓ mean fetal weight

# = This study was performed at the [redacted] in [redacted]
AMPA = Aminomethyl Phosphonic Acid
§: The category describes the acceptability of the study within the AIR 5 submission

Absorption, Distribution, Metabolism and Excretion

1. Information on the study

Data point:	CA 5.8.1/001
Report author	[redacted]
Report year	1973
Report title	Final Report on CP 67573 RESIDUE AND METABOLISM Part 11: The Metabolism of Aminomethylphosphonic Acid-^{14}C (CP 50435-^{14}C) in the Laboratory Rat

Report No	303
Document No	NA
Guidelines followed in study	None stated
Deviations from current test guideline	Yes, reporting deficiencies (no batch or purity of test material, only one dose, only 5 days)
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, study conducted prior to GLP
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

Executive summary

These studies were initiated to investigate the metabolic fate of aminomethylphosphonic acid-^{14}C ([^{14}C]-AMPA), in the rat following a single oral dose to rats.

Male Wistar rats received a single dose of approximately 6.7 mg [^{14}C]-AMPA/kg bw by oral gavage and were sacrificed 120 hours (5 days) after dosing. During this period, urine, faeces and expired gases were collected at 12 or 24 hour intervals and assayed for radioactivity. At sacrifice, blood and selected tissues were examined for radioactive residues.

74 % of the applied dose was detected in faeces and 20 % in urine with less than 0.1 % expired as $^{14}CO_2$ 120 h after application. Therefore, the recovery of administered radioactivity in excreta exceeded 90 %. Approximately 0.06 % of the original dose was recovered from the carcass at 120 hours post-administration, but liver, kidney and muscle exhibited residues of only 6, 6 and 3 ppb, respectively.

Chromatographic and spectral data have demonstrated that orally administered [C^{14}]-AMPA is excreted unchanged in the urine.

I. MATERIALS AND METHODS

A. MATERIALS

1. Radiolabelled test material: Aminomethylphosphonic acid-^{14}C

Identification:	Aminomethylphosphonic acid-^{14}C (CP 50435-^{14}C) (AMPA-^{14}C)
Position of radiolabel:	Not stated
Lot/Batch #:	Not reported
Purity:	Not reported
Specific activity:	8.9 mCi/mmol
Stability of test compound:	Not reported

2. Test animals:

Species:	Rat
Strain:	Wistar

Source:	[redacted]
Age:	Not stated, but about 6 to 8 weeks considering the body weight
Sex:	Male
Weight at dosing:	Approximately 150 g
Acclimation period:	Not reported
Diet/Food:	*ad libitum,* fasted 4 hours prior to dose
Water:	*ad libitum*
Housing:	Individually in Roth metabolism cages
Environmental conditions:	Temperature: not reported Humidity: not reported Air changes: not reported

B. STUDY DESIGN

Animal assignment and treatment

Male rats were fasted for four hours, before application of an aqueous solution containing approximately 1 mg of [^{14}C]-AMPA (corresponding to a dose of 6.7 mg/kg bw) by gavage. Urine, faeces and the 1N NaOH used to trap the expired gases were collected after 12, 24, 48, 72, 96 and 120 hours. At termination a heparinised blood sample was taken by cardiac puncture under light ether anaesthesia. The animal was sacrificed by continued ether anaesthesia and the following tissues removed: liver, kidney, spleen, heart, brain, testes, fat, muscle and the gastrointestinal tract (GIT) from the oesophagus to the rectum including the caecum.

Measurement of radioactivity in excreta and expired gases

One ml of the NaOH trapping solution was diluted with 2 mL water, and 15 mL phosphor solution (Insta-Gel-Packard Instrument Co., Downers Grove, Ill.) added and the sample analysed for $^{14}CO_2$ by liquid scintillation counting.

Urine was analysed for ^{14}C-activity by diluting 0.1 mL and 0.2 mL aliquots with 4 mL 0.1M NH_4HCO_3. 15 mL phosphor solution was added and the mixture shaken vigorously and chilled to form a gel. The samples were then analysed by liquid scintillation counting.

Faeces was homogenised in 30 % aqueous isopropyl alcohol. The faeces homogenate was lyophilized and 100 mg aliquots submitted for combustion. The trapped ^{14}C-activity was analysed by liquid scintillation counting.

Measurement of radioactivity in tissues

The collected tissue samples were weighed, frozen and lyophilized. The lyophilized samples were weighed, aliquoted and submitted for combustion and subsequent liquid scintillation counting.

Isolation of AMPA-^{14}C from rat urine

A cation-exchange column was prepared by pouring aqueous slurry of AG-50W-X8 (H^+ form; 200/400 m). The resin bed was washed with distilled water until the eluate was colourless. A 12 or 24 hour urine sample which had been diluted to 25-35 mL with distilled water was loaded onto this cation exchanger. The column was then eluted with 1500 mL distilled water and 20 mL fractions were collected. The eluent fractions

which contained radioactivity were pooled and loaded onto an anion-exchanger of AG-1-x8 (HCO_3^- form, 200/400 m). The resin was prepared from the Cl^- form by passing 1M NH_4 hCO$_3$ (2L 1M NH_4 hCO3/1b resin) through the resin. The resin was then washed with water until the eluate was neutral. An amount of resin equivalent to 20 g dry weight was slurried with water and poured into a glass column forming a resin bed. The sample was then applied in a volume of 200 mL. The column was eluted with 200 mL distilled water, followed by 300 mL 0.2N NH_4HCO_3. Fractions of 5 or 10 mL were collected.

The eluent fractions from the anion exchanger which contained radioactivity were pooled and reduced to 1 – 1.5 mL and loaded onto a Bio-Gel P-2 column for gel-filtration chromatography. The gel was prepared by suspension in 0.5N acetic acid, deaerator with a water aspirator and pouring the slurry into a column to form a bed of 1 x 108 cm. After the sample was charged onto the column, the sample flask was rinsed with 1 - 2 mL distilled water which was added to the column. The column was then eluted with 200 mL 0.5N acetic acid and 1 - 2 mL fractions collected.

Authentic standards of radiolabelled AMPA added to urine from untreated rats were eluted quantitatively from the cation exchange resin. Those fractions containing ^{14}C-activity were then pooled, reduced in volume and chromatographed on an anion exchanger and subsequently on a gel filtration column. Typically, overall recoveries of ^{14}C-activity from [^{14}C]-AMPA enriched rat urine were approximately 70 % of initial ^{14}C-activity. Urine from rats orally administered [^{14}C]-AMPA which was chromatographed in the same way resulted in approximately 60 % recovery of the initial ^{14}C-activity present in the crude urine.

Thin layer chromatography (TLC)

The Bio-Gel fractions which contained radioactivity were pooled reduced to small volume and an aliquot applied to a 20 x 20 cm thin-layer chromatography (TLC) plate with a 250 µ layer of microcrystalline cellulose. The plates were developed first in a phenol-water system of the following composition:

90 % Phenol (Fisher Certified)	84 mL	
Distilled Water		16 mL
Glacial Acetic Acid		1 mL
EDTA		37.2 mg

After the plates were air-dried, they were rotated 90° and developed in a modified semi-stench solvent system of the following composition:

EDTA		1.2 g
17N NH_4OH		100 mL
Distilled Water		475 mL
l-Propanol		350 mL
2-Propanol		75 mL
l-Butanol		75 mL
Iso-Butyric Acid	2500 mL	

Colorimetric visualization of the TLC plates was accomplished by spraying with or with a modified Hanes reagent. ^{14}C-activity on the TLC plates was detected by means of the Beta Camera, Model 6000. A permanent copy of the Beta Camera CRT image was reproduced using a Polaroid Pack Camera.

Nuclear Magnetic Resonance (^{1}H-NMR) of the isolated fractions

The samples were lyophilized and exchanged twice with 99.8 % deuterium oxide (D_2O). The sample was then dissolved in 0.1 mL 100 % D_2O. Immediately before running the spectra, the samples were dissolved in 10 µl 100 % D_2O and the solution filtered through a Flath-Ludin syringe filter directly into a capillary tube. An additional 5 µL D_2O was used to rinse the vial and was also filtered directly into the capillary tube. The capillary was inserted into a teflon chuck which was then placed in a 7 inch NMR tube (Wilmad, No.

529-PP).

High resolution proton spectra (60 mHz) were run on a Varian T-60 and/or a JEOL JNM C-60-HL spectrometer. The latter was equipped with a JNM-AS-1 resolution stabilizer, JRA-1 spectrum accumulator, Monsanto 100-A frequency counter, an external Hewlett Packard Model 200 CD wide range audio oscillator, a Hewlett Packard Model 5245L electronic counter, a hetero spin decoupler (JNM-5D-HC) and an RF oscillator adapter (JNM-OA-1). All ^{1}H-NMR spectra were calibrated using HOD as the internal reference and were decoupled using the hetero spin decoupler (JNM-5D-HC) and an RF oscillator adapter (JNM-OA-1).

Gas-Liquid Chromatography (GLC) of the isolated fractions

Following NMR analysis the samples were derivatized using trifluoroacetic acid, trifluoroacetic anhydride, diazobutane and acetic acid in benzene and examined by GLC on a Perkin-Elmer Model 900 which is a dual column instrument equipped with thermal conductivity (TC), flame ionization (FID) and phosphorus specific (FPD) detectors. The samples were analysed on a glass column packed with 1 – 5 % OV-17 on Chromosorb W-HP (80/100 m) programed from l20 – 240 °C at 10 °C/min.

Mass Spectrometry of the isolated fractions

The final analysis of the purified fractions was performed by coupled gas chromatography and mass spectrometry. A PE-900 GLC was coupled through a Rieman separator to a Perkin-Elmer Model 270 mass spectrometer (MS) operating at 70 ev in the GLC mode. A Honeywell 2106 Visicorder was employed to focus the instrument and record the mass spectra. The PE-270 MS was interfaced through a Hall probe to a Varian SS-100MS data system.

II. RESULTS AND DISCUSSION

A. DISTRIBUTION OF RADIOACTIVITY FOLLOWING ORAL ADMINISTRATION OF [^{14}C]-AMPA

The major route of excretion of orally administered [^{14}C]-AMPA is via the faeces. More than 50 % of the administered dose was excreted in the faeces within 24 hours and a total of 74 % within 120 hours.

13 % of the dose was recovered from the urine within 12 hours after administration, 4.5 % was detected urine collected 12 - 24 hours after application and less than 3 % was detected in urine collected from 24 - 120 hours. Thus, most of the absorbed material was excreted within 24 hours.

Very little of the absorbed material was catabolised. Less than 0.1 % of the administered ^{14}C-activity was recovered in expired air.

Table 5.8.1-2: Distribution of ^{14}C-activity in male rats orally administered [^{14}C]-AMPA

Time (Hour)	Percent dose recovered[1]					
	Urine	Faeces	CO_2	Washes[2]	Tissues	Cumulative
12	13.04	5.86	0.06			18.95
24	4.50	47.24	0.01			70.70
48	1.52	19.30	0.00			91.52
72	0.66	0.91	0.00			93.09
96	0.23	0.12	0.00			93.44
120	0.05	0.06	0.00			93.55
Total	**20.00**	**73.49**	**0.06**	**0.13**	**0.06**	**93.74**

[1]Dose was 6.7 mg/kg body weight
[2]Consists of 0.1M NH_4HCO_3 washes of cage and urine-faeces separator

Approximately 0.06 % of the administered ^{14}C-activity could be accounted for in the carcass 120 hours following a single oral dose. Of that ^{14}C-activity the muscle, due to its large relative mass, i.e., 38 % of the total live weight, accounted for approximately one-third of the ^{14}C-activity. However, the concentration calculated on the basis of AMPA-^{14}C equivalents was only 3 ppb in fresh tissue. In addition, the liver and kidney were only 6 ppb.

Table 5.8.1-3: Radioactivity in tissues 120 h after single oral dose of 6.7 mg [^{14}C]-AMPA/kg bw (in µg/g fresh tissue)

Tissue	% Dose	[^{14}C]-AMPA equivalents (µg/g Fresh tissue)
Liver	0.01	0.006
Kidney	<0.01	0.006
Muscle	0.02	0.003
Fat	0.01	0.004
Gut	0.02	0.008
Spleen	<0.01	0.004
Heart	<0.01	0.004
Brain	<0.01	0.001
Testes	<0.01	0.003
Blood	<0.01	0.003

B. CHARACTERIZATION OF THE EXCRETED RADIOACTIVITY.

The purification and spectral methods employed were defined on a urine sample from an untreated control rat. The urine was spiked with approximately 500 µg [^{14}C]-AMPA. The TLC screening procedure is capable of separating the following compounds: glyphosate, AMPA, N-methylaminomethylphosphonic acid, glycine, sarcosine, methylphosphonic acid and hydroxymethylphosphonic acid.

Recovery of ^{14}C-activity from [^{14}C]-AMPA enriched rat urine was approximately 70 % of initial ^{14}C-activity. Urine from rats orally administered [^{14}C]-AMPA which was chromatographed in the same way resulted in approximately 60 % recovery of the initial ^{14}C-activity present in the crude urine.

The purified urine samples were analysed by ^{1}H-NMR after being exchanged with D_2O. The sample isolated from the urine of rats administered [^{14}C]-AMPA exhibited the characteristic doublet due to the methylene protons, although it was not as well defined as in the sample enriched with [^{14}C]-AMPA. However, the chemical shifts for the methylene protons are consistent with those determined for authentic standards, and the ^{31}P-decoupled spectrum shows a clear enhancement in the intensity of the response.

The samples which had been analysed by ^{1}H-NMR were exchanged with water and derivatised for GLC/MS analysis. The mass spectrum of the Di-n-butyl-*N*-trifluoromethyl derivative of the sample isolated from urine enriched with [^{14}C]-AMPA yielded the same basic fragmentation pattern as the spectrum of the standard AMPA-Di-n-butyl-TFA.

Beside the peak of AMPA-Di-n-butyl-TFA additional peaks were observed in urine (P+2) spiked with [^{14}C]-AMPA contributing significantly to fragments containing the ^{14}C-enriched methylene group. In the sample in which [^{14}C]-AMPA was added to and isolated from an untreated urine sample, there are four fragments showing a significant P+2 peak.

The mass spectra of the sample isolated from rat urine have the same characteristic fragmentation pattern as the standard AMPA. The relative intensities of some of the lower mass fragments vary from the standard, but these are not as significant in identification as the higher mass fragments which agree well with the standard.

III. CONCLUSIONS

Orally administered AMPA is only moderately absorbed from the gut of the rat after single application of 6.7 mg/kg bw. Consequently, the faeces represent the major route of excretion of ingested AMPA. Approximately 20 % of the material is absorbed and most of that amount is rapidly excreted unchanged in the urine. Of the material absorbed, very small amounts are catabolized as evidenced by the facts that less than 0.1 % of the dose is expired in air and tissue residues are less than 10 ppb.

The absorption of [^{14}C]-AMPA from the gut appears to be slightly more rapid than glyphosate since the log unexcreted dose (i.e., body load) versus time plot of AMPA shows only a small distributive phase. However, the biological half-life of [^{14}C]-AMPA of 10-11 hours is of approximately the same magnitude as that of glyphosate which was 8-9 hours. But unlike glyphosate, [^{14}C]-AMPA did not show a redistribution phase at 48-96 hours which plateaued the excretion curve followed by resumption of first-order elimination. Rather at approximately 72 hours the elimination curve simply plateaued as if approaching an asymptote.

3. Assessment and conclusion

<u>Assessment and conclusion by applicant</u>:

The excreta from male rats orally administered [^{14}C]-AMPA were collected at various intervals through 120 hours post-administration and analysed for ^{14}C-activity.

It was found that a total of approximately 74 % of the dose appeared in the faeces and ca 20 % in the urine with less than 0.1 % expired in air. These figures suggest only limited absorption from the gastrointestinal tract. Elimination is rapid and nearly complete. More than 50 % was excreted in the faeces during the first 24 hours following dosing. About 13 % of the administered dose was found in the urine within the first 12 hours already. Approximately 0.06 % of the total dose only was recovered from the carcass at 120 hours post dose. Liver, kidney and muscle exhibited residues of 6, 6 and 3 ppb, respectively. Chromatographic and spectral data demonstrated that the orally administered AMPA was excreted unchanged in the urine. There was no indication of further metabolism.

The report does not contain any information on the limits of detection or limits of quantitation.

The reporting in this study is very brief and can be considered to be poor and therefore, it is not acceptable based on current regulatory standards.

<u>Assessment and conclusion by RMS</u>:

Acute oral toxicity

1. Information on the study

Data point	CA 5.8.1/002
Report author	[redacted]

Report year	1996
Report title	AMPA: Acute Oral Toxicity Study in Mice
Report No	[redacted] 96-0075
Document No	NA
Guidelines followed in study	OECD 401 (1987); US EPA FIFRA Guidelines Subdivision F (1994); Japan MAFF Guidelines 59 NohSan No. 4200 (1985)
Deviations from current test guideline	The study was conducted according to OECD 401 however the deviation was identified according to OECD 420 adopted in 2001 to replace OECD 401. Clinical signs of toxicity were measured 1 hour after initial dosing, not 30 minutes after initial dosing as recommended by OECD 420.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

AMPA was evaluated for its acute oral toxicity potential in male and female mice when administered as a gavage dose at a level of 5000 mg/kg bw. No mortality occurred during the 14-day observation period and no clinical signs were observed at necropsy following the observation period. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute oral LD_{50} was determined to be;

LD_{50}, oral, male and female mouse > 5000 mg/kg bw

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: AMPA

Identification: AMPA

Description: White powder

Lot/Batch #: 20080801

Purity: 99.33 %

Stability of test compound: Stable for 1 year at room temperature

2. Vehicle and/ or positive control: 1 % carboxymethylcellulose

3. Test animals:

Species: ICR mice

Strain / Stock: Crj:CD-1

Source: [redacted]

Age:	5 weeks
Sex:	Male and female
Weight at dosing:	30.5 – 34.6 g for males; 22.9 – 24.8 g for females
Acclimation period:	7 days
Diet/Food:	Certified pellet diet MF (Oriental Yeast Co., Ltd., Azusawa, Itabashi-ku, Tokyo), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Groups of 5 animals/sex/cage were housed in aluminium cages with wire-mesh floors
Environmental conditions:	Temperature: 22 ± 3°C Rel. humidity: 55 ± 15 % 12-hour light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 17 September 1996 to 8 October 1996

Animal assignment and treatment

Based on the results of a preliminary dose-range finding study, 5000 mg/kg bw/day was selected as the dose level for this study.

Table 5.8.1-4: AMPA: Acute Oral Toxicity Study in Mice ([redacted], 1996): animal distribution

	Dose (mg/kg bw/day)	Number of Animals Per Group	
		Males	Females
Test Group	5000	5	5

Animals were fasted for about 3 hours before and 3 hours after administration. The dosing volume was 20 mL/kg bw. Observations for clinical signs of toxicity were made 1, 3, and 6 hours after administration and then once daily thereafter until termination of the observation period. Individual body weights were recorded just prior to dosing, as well as 7 and 14 days after administration. On Day 14 after dosing, all animals were sacrificed under ether anaesthesia and subjected to necropsy.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. CLINICAL OBSERVATIONS
No clinical signs were observed during the study.

C. BODY WEIGHT
Body weight gain was unaffected by the administration of the test substance.

D. NECROPSY
The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The oral LD_{50} of the test material (AMPA) in mice was estimated to be greater than 5000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:

In a GLP study conducted in accordance with OECD 401 guidelines (1987), the acute oral LD_{50} for AMPA was determined to be greater than 5000 mg/kg bw in male and female mice. The study is considered reliable because of its adherence to current study guidelines.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.8.1/003
Report author	[redacted]
Report year	1993
Report title	AMPA: Acute oral toxicity (limit) test in rats
Report No	8763
Document No	NA
Guidelines followed in study	FIFRA, OECD 401 (1981)
Deviations from current test guideline (OECD 420, 2001)	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

The acute oral toxicity of glyphosate's metabolite AMPA was investigated in male and female rats (5 animals/sex/group) of the Sprague-Dawley strain. The test substance, suspended in 0.5 % carboxymethylcellulose, was administered by oral gavage to each animal at a dosage of 5000 mg/kg bw and a constant dose volume of 10 mL/kg bw. Mortality, body weight and clinical signs were recorded during the subsequent 14 days. All animals were subjected to a gross necropsy at the end of the study.

Clinical signs noticed were piloerection, diarrhea, subdued behavior, hunched appearance, and soiled anal and perigenital areas. Changes of body weight were in the normal range. No Mortality occurred. No abnormal necropsy findings were noted.

The acute oral LD_{50} was calculated to be;

LD_{50}, oral, female and male rat > 5000 mg/kg bw

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	Glyphosate metabolite
Identification:	AMPA
Description:	White powder
Lot/Batch #:	286-JRJ-73-4
Purity:	99.2 %
Stability of test compound:	Not specified
2. Vehicle and/ or positive control:	0.5 % carboxymethylcellulose (CMC)
3. Test animals:	
Species:	Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	6 – 8 weeks
Sex:	Male and female
Weight at dosing:	131 – 175 g
Acclimation period:	7 days
Diet/Food:	Rat and Mouse No. 1 Maintenance diet, *ad libitum* (except the night prior to dosing and 4 hours after dosing)
Water:	Tap water, *ad libitum*
Housing:	5 animals/sex/cage in Polypropylene cages with mesh floors suspended over absorbent paper lined trays
Environmental conditions:	Temperature: 18 °C - 21 °C Humidity: 39 % (Average) Air changes: 15 – 20 times per hour 12-hour light/dark cycle (light during 07:00 – 19:00)

B. STUDY DESIGN AND METHODS

In life dates:

01/04/1992 to 15/04/1992 (experimental phase)
Finalisation date: 28/01/1993.

Animal assignment and treatment:

Rats were housed by sex (5 males and 5 females) and starved the night prior to dosing.

The test material was suspended in 0.5 % CMC at concentration of 500 mg/mL and administered by a single oral gavage at dose level of 5000 mg/kg bw with an application volume of 10 mL/kg bw. After dosing, the animals were starved for a further 4 hours.

All animals were observed for clinical signs of toxicity at several time points on the day of administration and at least once a day, thereafter for a period of 14 days. Body weights were recorded prior to administration, day seven and prior to sacrifice on day 14.

At study termination all animals were sacrificed by carbon dioxide asphyxiation followed by a gross necropsy examination.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortality occurred during the 14-days observation period after administration.

The oral LD_{50} is above 5000 mg/kg bw for male and female rats.

B. CLINICAL OBSERVATIONS

Clinical signs observed, were piloerection, diarrhea, subdued behavior, hunched appearance, and soiled anal and perigenital areas. These observations appeared at 4 hours post dosing the first time. Females recovered after day 1, while piloerection in males lasted until day 4 of the study. All clinical signs are listed in table below.

Table 5.8.1-5: AMPA: Acute oral toxicity (limit) test in rats ([redacted], 1993): Clinical observations

Dose group		5000 mg/kg bw									
Sex		Males						Females			
Time after treatment		1 min - 2 h	4 h	1 d	2 d	3 d	4 – 14 d	1 min – 2 h	4 h	1 d	2 – 14 d
Total animals examined	n	5	5	5	5	5	5	5	5	5	5
Clinical sign	n	0	5	5	3	2	0	0	5	5	0
Piloerection				2	2	2			5	5	
Diarrhea			5	5					5		
Hunched appearance				1	1					3	
Soiled anal and perigenital areas				3						3	
Subdued behaviour									5		

C. BODY WEIGHT

Weight gain of all animals was within the normal range. Individual body weights are depicted in the table below.

Table 5.8.1-6: AMPA: Acute oral toxicity (limit) test in rats ([redacted], 1993): Body weights

Dose group		5000 mg/kg bw	
Sex		Males	Females
Body weight (g)			
Day 0	Mean	168	148
	± SD	5	12

Day 7	Mean	216	180
	± SD	12	12
Day 14	Mean	256	199
	± SD	18	10
Body weight gain	Mean	88	51
	± SD	18	7

D. NECROPSY

No abnormal necropsy findings were noticed.

III. CONCLUSIONS

The oral LD_{50} of the test material (AMPA) in rats was estimated to be greater than 5000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:

As no mortality occurred during the study period, the oral LD_{50} of glyphosate metabolite AMPA in rats is greater than 5000 mg/kg bw. No deviations from the current test guideline were identified, therefore the study is considered acceptable.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.8.1/004
Report author	[redacted]
Report year	1991
Report title	Assessment of acute oral toxicity of (N-methyl-N-phosphonomethyl)glycine to rats
Report No	12837
Document No	NA
Guidelines followed in study	US EPA, subdivision F, Serie 81-1 (1984)
Deviations from current test guideline (OECD 420, 2001)	Control group included in the study
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive summary

The acute oral toxicity of glyphosate's metabolite N-methyl-N-phosphonomethyl)glycine was investigated in male and female rats (5 animals/sex/group) of the Sprague-Dawley strain. The test substance, suspended in 1 % carboxymethylcellulose, was administered by oral gavage to each animal at a dosage of 5000 mg/kg bw and a constant dose volume of 20 mL/kg bw. The vehicle only was administered to rats of the control group at a dose volume of 20 mL/kg bw. Mortality, body weight and clinical signs were recorded during the subsequent 14 days. All animals were subjected to a gross necropsy at the end of the study.

Clinical signs noticed were piloerection, diarrhea, and pinched abdomen. Changes of body weight were in the normal range. No mortality occurred. No abnormal necropsy findings were noted.
The acute oral LD_{50} was calculated to be;

LD_{50}, oral, female and male rat > 5000 mg/kg bw

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	Glyphosate metabolite
Identification:	(N-methyl-N-phosphonomethyl)glycine
Description:	White powder
Lot/Batch #:	244-KMA-9.1
Purity:	97.3 %
Stability of test compound:	Not specified
2. Vehicle and/ or positive control:	1 % carboxymethylcellulose (CMC)
3. Test animals:	
Species:	Rat
Strain:	Wistar
Source:	[redacted]
Age:	6 – 7 weeks
Sex:	Male and female
Weight at dosing:	141 – 155 g
Acclimation period:	2 days
Diet/Food:	Complete rodent diet "Altromin 1314", *ad libitum* (except for 18 hours prior to dosing and 3 hours after dosing)
Water:	Water acidified with hydrochloric acid to pH 2.5, *ad libitum*
Housing:	2-3/sex/cage in macrolone cages Type III (42x26x15 cm) with pinewood sawdust bedding
Environmental conditions:	Temperature: 21 ± 3 °C Humidity: 55 ± 15 % (Average) Air changes: 6 times per hour 12-hour light/dark cycle (light during 06:00 – 18:00)

B. STUDY DESIGN AND METHODS

In life dates:

04/07/1991 to 18/07/1991 (experimental phase).
Finalisation date 14/10/1991.

Animal assignment and treatment

Rats were housed by sex (5 males and 5 females) and fasted 18 hours prior to dosing with the test material. The substance was suspended in 1 % CMC and administered by a single oral gavage at a dose level of 5000 mg/kg bw with an application volume of 20 mL/kg bw. After dosing, the animals were fasted for a further 3 hours. A control group was included in the study. The animals of this group received the vehicle only under the same conditions and dose volume as the test group.

All animals were observed for clinical signs of toxicity at several time points on the day of administration and at least once a day, thereafter for a period of 14 days. Body weights were recorded prior to administration, day seven and prior to sacrifice on day 14.

At study termination all animals were sacrificed by carbon dioxide asphyxiation followed by a gross necropsy examination.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortality occurred during the 14-days observation period after administration.

The oral LD_{50} is above 5000 mg/kg bw for male and female rats.

B. CLINICAL OBSERVATIONS

Clinical signs observed in the control group included piloerection and pinched abdomen.
The animals of the test group showed the same signs and diarrhea in addition. The observations in the test group had resolved on day 2 while observations in the test group persisted until day 3 of the study. All clinical signs are listed in the following table.

Table 5.8.1-7: Assessment of acute oral toxicity of (N-methyl-N-phosphonomethyl)glycine to rats ([redacted], 1992): Clinical observations

Dose group (mg/kg bw)		0					5000										
Time after treatment		1 h	3 h	5 h	1 d	2 – 14 d	1 h	3 h	5 h	1 d	2 d	3 d	4 d	5 d	6 d	7 d	8 – 14 d
Total animals examined	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Clinical sign	n	10	10	10	3	0	10	10	10	10	9	0	2	1	0	1	0
Piloerection		10	10	10	3F		10	10	10	10	5M/4F		2F	1F		1F	
Pinched abdomen		1M/2F					10	5M/2F	1M								
Diarrhea											4M						

M = males; F = females

C. BODY WEIGHT

Weight gain of all animals was within the normal range. Individual body weights are depicted in the following table.

Table 5.8.1-8: Assessment of acute oral toxicity of (N-methyl-N-phosphonomethyl)glycine to rats ([redacted], 1991): Body weights

Dose group (mg/kg bw)		0	5000	0	5000
Sex		Males		Females	
Body weight (g)					
Day 0	Mean	143	153	150	153
	± SD	2	2	2	2
Day 7	Mean	200	197	187	186
	± SD	4	10	4	4
Day 14	Mean	251	248	207	206
	± SD	4	9	4	5

D. NECROPSY

No abnormal necropsy findings were noticed.

III. CONCLUSIONS

The oral LD_{50} of the test material (N-methyl-N-phosphonomethyl)glycine in rats was estimated to be greater than 5000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:

As no mortality occurred during the study period, the oral LD_{50} of the test material was estimated to be greater than 5000 mg/kg bw. A control group was used in this study, deviating from the current test guideline. However, this is not interfering with the obtained results and the study is considered acceptable.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.8.1/005
Report author	[redacted]
Report year	1988
Report title	Aminomethyl Phosphonic Acid: Acute Oral Toxicity to the Rat
Report No	[redacted]/P/2266
Document No	NA
Guidelines followed in study	No guideline specified in the report
Deviations from current test guideline	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised	Yes

testing facilities	
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive summary

Aminomethyl phosphonic acid was evaluated for its acute oral toxicity potential in male and female rats when administered as a gavage dose at a level of 5000 mg/kg bw. No mortality occurred during the 14-day observation period. Slight clinical signs of toxicity were observed following dosing but were resolved by Day 4. All animals exhibited a decrease in body weight due to the pre-dose fast; however, body weights normalized by Day 6. Decreased body weights were again observed on Days 6 and 8 for one male, as well as on Days 8 through 15 for one male and three females. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute oral LD_{50} was determined to be;

LD_{50}, oral, male and female rat > 5000 mg/kg bw

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	Aminomethyl phosphonic acid
Identification:	Aminomethyl phosphonic acid
Description:	White powder
Lot/Batch #:	Y06384/001/001
Purity:	100 %
Stability of test compound:	Not reported
2. Vehicle and/ or positive control:	0.5 % (w/v) aqueous polysorbate 80
3. Test animals:	
Species:	Wistar-derived albino rats
Strain / Stock:	Alpk:APfSD
Source:	[redacted]
Age:	8 - 9 weeks
Sex:	Male and female
Weight at dosing:	280 - 312 for males; 204 - 214 g for females
Acclimation period:	Maximum of 6 days
Diet/Food:	Porton Combined Diet, Special Diets Services Ltd, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	A maximum 5 animals/sex/cage were housed in suspended cages with stainless steel mesh floor and back
Environmental conditions:	Temperature: 15 – 24 °C Rel. humidity: 50 ± 10 % Air changes: 20 – 30 air changes per hour 12-hour light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: Not reported

Animal assignment and treatment

Based on the results of a preliminary dose-range finding study, 5000 mg/kg bw/day was selected as the dose level for this study.

Table 5.8.1-9: Aminomethyl Phosphonic Acid: Acute Oral Toxicity to the Rat ([redacted], 1998): animal distribution

	Dose (mg/kg bw/day)	Number of Animals Per Group	
		Males	Females
Test Group	5000	5	5

Animals were fasted overnight for a period of up to 24 hours prior to dosing. One female was accidentally killed on Day 1 and was therefore substituted with another animal that was dosed one day later. The dosing volume was 10 mL/kg bw. Observations for clinical signs of toxicity were made once 30 and 90 minutes after dosing and again between 4 and 6 hours after dosing. Individual body weights were recorded one the day prior to dosing, the day of dosing and on Days 3, 5 or 6, 8, and 15. At the end of the study, all animals were sacrificed via inhalation of halothane BP and examined for gross abnormalities.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

Slight signs of clinical toxicity were observed in all animals; however, all animals recovered by Day 3 or 4.

Table 5.8.1-10: Aminomethyl Phosphonic Acid: Acute Oral Toxicity to the Rat ([redacted], 1998): clinical signs of toxicity

	From Days 1 through 15	
	Males	Females
Diarrhea	2/5	1/6
Signs of diarrhea	4/5	2/6
Chromodacryorrhea	2/5	2/6
Piloerection	1/5	1/6
Stains around nose	2/5	4/6
Signs of urinary incontinence	1/5	5/6
Ungroomed	5/5	4/6
Reduced splay reflex	0/5	2/6

C. BODY WEIGHT

All animals exhibited decreased body weights due to the pre-dosing fast; however, all weights were

normalized by Day 6. After Day 6, one male lost weight between Days 6 and 8. Three females and one male also lost weight between Days 8 through 15.

Table 5.8.1-11: Aminomethyl Phosphonic Acid: Acute Oral Toxicity to the Rat ([redacted], 1998): body weight (mean ± standard deviation)

	From Days 1 through 15	
	Males	**Females**
Pre-dosing	293.2 ± 12.1	207.7 ± 3.7
Day 1	263.6 ± 12.3	186.8 ± 2.7
Day 3	285.4 ± 9.4	209.6 ± 3.8
Day 5	n/a	217.0 ± 0.0
Day 6	311.0 ± 9.7	223.0 ± 4.0
Day 8	325.0 ± 51.6	248.2 ± 27.4
Day 15	364.0 ± 12.7	245.0 ± 7.1

n/a: not applicable

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The oral LD_{50} of aminomethyl phosphonic acid in rats was estimated to be greater than 5000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:

In a GLP study conducted in a similar to OECD 420, the acute oral LD_{50} for aminomethyl phosphonic acid was determined to be greater than 5000 mg/kg bw in male and female rats. The study is considered reliable because of its adherence to current study guidelines.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.8.1/006
Report author	[redacted]
Report year	1973
Report title	Toxicological investigation of: CP 50435 – Lot: XHD-16
Report No	Y-73-19
Document No	NA
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised	No, conducted prior to GLP

testing facilities	
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

Executive summary

The acute oral toxicity of glyphosate's metabolite CP 50435 was investigated in male and female rats (5 animals/group). The test substance, suspended in corn oil, was administered by a single oral gavage to each animal at a dosage of 5010 mg/kg bw, 6310 mg/kg bw, 7940 mg/kg bw and 10000 mg/kg bw. Mortality and clinical signs were recorded during the subsequent 7 days. All animals were subjected to a gross necropsy at the end of the study.

Clinical signs noticed were reduced appetite, reduced activity, increasing weakness, slight diarrhoea and collapse. Mortality occurred in 1/5 rats at 6310 mg/kg bw, 2/5 rats at 7940 mg/kg bw and 4/5 rats at 10000 mg/kg bw. No abnormal necropsy findings were noted in animals that survived until the end of the study period.

The acute oral LD_{50} was calculated to be;

LD_{50}, oral, female and male rat 8300 mg/kg bw (CI: 7300 mg/kg bw – 9460 mg/kg bw)

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	Glyphosate metabolite
Identification:	CP 50435
Description:	Not specified
Lot/Batch #:	XHD – 16
Purity:	Not specified
Stability of test compound:	Not specified
2. Vehicle and/ or positive control:	Corn oil
3. Test animals:	
Species:	Rat
Strain:	Not specified
Source:	Not specified
Age:	Not specified
Sex:	Male and female
Weight at dosing:	205 g – 250 g
Acclimation period:	Not specified
Diet/Food:	Not specified
Water:	Not specified
Housing:	Not specified

Environmental conditions: Temperature: Not specified
Humidity: Not specified

B. STUDY DESIGN AND METHODS

In life dates:
Not specified.
Finalisation date 07/03/1973

Animal assignment and treatment

The test substance was suspended in corn oil and administered by a single oral gavage to 5 rats (mixed gender) per dose group at a dose level of 5010 mg/kg bw, 6310 mg/kg bw, 7940 mg/kg bw and 10000 mg/kg bw.

All animals were observed for clinical signs of toxicity for a period of 7 days.

At study termination all animals were sacrificed and subjected to a gross necropsy examination.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortality was observed in lowest dose group (5010 mg/kg bw). Mortality occurred in 1/5 rats at 6310 mg/kg bw, 2/5 at 7940 mg/kg bw and 4/5 at 10000 mg/kg bw (see table below).
The oral LD_{50} is 8300 mg/kg bw for male and female rats (CI: 7300 mg/kg bw – 9460 mg/kg bw).

Table 5.8.1-12: Toxicological investigation of: CP 50435 – Lot: XHD-16 ([redacted], 1973): Mortality for all dose groups

Dose level (mg/kg bw)	Animal No.		No. of deaths/No. of animals examined (Day of death)	
	Males	Females	Males	Females
5.010	2, 4	1, 3, 5	-	-
6.310	6, 8, 10	7, 9	-	1/2([a])
7.940	12, 14	11, 13, 15	1/2([a])	1/3([a])
10.000	16, 18, 20	17, 19	3/3([a])	1/2([a])

- = No death
[a] Day of death not mentioned in the study report

B. CLINICAL OBSERVATIONS

Clinical signs observed included reduced appetite, reduced activity on days 1 to 3 after treatment. Furthermore, increased weakness, slight diarrhea and collapse were observed.

C. BODY WEIGHT

Body weight was not measured after treatment.

D. NECROPSY

Animals that died during the study showed slight liver discoloration and acute gastrointestinal

inflammation. Animals that were sacrificed at the end of the study period did reveal abnormal necropsy findings.

III. CONCLUSIONS

The oral LD_{50} of the test material in rats was estimated to be 8300 mg/kg bw with a lower and upper limit of 7300 to 9460 mg/kg.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Based on mortality data, the LD_{50} was estimated to be 8300 mg/kg bw. However due to the very brief reporting of the experimental design and results, the study was considered as supplementary.

Assessment and conclusion by RMS:

Acute dermal toxicity

3. Information on the study

Data point	CA 5.8.1/007
Report author	[redacted]
Report year	2002
Report title	Acute Toxicity Study of AMPA (Aminomethyl Phosphonic Acid) in CD Rats by Dermal Administration – Limit Test
Report No	16168/02
Document No	NA
Guidelines followed in study	OECD 402 (1987); Commission Directive 84/449/EEC B.3 (1992)
Deviations from current test guideline	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid[#]
Category study in AIR 5 dossier (L docs)	Category 2a

#: Following the feedback from the Assessment Group Glyphosate in March 2020, the Notifier is aware of concerns regarding work conducted at this specific contract laboratory ([redacted]). For the sake of completeness, this study is included in the dossier submission since it was part of the data package of previous EU evaluation processes.

2. Full summary

Executive Summary

Aminomethyl phosphonic acid (AMPA) was evaluated for its acute dermal toxicity potential in male and female rats when administered at a dose level of 2000 mg/kg bw. No mortality occurred during the observation period and no clinical signs were observed at necropsy following the observation period. With regard to skin reactions, no erythema or oedema were observed during the study. There was no effect on

body weight gain. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute dermal LD_{50} was determined to be;

LD_{50}, dermal, male and female rats > 2000 mg/kg bw

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	Aminomethyl phosphonic acid (AMPA)
Identification:	AMPA
Description:	White powder
Lot/Batch #:	FA005563
Purity:	98.0 %
Stability of test compound:	Stable until December 21st, 2004
2. Vehicle and/ or positive control:	0.5 % aqueous hydroxypropylmethyl cellulose gel
3. Test animals:	
Species:	Rat
Strain / Stock:	CD® / Crl: CD®
Source:	[redacted]
Age:	20 – 22 days
Sex:	Male and female
Weight at dosing:	214 - 238 g for males; 213 - 223 g for females
Acclimation period:	At least 5 days
Diet/Food:	ssniff® R/M-H V1530 (sniff Spezialdiäten GmbH, D59494 Soet); food was discontinued approximately 16 hours before administration
Water:	Tap water, *ad libitum*
Housing:	Individually housed in Makrolon cages
Environmental conditions:	Temperature: 22 ± 3°C Rel. humidity: 55 ± 15 % 12-hour light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 21 October 2002 to 1 November 2002

Animal assignment and treatment

The test substance was suspended to the appropriate concentration (i.e., 2000 mg/kg bw) in 0.5 % aqueous hydroxypropylmethyl cellulose gel was use applied once for 24 hours on shaved intact dorsal skin of rats.

Table 5.8.1-13: Acute Toxicity Study of AMPA (Aminomethyl Phosphonic Acid) in CD Rats by Dermal Administration – Limit Test ([redacted], 2002): animal distribution

	Dose (mg/kg bw/day)	Number of Animals Per Group	
		Males	Females
Test Group	2000	5	5

The test material was applied to the skin using gauze. The gauze was covered with an occlusive dressing. The exposure time was 24 hours. At the end of the exposure period, possible residual substance was removed. Observations for mortality were made daily. Observations for clinical signs of toxicity were made before and immediately after application. Additional observations were mad 5, 15, 30, and 60 minutes after application, as well as 3, 6, and 24 hours after application. Surviving animals were observed for a total of 14 days. During the two-week follow up period, changes in skin and fur, eyes and mucous membranes, and the respiratory, circulatory, autonomic and central nervous system and somatomotorical activity and behaviour pattern were observed at least once a day until all symptom subsided, thereafter each working day. Attention was given to possible tremor, convulsions, salivation, diarrhoea, lethargy, sleep and coma. Skin was observed for signs of erythema and oedema. Individual body weights were recorded prior to dosing and in weekly intervals thereafter. At the end of the study, all surviving animals were sacrificed, dissected, and inspected macroscopically. Gross pathological changes were recorded.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

No clinical signs were observed during the study. No influence on animal behaviour were noted. No skin reactions were observed.

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance.

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The dermal LD_{50} of the test material (AMPA) in rats was estimated to be greater than 2000 mg/kg bw.

2. Assessment and conclusion

Assessment and conclusion by applicant:

In a GLP study conducted in accordance with OECD 402 guidelines (1987), the acute dermal LD_{50} for AMPA was determined to be greater than 2000 mg/kg bw in male and female rats. The study is considered reliable because of its adherence to current study guidelines.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.8.1/008
Report author	[redacted]
Report year	1993
Report title	AMPA: Acute Dermal Toxicity (Limit) Test in Rats
Report No	8764
Document No	118-GLY
Guidelines followed in study	OECD 402 (1987); Commission Directive 84/449/EEC B.3
Deviations from current test guideline (OECD 402, 2017)	Temperature was outside of the guideline recommendation; it was not specified whether clinical observations were measured 30 minutes after treatment; specific endpoints measured during the observation period were not reported
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive Summary

Aminomethyl phosphonic acid (AMPA) was evaluated for its acute dermal toxicity potential in male and female rats when administered at a dose level of 2000 mg/kg bw. No mortality occurred during the observation period and no clinical signs were observed at necropsy following the observation period. Skin reactions were not reported in the study. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute dermal LD_{50} was determined to be;

LD_{50}, dermal, male and female rats > 2000 mg/kg bw

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	Aminomethyl phosphonic acid (AMPA)
Identification:	AMPA
Description:	White powder
Lot/Batch #:	286-JRJ-73-4
Purity:	99.2 %
Stability of test compound:	Not reported
2. Vehicle and/ or positive control:	Distilled water
3. Test animals:	
Species:	Rat

Strain / Stock:	Sprague-Dawley
Source:	[redacted]
Age:	8-10 weeks old
Sex:	Male and female
Weight at dosing:	218-289 g for both sexes combined
Acclimation period:	7 days
Diet/Food:	Rat and Mouse No. 1 Maintenance Diet, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Housed with a maximum of 5 animals/cage in polypropylene cages
Environmental conditions:	Temperature: 18 – 21 °C Rel. humidity: 39 % Air changes: 15-20/hour 12-hour light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 1 April 1992 through 15 April 1992

Animal assignment and treatment

The test substance at a concentration of 2000 mg/kg bw was use applied once for 24 hours on shaved intact dorsal skin of rats.

Table 5.8.1-14: AMPA: Acute Dermal Toxicity (Limit) Test in Rats ([redacted], 1993): Animal distribution

	Dose (mg/kg bw/day)	Number of Animals Per Group	
		Males	Females
Test Group	2000	5	5

The test material was applied to the skin using gauze, which was moistened with distilled water. The gauze was covered with an occlusive dressing. The exposure time was 24 hours. At the end of the exposure period, residual substance was removed. Animals were observed once daily for clinical signs of toxicity and observations continued for 14 days following dosing. Body weights were recorded immediately prior to dosing, 7 days after dosing, and at sacrifice at the end of the 14-day observation period. At the end of the observation period, animals were sacrificed by carbon dioxide asphyxiation and subjected to necropsy.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

No clinical signs were observed during the study.

C. BODY WEIGHT

Body weight weights were considered acceptable.

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The dermal LD_{50} of the test material (AMPA) in rats was estimated to be greater than 2000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:

In a GLP study conducted in accordance with OECD 402 guidelines (1987), the acute dermal LD_{50} for AMPA was determined to be greater than 2000 mg/kg bw in male and female rats. The study is considered reliable because of its adherence to current study guidelines.

Assessment and conclusion by RMS:

Skin and Eye irritation

1. Information on the study

Data point	CA 5.8.1/009
Report author	[redacted]
Report year	1973
Report title	Toxicological Investigation of: CP 50435 – Lot XHD - 16
Report No	Y-73-19 (Bayer)
Document No	NA
Guidelines followed in study	None followed
Deviations from current test guideline (OECD 404, 2002)	Exposure period was 24 hours not the recommended 4 hours; experimental procedures are only briefly described
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, conducted prior to GLP
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

Aminomethyl phosphonic acid (AMPA) was tested for its dermal irritating potential. In an *in vivo* study, the 0.5 grams of the test material was applied as a finely ground powder moistened with water to the intact skin of male and female rabbits. The exposure period was 24 hours. The average maximum score was 0.0

out of a possible 8 during the seven-day observation period. Thus, indicating no skin irritating potential.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	Aminomethyl phosphonic acid (AMPA)	
Identification:	Not reported	
Description:	Not reported	
Lot/Batch #:	XHD - 16	
Purity:	Not reported	
Stability of test compound:	Not reported	
2. Vehicle and/ or positive control:	None used	
3. Test animals:		
Species:	Rabbits	
Strain:	Albino	
Source:	Not reported	
Age:	Not reported	
Sex:	Male and female	
Weight at dosing:	Not reported	
Acclimation period:	Not reported	
Diet/Food:	Not reported	
Water:	Not reported	
Housing:	Not reported	
Environmental conditions:	Temperature:	Not reported
	Humidity:	Not reported
	Air changes:	Not reported
	Light/dark cycle not reported	

B. STUDY DESIGN AND METHODS

In life dates: Not reported

Animal assignment and treatment:
Animal assignment was not reported in the study report.

The test material (0.5 grams) was applied to intact rabbit skin as a finely ground powder moistened with water. The exposure period was 24 hours.

II. RESULTS AND DISCUSSION

A. MORTALITY
Mortality was not reported.

B. CLINICAL OBSERVATIONS
Clinical signs of toxicity were not reported.

C. BODY WEIGHT
Body weights were not reported.

D. NECROPSY
A necropsy performance was not reported.

E. SKIN REACTIONS
No skin reactions were observed after 24 hours of exposure during the 7-day observation period.

III. CONCLUSIONS

Based on the EU classification criteria, AMPA is not to be classified for skin irritation.

2. Assessment and conclusion

Assessment and conclusion by applicant:

The study is considered supplementary information only as just basic information is given and no individual skin scores are provided. The treatment period was 24 hours instead of 4 hours according to the most recent guideline. Therefore, the result can be considered as worst case. Based on the results of the study, no skin reactions were observed after 24 hours of exposure during the 7-day observation period. Therefore, the test material is not considered to be irritating to the skin.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.8.1/010
Report author	[redacted]
Report year	1973
Report title	Toxicological Investigation of: CP 50435 – Lot XHD - 16
Report No	Y-73-19
Document No	NA
Guidelines followed in study	None
Deviations from current test guideline (OECD 405, 2017)	Individual scores for eye irritation not included in the report, therefore no mean values could be obtained.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, conducted prior to GLP
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary
Executive summary

In an eye irritation study, three albino rabbits were treated with glyphosate's metabolite. Therefore, the test substance was instilled into the conjunctival sac of all animals.

Following treatment, eye irritation examined at 1, 24, 48, 72, 120, and 168 hours.

Application of the test substance into the rabbit eye resulted in effects on the conjunctivae (slight to moderate erythema), which all resolved within 120 hours. The individual mean irritation scores could not be calculated as no individual irritation scores were given in the study report.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate metabolite
Description:	White powder
Lot/Batch #:	Y-73-19
Purity:	Not specified
Stability of test compound:	Not specified

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rabbit
Strain:	Not specified
Source:	Not specified
Age:	Not specified
Sex:	Male and Female
Body weight at dosing:	Not specified
Acclimation period:	Not specified
Diet/Food:	Not specified
Water:	Not specified
Housing:	Not specified
Environmental conditions:	Not specified

B. STUDY DESIGN AND METHODS

In life dates: Not specified
Finalisation date: 07/03/1973

Animal assignment and treatment

An amount of 100 mg of the test substance was placed into the conjunctival sac of one eye of each animal.

At 1, 24, 48, 72, 120, and 168 hours all eyes were examined for signs of irritation.

II. RESULTS AND DISCUSSION

A. MORTALITY
No mortality occurred.

B. CLINICAL OBSERVATIONS
No clinical signs of systemic toxicity were observed during the study.

C. EYE OBSERVATIONS

Signs of oedema, erythema and discharge were seen in all animals. However, all observations had resolved until 120 hours after treatment (see below).

Table 5.8.1-15: Eye irritation in rabbits after application of 'CP 50435' – Lot: XHD-16 ([redacted] 1973): Eye irritation observations

Animal number		10 min	1 h	24 h	48 h	72 h	120 h	168 h
Cornea	1-3	-	-	-	-	-	-	-
Iris	1-3	-	-	-	-	-	-	-
Conjunctiva Redness	1-3	Slight erythema	Slight erythema	Slight to moderate erythema	Slight to moderate erythema	Slight erythema in one instance	-	-
Conjunctiva Chemosis	1-3	Very slight oedema	Very slight oedema	No oedema	No oedema	No oedema	-	-
Conjunctiva Discharge	1-3	Copius discharge	Copius discharge	Moderate discharge containing whitish exudate	No discharge	No discharge	-	-

III. CONCLUSIONS

Effects on the conjunctiva were observed in all animals. These effects had resolved until 120 hours in all animals. The compound was classified as a slight eye irritant in male and female rabbits.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The classification criteria laid down in the CLP regulation (EC No. 1272/2008) could not be applied to classify the test substance, based on the given study report as there were no individual values given. However, it can be concluded that the test substance is not corrosive as all observations on the eye were reversible within 120 hours after test item application. Detailed reporting of individual animal data is missing, therefore the study is considered as supplementary.

Assessment and conclusion by RMS:

Skin sensitisation

1. Information on the study

Data point	CA 5.8.1/011
Report author	[redacted]
Report year	2002
Report title	Examination of AMPA (Aminomethyl Phosphonic Acid) in the Skin Sensitation Test in Guinea Pigs According to Magnusson and Kligman (Maximisation Test)
Report No	16169/02
Document No	NA
Guidelines followed in study	OECD 406; Commission Directive 84/449/EEC B.6
Deviations from current test guideline	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid#
Category study in AIR 5 dossier (L docs)	Category 2a

#: Following the feedback from the Assessment Group Glyphosate in March 2020, the Notifier is aware of concerns regarding work conducted at this specific contract laboratory ([redacted]). For the sake of completeness, this study is included in the dossier submission since it was part of the data package of previous EU evaluation processes.

2. Full summary

Aminomethyl phosphonic acid (AMPA) was tested for its sensitising effect on the skin of the guinea pig in the Maximisation Test. The test-substance concentrations for the main test were selected based on the results of the pre-test. The intradermal induction was performed with a 5 % dilution of the test item in a*qua ad iniectabilia*. The epidermal induction was conducted under occlusion with the test item at 50 % one week after the intradermal induction. Two weeks after induction the animals were challenged by epidermal application of the test item at 50 % under occlusive dressing.

The study was performed using one control group consisting of 5 animals, and one test group consisting of 10 animals. None of the animals exhibited a positive skin reaction after the challenge treatment.

II. MATERIALS AND METHODS

A: Materials

1. Test material:	Aminomethyl phosphonic acid (AMPA)
Identification:	Not reported
Description:	White solid powder
Lot/Batch #:	FA005563
Purity:	98 %
Stability of test compound:	Expires December 31st, 2004
2. Vehicle and/ or positive control:	*Aqua ad iniectabilia* / benzocaine
3. Test animals:	
Species:	Guinea pig
Strain:	Dunkin-Hartley

Source:	[redacted]
Age:	22 days
Sex:	Male
Weight at dosing:	252 – 307 g (positive control 228 – 341 g)
Acclimation period:	At least 5 days
Diet/Food:	ssniff® Ms-H (ssniff Spezialdiäten GmbH, D-59494 Soest), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Housed in pairs in Makrolon cages
Environmental conditions:	Temperature: 22 ± 3°C Humidity: 55 ± 15 % Air changes: not reported 12 hours light/dark cycle

B: Study design and methods

In life dates: 21 October 2002 to 26 November 2002

Animal assignment and treatment

AMPA was tested for its sensitising effect on the skin of the guinea pig using the Maximisation test according to Magnusson and Kligman. Male Dunkin Hartley guinea pigs with body weights ranging from 252 to 307 g were used. The test substance concentrations for the main study were selected based on the results of the pre-testing. The main study was performed in 10 test animals.

Table 5.8.1-16: Examination of AMPA (Aminomethyl Phosphonic Acid) in the Skin Sensitation Test in Guinea Pigs According to Magnusson and Kligman (Maximisation Test) ([redacted], 2002): animal distribution

	Number of Animals Per Group
Pretest	
Intradermal Pretest	2
Epidermal Pretest	6
Main Study	
Negative Control Group	5
Test Group	10
Positive Control Group	20

The induction phase consisted of 3 injections to the intra-scapular region beginning at the cranial and ending at the caudal limits. Two of the injections consisted of 0.1 mL Freund's Complete Adjuvant (FCA) (diluted 1:1 with 0.9 % sodium chloride; 0.1 mL of the test material suspended in *aqua ad iniectabilia*; and 0.1 mL of a mixture of the test material and FCA (1:1). The third injection consisted of a final concentration of the test substance that was equal to that in the second injection. Six days after the injection phase, the application area was shaved and exposed skin was coated with 0.5 mL sodium lauryl sulfate 10 % in Vaseline in order to induce local irritation. On Day 7, an epidermal application was made and the test material was topically applied at a concentration of 50 % to the same shoulder area and covered with an occlusive dressing, which was left in place for 48 hours.

Two weeks after the topical indication, test and control animals were challenged with an occlusive patch

containing the test material at a concentration of 50 %.

For control animals, topical applications used the same procedures as those noted for test animals except that the vehicle (i.e., *aqua ad iniectabilia*) alone was applied. During the challenge phase, the left flank of vehicle control animals was treated with the test material and the right flank was treated with vehicle. Positive control animals were treated with 2 % (w/v) benzocaine solution intracutaneously during the intradermal stage of induction. A 5 % (w/v) benzocaine solution was topically applied during the epidermal stage of induction and during the challenge.

Skin reactions were evaluated at 25 and 28 hours during the intradermal stage of induction and again at 49 and 72 hours during the epidermal stage of induction. Mortality and clinical signs of toxicity were observed daily during the observation period. Body weights were measured at the start of the study and at study termination. Body weights were statistically analysed suing the Student's t-test ($p \leq 0.01$).

Any animal showing erythema at the site of challenge was considered to have shown a positive response.

II. RESULTS

A. MORTALITY

One control animal died during the study.

B. CLINICAL OBSERVATIONS

No clinical signs, other than skin reactions induced by treatment, were noted.

C. BODY WEIGHT

Body weights were considered acceptable.

D. NECROPSY

A necropsy was not performed.

E. SKIN REACTIONS

No skin reactions were observed 24 or 48 h after the challenge treatment with AMPA in the control or test group.

Table 5.8.1-17: Examination of AMPA (Aminomethyl Phosphonic Acid) in the Skin Sensitation Test in Guinea Pigs According to Magnusson and Kligman (Maximisation Test) ([redacted], 2002): number of animals with positive signs following challenge

	Challenge at 25 %	
	24 hours	48 hours
Test group	0/10	0/10
Negative control group	0/5	0/5
Positive control group	20/20	20/20

III. STUDY CONCLUSION

Based on the results of the study, AMPA is not considered a skin sensitiser.

3. Assessment and conclusion

Assessment and conclusion by applicant:

In a study conducted in accordance with OECD 406 guidelines, AMPA is not considered a skin sensitiser based on the results of a guinea pig maximisation test. The study is considered reliable because of its adherence to current study guidelines.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.8.1/012
Report author	[redacted]
Report year	1993
Report title	AMPA: Magnusson-Kligman Maximisation Test in Guinea Pigs
Report No	8765
Document No	NA
Guidelines followed in study	OECD 406 (1982); Commission Directive 84/449/EEC B.6 (1989)
Deviations from current test guideline (OECD 406, 1992)	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive summary

Aminomethyl phosphonic acid (AMPA) was tested for its sensitizing effect on the skin of the guinea pig in the Maximisation Test. The test-substance concentrations for the main test were selected based on the results of the pre-test. The intradermal induction was performed with a 10 % concentration of the test item in 0.5 % carboxymethylcellulose (CMC). The epidermal induction was conducted under occlusion with the test item at 25 % in 0.5 % CMC one week after the intradermal induction. Two weeks after induction the animals were challenged by epidermal application of the test item at 25 % in 0.5 % CMC.

The study was performed using one control group consisting of 20 animals, and one test group consisting of 20 animals. The sensitivity of the strain used was assessed at 6 monthly intervals using a known skin sensitizer e.g. 2,4-dinitro-chlorobenzene. None of the animals in the control and test group exhibited a positive skin reaction after the challenge treatment. Animals treated with the positive control exhibited a skin sensitizing reaction in all animals. Therefore, there is no evidence from the test results that AMPA is a skin sensitizer in guinea pigs.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	Aminomethyl phosphonic acid (AMPA)
Identification:	Not reported
Description:	White solid powder
Lot/Batch #:	286-JRJ-73-4
Purity:	99.2 %
Stability of test compound:	Not reported
2. Vehicle and/ or positive control:	0.5 % Carboxymethylcellulose (CMC) / 2,4-dinitrochlorobenzene
3. Test animals:	
Species:	Guinea pig
Strain:	Dunkin-Hartley
Source:	[redacted]
Age:	Less than one year old
Sex:	Female
Weight at dosing:	379-484 g
Acclimation period:	At least 7 days
Diet/Food:	FDI Guinea Pig Diet, supplied by Special Diets Services, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Housed 5 per cage in aluminium cages
Environmental conditions:	Temperature: 19-21 °C Humidity: 44 % Air changes: not reported 12 hours light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 1 April 1992 through 1 May 1992

Animal assignment and treatment

AMPA was tested for its sensitising effect on the skin of the guinea pig using the Maximisation test according to Magnusson and Kligman. Female Dunkin Hartley guinea pigs with body weights ranging from 379 to 484 grams were used. The test substance concentrations for the main study were selected based on the results of the pre-testing. The main study was performed in 20 test animals.

Table 5.8.1-18: AMPA: Magnusson-Kligman Maximisation Test in Guinea Pigs ([redacted], 1993): Animal distribution

	Number of Animals Per Group
Pretest	
Intradermal Pretest	2

Epidermal Pretest	2
Main Study	
Negative Control Group	20
Test Group	20
Challenge Dose Ranging Group	4

The induction phase consisted of 6 injections consisting of two lines of three injections on each side of and parallel to the mid-line. The injections consisted of 0.1 mL Freund's Complete Adjuvant (FCA) (anterior injection), 0.1 mL test material (middle injection), and 0.1 mL of 50:50 emulsion of test material in Freund's Complete Adjuvant (posterior injection). The test material was injected at a concentration of 10 % w/v in CMC. The concentration of AMPA in Freund's Complete Adjuvant was also 10 %. Six days after the injection phase, the application area was shaved and exposed skin was wetted with 10 % aqueous sodium lauryl sulfate in order to induce local irritation. On Day 7, an epidermal application was made and the test material was topically applied at a concentration of 25 % w/v in CMC to the same pre-treated area and covered with an occlusive dressing, which was left in place for 48 hours.

Two weeks after the topical induction, test and control animals were challenged with an occlusive patch containing the test material at a concentration of 25 % w/v in CMC and with vehicle (i.e., CMC). Patches were held in place for 24 hours using the same method as topical induction.

The 4 animals intended for the dose ranging for challenge were each administered 0.1 mL intradermal injection of Freund's Complete Adjuvant on either side of the mid-line of the shaved scapular region. During the challenge period, these animals remained untreated.

For the control group, animals were treated similarly to test animals but were administered CMC instead of the test material.

According to the study report, the sensitivity of this strain of guinea pig to a known sensitizer, 2,4-dinitrochlorobenzed, is checked at 6 month intervals. The most recent positive control test was completed on 23 December 1991, in which 67 % of the test group reacted positively.

Body weights were measured at the start of the study and at study termination. Clinical signs of toxicity also were recorded

Any animal showing erythema at the site of challenge was considered to have shown a positive response.

II. RESULTS AND DISCUSSION

A. MORTALITY

One control animal died during the study.

B. CLINICAL OBSERVATIONS

Clinical signs of toxicity were limited to pale extremities.

C. BODY WEIGHT

Body weights were considered acceptable.

D. NECROPSY

A necropsy was not performed.

E. SKIN REACTIONS

No skin reactions were observed 24 or 48 h after the challenge treatment with AMPA in the control or test group.

Table 5.8.1 19: AMPA: Magnusson-Kligman Maximisation Test in Guinea Pigs ([redacted], 1993): Number of animals with positive signs following challenge

	Challenge at 25 %	
	24 hours	**48 hours**
Test Group	0/20	0/20
Negative Control Group	0/20	0/20
Challenge Dose Ranging Group	0/4	0/4

III. CONCLUSIONS

There is no evidence from the test results that AMPA is a skin sensitiser in guinea pigs. AMPA is classified as a weak sensitiser according to the Magnusson-Kligman classification.

3. Assessment and conclusion

Assessment and conclusion by applicant:

In a study conducted in accordance with OECD 406 guidelines, AMPA is not considered a skin sensitiser based on the results of a guinea pig maximisation test. The study is considered reliable because of its adherence to current study guidelines.

Assessment and conclusion by RMS:

Short-term toxicity

1. Information on the study

Data point	CA 5.8.1/013
Report author	[redacted]
Report year	1978
Report title	Fourteen Day Rat Feeding
Report No	401-026
Document No	NA
Guidelines followed in study	No guideline followed.
Deviations from current test guideline (OECD 407, 2008)	No guidelines exist for 14 day feeding studies. When compared with OECD 407 (28 day oral toxicity) there was an absence of details for housing conditions and the certificates of analysis were not presented. In addition, stability and the certificate of analysis for the test substance

	were not identified. Only 5 animals per sex / group were employed. Oestrus cycle of females was not determined at necropsy. Haematology and clinical chemistry were not investigated. Full detailed gross necropsies and histopathology were not performed.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No formal claim of compliance with GLP or specific guidelines since the study was performed pre-GLP.
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

Executive summary

In a 14 day dietary study in Charles River CD rats, the test material (CP 50435) was administered at dosage levels of 1000, 2000 and 4000 mg/kg bw/day. Five male and 5 female rats were used at each dose level and also in the control group. Rats were observed twice daily for overt signs of toxicity or mortality. Detailed observations were recorded weekly. Individual body weights and food consumption were recorded weekly.

Red coloured material in the urine was noted for 1 male rat at the 4000 mg/kg bw/day dosage level. No other changes were seen in general behaviour and appearance.

Male rats at the 4000 mg/kg bw/day dosage level showed slight to moderate decreases in body weight gain when compared with control and other treated rats. Food consumption was very slightly decreased for rats at the 4000 mg/kg bw/day dosage level.

None of the rats died during the study.

No gross lesions considered to be related to compound intake were seen at necropsy.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	"CP 50435; Aminomethyl Phosphonic Acid; Notebook Page 1219342-B; 99.9 % assay 7/7/77"
Description:	White granular material with a few yellowish crystalline chunks
Lot/Batch #:	Not specified
Purity:	99.9 %
Stability of test compound:	Not reported

2. Vehicle and/ or positive control: Purina® Laboratory Chow® / none

3. Test animals:

Species:	Rat
Strain:	Charles River CD®
Source:	[redacted]
Age:	Not specified.

Sex:	Male and female	
Weight at dosing:	♂ 90 – 113 g; ♀ 85 – 98 g	
Acclimation period:	Not specified.	
Diet/Food:	Purina® Laboratory Chow®, *ad libitum*	
Water:	Tap water, *ad libitum*	
Housing:	Individual housing in hanging wire-mesh cages	
Environmental conditions:	Temperature:	Not reported
	Humidity:	Not reported
	Air changes:	Not reported
	Light cycle:	Not reported

B. STUDY DESIGN AND METHODS

In life dates: 1977-11-25 (start of dosing) to 1977-12-09 (terminal necropsies)

Animal assignment and treatment

The test material (CP 50435) was administered in the diet at varying concentrations to provide dosage levels of 1000, 2000 and 4000 mg/kg bw/day. Five male and 5 female rats were used at each dose level and also in a control group. Control rats received the basal diet, Purina® Laboratory Chow® on the same regimen as treated rats.

Table 5.8.1-19: Fourteen Day Rat Feeding Study [redacted]-77-309 ([redacted], 1978): Study design

Test group	Dose Group [mg AMPA/kg bw/day]	Number of Rats	
		Males	Females
Control	0	5	5
Low	1000	5	5
Intermediate	2000	5	5
High	4000	5	5

The compound was ground in a mortar with pestle prior to weighing. The appropriate amount of compound was then mixed with a small amount of basal diet in a Hobart food mixer. This premix was mixed with the total amount of diet in a twin shell blender (with an intensifier bar). The diets were mixed weekly.

Diet Samples

A sample of the compound of approx. 42.5 g (equivalent to 1.5 ounce) was taken at the start of the study and at the end of the study. Weekly samples of control feed and each treatment diet were taken on day 1 of feeding. Additional containers with feed from each diet level were placed in an empty cage at the beginning of each feeding week and sampled at the end of the week. The diet samples were immediately frozen for shipping to the sponsor after termination of the study.

Mortality

The animals were observed twice daily for mortality and signs of overt toxicity.

Clinical observations

Detailed observations of each rat were recorded weekly.

Body weight

Individual body weights were recorded weekly.

Food consumption

Individual food consumption was recorded weekly.

II. RESULTS AND DISCUSSION

A. GENERAL BEHAVIOUR, APPEARANCE AND SURVIVAL

Red coloured material in the urine was noted for 1 male rat at the 4000 mg/kg bw/day dosage level. No other changes were seen in general behaviour and appearance. No rats died during the study.

B. BODY WEIGHT

Male rats at the 4000 mg/kg bw/day dosage level showed slight to moderate decreases in body weight gain when compared with control and other treated rats. Changes in body weight were similar for control and treated female rats. Group mean body weights at study initiation and termination are presented below (see table below).

Table 5.8.1 21: Fourteen Day Rat Feeding Study [redacted]-77-309 ([redacted], 1978): Intergroup comparison of group mean body weights and body weight gain

Dosage Group [mg/kg bw/day]	Mean body weight [g]		
	Week 0	Week 2	Percentage Increase [%]
	Males		
0 (control)	99	193	94.9
1000	101	203	101.0
2000	103	193	87.4
4000	105	183	74.3
	Females		
0 (control)	94	152	61.7
1000	92	149	62.0
2000	95	156	64.2
4000	91	143	57.1

C. FOOD CONSUMPTION

Food consumption was very slightly decreased for rats at the 4000 mg/kg bw/day dosage level. Group mean food and compound consumptions for the study are presented below:

Table 5.8.1-20: Fourteen Day Rat Feeding Study [redacted]-77-309 ([redacted], 1978): Intergroup comparison of mean food consumption and test substance intake

Dosage Group [mg/kg bw/day]	Average Food Consumption [g/rat/day]		Compound Consumption [mg/kg bw/day]	
Males	**Week 1**	**Week 2**	**Week 1**	**Week 2**
0 (control)	19.7	23.4	-	-
1000	20.6	24.2	1068	1090
2000	20.1	24.2	2195	2232
4000	18.5	21.1	4152	4337
Females				
0 (control)	16.4	19.1	-	-
1000	16.9	19.2	1063	1059
2000	17.4	19.0	2139	1990
4000	15.6	17.9	4070	4303

D. NECROPSY FINDINGS

Raised, opaque corneal foci were seen in one male rat of the Low dose group and hydrometra was observed in the uterus of one female rats of the Intermediate group. Both findings were considered to be of no toxicological relevance.
There were no other treatment-related gross pathological findings.

3. Assessment and conclusion

Assessment and conclusion by applicant:

In this 14 day dietary study in Charles River CD rats, the test material AMPA was administered at dosage levels of 1000, 2000 and 4000 mg/kg bw/day.

The study was not conducted according to any guideline or in compliance with GLP (pre-GLP). Therefore, the major deviations listed above are from the current version of OECD 407 (2008): no haematology, no clinical chemistry, no full detailed gross necropsies and histopathology.

Apart from these major deviations, the study was well conducted and can provide supplemental information for the assessment of the metabolites of glyphosate. The study is therefore considered to be only supplementary.

Dosing CD rats with AMPA via the diet did not produce any changes in general behaviour or appearance. Slight to moderate decrease in body weight gain was observed in males at 4000 mg/kg bw/day. Food consumption was very slightly decreased in male and female rats at the 4000 mg/kg bw/day dose level. One male rat at the high dose showed red coloured material in the urine.

No gross lesions considered to be compound related were observed. Due to the absence of relevant examinations such as haematology, clinical chemistry or histopathology, no reliable NOAEL could be derived from this study.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.8.1/014

Report author	[redacted]
Report year	1993
Report title	4 Week Dose Range Finding Study in Rats with Administration by Gavage
Report No	7803
Document No	148-GLY
Guidelines followed in study	No guideline followed.
Deviations from current test guideline	This study was essentially a dose range-finding study for which there are no guidelines. Haematology and clinical biochemistry parameters were not measured or evaluated. General gross pathology of organs and tissues was not performed - only selected organs and tissues were weighed, fixed or examined as detailed below. Histopathology was only performed on urinary bladder, mandibular salivary gland, sublingual salivary gland and parotid salivary gland.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive summary

Groups of 5 male and 5 female Sprague-Dawley rats were dosed orally via a steel dosing cannula with AMPA (aminomethylphosphonic acid) at dose levels of 0, 10, 100, 350 or 1000 mg/kg bw/day.
After 4 weeks of dosing all surviving animals were killed, subjected to necropsy and had selected organs weighed.
All animals underwent histological examinations of the urinary bladder, mandibular salivary gland, sublingual salivary gland and parotid salivary gland.
The results are summarised as follows:

Mortality: There were no premature decedents.
Clinical Signs: There were no clinical signs.
Body Weight: There were no notable intergroup differences.
Food Consumption: There were no notable intergroup differences.
Water Consumption: There were no notable intergroup differences.
Organ weights: There was a slight equivocal increase in kidney weight at 350 and 1000 mg/kg bw/day in males.
Necropsy Findings: There were no notable intergroup differences.
Histological Findings: There were no notable intergroup differences.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification: AMPA (Aminomethylphosphonic acid)
Description: White Powder

Lot/Batch #:	286-JRJ-73-4
Purity:	99.2 %
Stability of test compound:	The test substance is stable at least 3 years from the date of analysis at ambient temperature in the dark.
2. Vehicle and/ or positive control:	0.5 % Carboxymethylcellulose (CMC) in distilled water / none
3. Test animals:	
Species:	Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	Approx. 4 weeks
Sex:	Male and female
Weight at dosing:	♂ 209 – 230 g; ♀ 156 – 162 g
Acclimation period:	16 days
Diet/Food:	SQC Expanded Maintenance Diet No. 1 Rat and Mouse (pelleted), *ad libitum,*
Water:	Tap water, *ad libitum*
Housing:	2 of one sex or 1 animal/cage in polypropylene cages with stainless steel wire grid tops and bottoms (420 × 270 × 200 mm).
Environmental conditions:	Temperature: 20 ± 2 °C Humidity: 50 ± 15 % Air changes: 15 – 20 / hour 12 hours light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 1992-02-20 (start of dosing) to 1992-03-19 (terminal necropsies)

Animal assignment and treatment

In a 4 week dose range finding study groups of 5 Sprague-Dawley rats per sex received daily doses of 0, 10, 100, 350 or 1000 mg/kg bw/day, orally via steel dosing cannula, at a dose volume of 10 mL/kg bw for 28 consecutive days. The dose formulations were prepared fresh daily using 0.5 % carboxymethylcellulose (CMC) in distilled water as vehicle. Samples of formulations for all dosing groups (including Control) were analysed during Weeks 1 and 4 of dosing for the concentration of test item in the suspension. In addition, data demonstrating 24 hour stability of dosing suspensions were generated during the study.

Table 5.8.1-21: 4 Week Dose Range Finding Study in Rats with Administration by Gavage ([redacted], 1993): Study design

Test group	Dose Group [mg AMPA/kg bw/day]	Animal Numbers	
		Males	Females
Control	0	1 – 5	26 – 30
Low	10	6 – 10	31 – 35
Intermediate I	100	11 – 15	36 – 40
Intermediate II	350	16 – 20	41 – 45
High	1000	21 – 25	46 – 50

Mortality
All animals were checked for viability/mortality early each morning and as late as possible each day.

Clinical observations
All animals were examined for signs of reaction to treatment each day. All animals received a detailed clinical examination once each week.

Body weight
The body weight of each animal was recorded on three occasions over a 10 day pre-trial period and daily from the start of treatment until the end of the study.

Food consumption
The quantity of food consumed by each cage of animals was recorded on three occasions over a 10 day pre-trial period and twice each week from the start of treatment until the end of the study.

Water consumption
Water consumption was monitored by visual inspection throughout the treatment period.

Terminal studies
After 28 consecutive days of treatment, all surviving animals were killed by carbon dioxide asphyxiation followed by exsanguination. Gross dissection and necropsy were performed under the supervision of a pathologist.

The following organs were weighed and fixed: Liver, heart, kidneys, lung, spleen, testes, ovaries, mandibular salivary gland, sublingual salivary gland and parotid salivary gland.

The following organs were fixed and examined histologically: Urinary bladder, mandibular salivary gland, sublingual salivary gland and parotid salivary gland.

The following organs were fixed: Abnormal tissue and ears.

The above tissues were fixed in 10 % neutral buffered formalin. Lungs were fixed in their entirety by perfusion with 10 % neutral buffered formalin.
Ears were fixed for identification purposes.

Histological evaluation of the urinary bladder, mandibular salivary gland, sublingual salivary gland and parotid salivary gland was performed for all animals.

Carcasses of all animals were discarded immediately following necropsy and placing of all tissues in fixative as identified above.

Statistics
Organ weight and body weight data were statistically analysed for homogeneity of variance using the F-max test. If group variances appeared homogeneous a parametric ANOVA was used and pairwise comparisons made via Student's t-test using Fisher's F-protected LSD. If variances were heterogeneous log or square root transformations were used in an attempt to stabilise the variances. If variances were still heterogeneous, a non-parametric test such as a Kruskal-Wallis ANOVA was used and pairwise comparisons made via Dunn Z test where considered appropriate.

Organ weights were also analysed conditional on body weight (i.e. analysis of covariance).

Histological data were analysed using Fisher's Exact Probability test.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no premature decedents.

B. CLINICAL OBSERVATIONS
There were no clinical signs observed throughout the dose groups in either sex.

C. BODY WEIGHT
There were no notable intergroup differences in male animals.

A slight reduction in overall group mean body weight gain (13 %) was noted in High dose females (see table below), however, due to the small magnitude of difference and lack of statistical significance at any time point, this reduction was considered not to be related to treatment with AMPA.

Table 5.8.1-22: 4 Week Dose Range Finding Study in Rats with Administration by Gavage ([redacted], 1993): Intergroup comparison of group mean body weights and body weight gain

Time point	Mean body weight or body weight gain [g]		
	Initial body weight	Final body weight	Total weight gain Day 0 – 28
Dose [mg/kg bw/day]	**Males**		
0	215	406	191
10	220	399	179 (94 % of Control)
100	209	420	211 (110 % of Control)
350	230	420	190 (99 % of Control)
1000	220	414	194 (102 % of Control)
	Females		
0	162	254	92
10	159	258	99 (108 % of Control)
100	156	244	88 (96 % of Control)
350	162	257	95 (103 % of Control)
1000	160	240	80 (87 % of Control)

D. FOOD CONSUMPTION
There were no notable intergroup differences for food consumption in either sex.

E. WATER CONSUMPTION
There were no notable intergroup differences for water consumption in either sex.

F. TERMINAL STUDIES

Organ weights
In males, absolute organ weights showed no notable intergroup differences.
Following covariance analysis a slight increase in kidney weight was observed for the Intermediate II and High dose groups (7 %, $p<0.05$ and 8 %, $p<0.05$, respectively). In addition, a slight increase in liver weight (10 %, $p<0.05$) was noted in the Intermediate II dose group, however, due to an absence of similar effect observed in the High dose group, this increase was considered not to be related to treatment with AMPA.

In females, there were no notable intergroup differences.

Table 5.8.1-23: 4 Week Dose Range Finding Study in Rats with Administration by Gavage ([redacted], 1993): Intergroup comparison of selected relative mean organ weights (mean ± SE)

Organ [g]	Dose Groups [mg AMPA/kg bw/day]									
	Males					Females				
	0	10	100	350	1000	0	10	100	350	1000
Kidney (covariance analysis)	3.16 ± 0.06	3.26 ± 0.06	3.01 ± 0.06	**3.37* ± 0.06**	**3.40* ± 0.06**	2.03 ± 0.09	2.13 ± 0.09	2.16 ± 0.09	2.12 ± 0.09	2.19 ± 0.09
Liver (covariance analysis)	17.67 ± 0.54	18.91 ± 0.54	17.18 ± 0.54	**19.44* ± 0.54**	18.06 ± 0.53	10.78 ± 0.39	11.10 ± 0.40	10.98 ± 0.40	10.41 ± 0.40	9.88 ± 0.40

*: Statistically significant from controls, p<0.05

Gross pathology
Enlargement of the submandibular lymph node was seen in all dose groups (including Controls) except for male animals in the Intermediate II dose group. The incidence varied and despite being highest in the High dose females (5 / 5 compared with 1/5 in the Control) this finding was considered not to be related to treatment with AMPA.
There were no other notable intergroup differences.

Table 5.8.1-24: 4 Week Dose Range Finding Study in Rats with Administration by Gavage ([redacted], 1993): Summary of necropsy findings

Finding	Dose Groups [mg AMPA/kg bw/day]									
	Males					Females				
	0	10	100	350	1000	0	10	100	350	1000
Submandibular salivary lymph node: enlarged	3 / 5	1 / 5	2 / 5	0 / 5	1 / 5	1 / 5	3 / 5	3 / 5	3 / 5	5 / 5

Histopathology
In males, very mild reduced serous secretion in the mandibular salivary gland was noted in 1 / 5 animals in the High dose group. The change was seen as reduced eosinophilic droplets in the epithelial cells of the serous duct acini. The finding was confirmed by staining with the PAS-Alcian blue method for mucopolysaccharides. This very mild reduced serous secretion does not resemble salivary gland changes seen in long-term studies with Glyphosate (parent compound of AMPA) and was considered not to be related to treatment with AMPA.

In females, no histological changes were seen.

Table 5.8.1-25: 4 Week Dose Range Finding Study in Rats with Administration by Gavage ([redacted], 1993): Summary of histological findings

Finding	Dose Groups [mg AMPA/kg bw/day]									
	Males					Females				
	0	10	100	350	1000	0	10	100	350	1000
Salivary gland: Mandibular: reduced serous secretion	0 / 5	0 / 5	0 / 5	0 / 5	1 / 5	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5

III. CONCLUSIONS

Dosing of Sprague-Dawley rats orally by gavage for 4 weeks with AMPA did not produce in-life signs of

toxicity. Organ weight analysis revealed a slight equivocal increase in kidney weight after covariance analysis at 350 and 1000 mg/kg bw/day in males. There were no toxicologically significant effects seen at 10 or 100 mg/kg bw/day.

3. Assessment and conclusion

Assessment and conclusion by applicant:

In this 4 week dose range finding study groups of 5 Sprague-Dawley rats per sex received daily doses of AMPA at 0, 10, 100, 350 or 1000 mg/kg bw/day by gavage for 28 consecutive days.
The study was conducted in compliance with GLP regulations. Deviations could not be applied as no guideline was followed.
However, the study was well conducted, in compliance with GLP and thus, can provide useful information for the assessment of the metabolites of glyphosate. The study is therefore considered valid.

Dosing of Sprague-Dawley rats orally by gavage for 4 weeks with AMPA did not produce in-life signs of toxicity. Organ weight analysis revealed a slight equivocal increase in kidney weight after covariance analysis at 350 and 1000 mg/kg bw/day in males.

There were no toxicologically significant effects seen at 10 or 100 mg/kg bw/day. Based on the slightly higher kidney weight in male rats, the NOAEL for AMPA can be set at 100 mg/kg bw/day in this dose range finding study.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.8.1/015
Report author	[redacted]
Report year	1991
Report title	One Month Study of AMPA Administered by Capsule to Beagle Dogs
Report No	[redacted]-11127
Document No	NA
Guidelines followed in study	US EPA Pesticide Assessment Guidelines, Subdivision F, FIFRA, Hazard Evaluation: Human and Domestic Animals, Section 82-1 and also the OECD Toxicity Test Guidelines (ISBN 92-64-12221-4, Section 409; 1981)
Deviations from current test guideline (OECD 409, 1998)	Ophthalmology not performed. Urinalysis not investigated. The following organ weights were not determined: Gall bladder, epididymides, ovaries, uterus and thymus. No histopathological examination of relevant tissues performed (all gross lesions, liver with gall bladder, kidneys, adrenals, testes, epididymides, ovaries, uterus, thyroid (with parathyroids), thymus, spleen, brain, heart, pituitary, eyes, oesophagus, salivary glands, stomach, small and large intestines, pancreas, trachea and lungs, aorta, accessory sex organs, female mammary gland, prostate, lymph nodes, peripheral nerve and bone marrow).
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised	Yes

testing facilities	
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary
Executive summary

AMPA was administered by capsule to groups of 2 beagle dogs/sex at dosages of 0, 10, 30, 100, 300 or 1000 mg/kg bw/day. Clinical observations were performed at least weekly with additional clinical signs documented by exception. Body weights and food consumption were determined weekly. Clinicopathologic examinations including haematology and clinical blood chemistry were performed at termination (1 month). All survivors were sacrificed at termination and given a complete necropsy (including weighing of selected organs). No tissues were retained.
Haematological changes at 100 mg/kg bw/day included decreased RBC counts, increased reticulocyte counts, decreased haemoglobin and decreased haematocrit in both sexes. Haematologic changes at 300 mg/kg bw/day included decreased reticulocyte counts, haematocrit and haemoglobin in females. These changes were indicative of a mild to moderate anaemia which was of undetermined aetiology.
No other signs of toxicity were observed in this study.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	AMPA (Aminomethylphosphonic acid) / EHL Substance Identification Code: T900031
Description:	White powder
Lot/Batch #:	HET-9001-1463T
Purity:	94.38 %
Stability of test compound:	Not reported.
2. Vehicle and/or positive control:	Empty gelatine capsule / none
3. Test animals:	
Species:	Dog
Strain:	Beagle
Source:	[redacted]
Age:	Approx. 6 months
Sex:	Male and female
Weight at dosing:	♂ 7.3 – 9.8 kg; ♀ 5.9 – 8.4 kg
Acclimation period:	41 days
Diet/Food:	Purina Mills Certified Canine Diet Meal #5007 (offered 1 – 2 hours each day)
Water:	Tap water, *ad libitum*
Housing:	Individual housing in stainless steel cages

Environmental conditions:	Temperature:	Not reported
	Humidity:	Not reported
	Air changes:	Not reported
	Light cycle:	Not reported

B. STUDY DESIGN AND METHODS

In life dates: 1990-04-04 (start of dosing) to 1990-05-03 (terminal necropsies)

Animal Assignment and Treatment:

AMPA was administered to groups of 2 beagle dogs/sex at dosages of 0, 10, 30, 100, 300 or 1000 mg/kg bw/day.

Neat test material was put into gelatine capsules and administered orally.

Table 5.8.1-5.8.1-26: One Month Study of AMPA Administered by Capsule to Beagle Dogs ([redacted], 1991): Study design

Group Number	Dose Group [mg AMPA/kg bw/day]	Number of Animals	
		Males	Females
1	0	2	2
2	10	2	2
3	30	2	2
4	100	2	2
5	300	2	2
6	1000	2	2

No analysis was performed to verify identity, purity or stability or otherwise characterise the test material.

In-life Observations

Checks for mortality, moribundity and noteworthy signs of toxicity were performed twice daily (AM and PM; documented by exception). Detailed observations for signs of toxicity were performed once weekly.

Body Weight

Body weights were recorded prior to randomisation and once weekly thereafter.

Food Consumption

Food consumption was analysed on a weekly basis by extrapolation from four or five-day determination of actual food consumption each week.

Ophthalmoscopic Examination

Not performed.

Clinical Pathology

At termination, all animals had blood samples collected from the jugular vein for haematology and blood chemistry determinations.

Haematology

The following parameters were evaluated: Total erythrocyte count, total leukocyte count, platelet count, haematocrit, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and activated partial thromboplastin time (APTT).

For leukocyte differential, blood smears were prepared on glass slides, stained with Wright's stain, and examined microscopically.

For reticulocyte count, a portion of the EDTA-treated blood sample was mixed with vital stain (methylene blue) and a slide was prepared and examined microscopically.

Blood Chemistry
The following parameters were evaluated: Albumin, total protein, blood urea nitrogen, total bilirubin, direct bilirubin, glucose, glutamic pyruvic transaminase, alkaline phosphatase, glutamic oxaloacetic transaminase, gamma glutamyl transpeptidase, creatinine, cholesterol, calcium, phosphorus, chloride, sodium, potassium and globulin.

Gross Pathology
All animals were subjected to an external and internal examination. Internal cavities were opened and the organs were examined in situ and then removed. Hollow organs were opened and examined.

Organ Weights
The following organs were weighed: Adrenals, brain, heart, kidneys, liver, spleen, testes and thyroids.
No tissues were retained or fixed.

Statistics
Because there were only two animals of each sex in each treatment group, statistical analysis often could not be performed or was inappropriate. When it was, the following statistical procedures were used to detect statistically significant differences between treated animals and their respective controls:

Dunnett's Multiple Comparison Test (two-tailed): In-life body weights, cumulative body weight changes, food consumption and APTT.

EHL decision tree analysis: Haematology data, clinical chemistry data, terminal body weights, absolute organ weights and organ/body weight ratios were evaluated by decision-tree statistical analyses which, depending on the results of tests for normality and homogeneity of variances, chose either parametric or nonparametric routines to detect differences and analyse for trend.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no unscheduled deaths in this study.

B. CLINICAL OBSERVATIONS
Diarrhoea was observed in both males and one female at 100 mg/kg bw/day (total of 18 incidents) and emesis was observed in both females and one male at 1000 mg/kg bw/day (total of 4 incidents).

C. BODY WEIGHT
There were no effects on body weight.

D. FOOD CONSUMPTION
There were no effects on food consumption.

E. CLINICAL PATHOLOGY

Haematology
Haematologic changes at 1000 mg/kg bw/day included decreased red blood cell counts, increased reticulocytes, decreased haemoglobin and decreased haematocrit in both sexes. Haematologic changes at 300 mg/kg bw/day included decreased reticulocyte counts, haematocrit and haemoglobin in females.
Statistically significant decreases in APTT in females at all dose levels were minor in magnitude and were not considered toxicologically significant.

Table 5.8.1-5.8.1-27: One Month Study of AMPA Administered by Capsule to Beagle Dogs ([redacted], 1991): Intergroup comparisons of selected group mean haematology parameters

Dose Group [mg/kg bw/day]	Group Mean Haematology Parameters				
	Total Erythrocyte Count [10^6/mm^3]	Reticulocytes [10^3/mm^3]	Haemoglobin [g/dL]	Haematocrit [%]	APPT [s]
	Males				
0	6.3200 ± 0.1131	31.8000 ± 22.9103	14.4000 ± 0.9899	41.3000 ± 2.9689	15.9 ± 0.4
10	6.0350 ± 0.0778	22.7825 ± 23.7623	14.5000 ± 0.1414	41.4000 ± 0.7071	15.9 ± 0.4
300	6.2600 ± 0.3818	55.5425 ± 73.9315	14.8500 ± 0.9192	42.4000 ± 2.6870	16.8 ± 0.6
100	6.8150 ± 0.1626	18.9425 ± 17.3135	15.5000 ± 0.4243	44.7500 ± 1.3435	16.8 ± 0.6
300	6.0750 ± 0.4031	21.1200 ± 2.8850	14.4500 ± 1.3435	40.9500 ± 3.7477	15.4 ± 0.4
1000	**5.2000* ± 0.0424**	71.3725 ± 30.6708	12.5000 ± 0.1414	35.4000 ± 0.7071	15.0 ± 0.6
	Females				
0	6.3600 ± 0.4384	22.1050 ± 2.9628	15.6500 ± 0.7778	44.1000 ± 1.9799	17.4 ± 0.0
10	6.7300 ± 0.0283	20.1500 ± 18.9505	15.7000 ± 0.0000	44.8500 ± 0.4950	**16.1* ± 0.1**
30	6.5600 ± 0.1838	23.0900 ± 9.9207	15.2500 ± 0.3536	43.5000 ± 1.2728	**15.7** ± 0.4**
100	6.1200 ± 0.4667	23.3625 ± 12.5688	14.6000 ± 0.0000	41.8500 ± 0.0707	**16.1* ± 0.1**
300	5.6500 ± 0.0000	7.0625 ± 1.9976	**13.6000* ± 0.5657**	**38.5500* ± 1.3435**	**16.0* ± 0.0**
1000	**5.0500* ± 0.1838**	**101.4550** ± 28.8732**	**12.1000** ± 0.8485**	**34.2500** ± 2.1920**	**15.3** ± 0.7**

*: Statistically significant from controls, p<0.05; **: Statistically significant from controls, p<0.01

Blood chemistry

There were no effects on blood chemistry parameters.

G. GROSS PATHOLOGY

There were no effects on organ weights and there were no necropsy observations which were considered related to administration of the test material.

3. Assessment and conclusion

Assessment and conclusion by applicant:

AMPA was administered orally (via capsule) to groups of 2 beagle dogs/sex at dosages of 0, 10, 30, 100, 300 or 1000 mg/kg bw/day for a period of one month.
The study was conducted according to OECD 408 (1981) and in compliance with GLP. However, there were major deviations to current standards: no ophthalmology, no urinalysis, no histopathology.
Apart from these major deviations, the study was well conducted and can provide supplemental information for the assessment of the metabolites of glyphosate. The study is therefore considered to be only supplementary.
There were no mortalities or treatment related effects upon clinical observations, body weight, food

consumption, blood chemistry, organ weights or necropsy in either sex.
At 1000 mg/kg bw/day decreased erythrocyte counts, increased reticulocyte counts and decreased haematocrit was observed in both sexes.
At 300 mg/kg bw/day decreased reticulocyte counts, haematocrit and haemoglobin was observed in females. These changes were indicative of mild to moderate anaemia of undetermined aetiology.
No other signs of toxicity were observed in this study.
Under the conditions of this study, the No-Observed-Adverse-Effect-Level (NOAEL) was considered to be 300 mg/kg bw/day in male and 100 mg/kg bw/day in female Beagle dogs, respectively.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.8.1/016
Report author	[redacted]
Report year	1993
Report title	13 Week Toxicity Study in Rats with Administration by Gavage
Report No	7866
Document No	152-GLY
Guidelines followed in study	US EPA Pesticide Assessment Guidelines Subdivision, FIFRA, 82-1, in general accordance with OECD 408 (1981)
Deviations from current test guideline (OECD 408, 2018)	Functional observation and/or sensor reactivity assessments were not performed. Platelet count not included within haematology parameters. Total cholesterol, HDL, LDL and urea were not evaluated as part of clinical chemistry parameters. In addition, only 2 liver enzymes (AST and ALT) were measured. T4, T3 and TSH were not measured. Vaginal smears for oestrus cycle determination of females not performed at necropsy.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive summary

Groups of 10 male and 10 female Sprague-Dawley rats were dosed orally each day for 13 weeks with AMPA (aminomethylphosphonic acid) at dose levels of 0, 10, 100 or 1000 mg/kg bw/day via a steel dosing cannula.

Blood samples were taken from all animals during Week 13 for investigations of haematology and clinical chemistry parameters. An ophthalmoscopic examination was undertaken on all animals during pre-trial and on all Control and High dose animals during Week 12.
On completion of 13 weeks dosing all surviving animals were killed and necropsied. Premature decedents were also necropsied. Histological examination was carried out on a full list of organs from all Control and

High dose animals and all premature decedents. In addition, histological examination was carried out on the kidneys, liver, lungs, submaxillary salivary gland, sublingual salivary gland and parotid salivary gland of all other animals.

There were 4 premature deaths (one intermediate dose male, one low dose female and two intermediate dose females). None of these deaths were due to administration of AMPA.

Clinical Signs:	There were no notable clinical signs.
Body Weight:	There were no notable intergroup differences.
Food Consumption:	There were no notable intergroup differences.
Water Consumption:	There were no notable intergroup differences.
Haematology:	There were no notable intergroup differences.
Clinical Chemistry:	There were no notable intergroup differences.
Organ weights:	There were no notable intergroup differences.
Necropsy Findings:	There were no necropsy findings which were considered to be due to treatment with AMPA.
Histological Findings:	There were no histological findings which were considered to be due to treatment with AMPA.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	AMPA (aminomethylphosphonic acid)
Description:	White Powder
Lot/Batch #:	286-JRJ-73-4
Purity:	99.2 %
Stability of test compound:	The test substance is stable at least 3 years from the date of analysis at ambient temperature in the dark.

2. Vehicle and/or positive control: 0.5 % Carboxymethylcellulose (CMC) in distilled water / none

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	approx. 4 weeks
Sex:	Male and female
Weight at dosing:	♂ 144 – 168 g; ♀ 106 – 129 g
Acclimation period:	13 days
Diet/Food:	Rat and Mouse (modified) No. 1 Diet SQC (pelleted), *ad libitum,* (except for laboratory investigation sampling during Week 13 where animals were deprived of food overnight)
Water:	Tap water, *ad libitum*
Housing:	2 of one sex per cage in polypropylene cages with stainless steel wire grid tops and bottoms (420 × 270 × 200 mm).

Environmental conditions: Temperature: 20 °C
Humidity: 50 ± 15 %
Air changes: 15 – 20 / hour
12 hours light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 1992-04-16 (start of dosing) to 1992-07-17 (terminal necropsies)

Animal assignment and treatment:
In a 13 week oral toxicity study groups of 10 Sprague-Dawley rats per sex received daily doses of 0, 10, 100 or 1000 mg/kg bw/day, via gavage, at a dose volume of 10 mL/kg bw. The dose formulations were prepared fresh daily using 0.5 % carboxymethylcellulose (CMC) in distilled water as vehicle. Samples of formulations for all dosing groups (including Control) were analysed during Weeks 1, 6 and 13 of dosing for the concentration of test item in the suspension.

Table 5.8.1-28: 13 Week Toxicity Study in Rats with Administration by Gavage ([redacted], 1993): Study design

Test group	Dose Group [mg AMPA/kg bw/day]	Animal Numbers	
		Males	Females
Control	0	1 – 10	41 – 50
Low	10	11 – 20	51 – 60
Intermediate	100	21 – 30	61 – 70
High	1000	31 – 40	71 – 80

Mortality
All animals were checked for viability/mortality early each morning an as late as possible each day.

Clinical observations
All animals were examined for signs of reaction to treatment each day. All animals received a detailed clinical examination once each week.

Body weight
The body weight of each animal was recorded once each week before treatment and weekly from the start of treatment until the end of the study.

Food consumption
The quantity of food consumed by each cage of animals was recorded once each week before treatment and weekly from the start of treatment until the end of the study.

Water consumption
Water consumption was monitored by visual inspection throughout the treatment period.

Ophthalmoscopic examination
The eyes of all animals from all dose groups were examined during pretrial and of all Control and High dose animals during Week 12 of dosing. Ophthalmoscopic examinations of the anterior, lenticular and fundic areas were evaluated.

Haematology and clinical chemistry
Samples for laboratory investigation were taken from all animals during Week 13 of dosing after overnight deprivation of food. Samples for haematology were measured on whole blood taken into tubes containing EDTA. Samples for clinical chemistry were measured from plasma of whole blood taken into heparin. The

following haematological parameters were measured: Haemoglobin (Hb), red blood cell count, haematocrits, white blood cell count, clotting time, mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), neutrophils, lymphocytes, monocytes, eosinophils, basophils and large unstained cells.

For clinical chemistry analysis the following parameters were measured: blood urea nitrogen, glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), sodium, potassium, chloride, total protein, albumin, albumin/globulin ratio, creatinine, calcium, phosphate and total bilirubin.

Terminal studies

After 13 weeks of consecutive treatment, all surviving animals were killed by carbon dioxide asphyxiation followed by exsanguination. Gross dissection and necropsy were performed under the supervision of a pathologist.

The following organs were weighed, fixed and examined histologically: Liver, heart, kidneys, lung, spleen, adrenal glands, thymus, testes, ovaries, prostate, uterus, brain, submaxillary (mandibular) salivary gland, sublingual salivary gland, parotid salivary gland and pituitary.

The following organs were fixed and examined histologically: Seminal vesicles, vagina, cervix uteri, spinal cord, thigh muscle, pancreas, submandibular lymph node, skin and mammary gland, urinary bladder, eyes, optic nerves, tongue, aortic arch, mesenteric lymph node, thyroids, parathyroids, trachea, oesophagus, stomach, duodenum, jejunum, ileum, caecum, colon, sciatic nerve, sternum and rib and any abnormal tissue.

The following organs were fixed: Rectum, smooth muscle (large intestine), nasal cavity and ears.

The above tissues were fixed in 10 % neutral buffered formalin. Lungs were fixed in their entirety by perfusion with 10 % buffered formalin. Testes were weighed with epididymides. Submaxillary and sublingual salivary glands were weighed together. Contracted bladders were distended with fixative with the epithelial surface examined after fixation. Optic nerves and eyes were fixed in Davidson's fluid. Thyroids (with parathyroids) were weighed after fixation. Ears were fixed for identification purposes.

Histological evaluation was performed on all animals from the Control and High dose groups in addition to any premature decedents. In addition, kidneys, livers, lungs, submaxillary salivary glands, sublingual salivary glands and parotid salivary glands were examined from the Low and Intermediate dose groups.

The stomach and intestines were opened at necropsy and the mucosal surface examined. Carcasses of all animals were discarded immediately following necropsy and placing of all tissues in fixative as identified above.

Statistics

Body weight, haematology, clinical chemistry and organ weight data were statistically analysed for homogeneity of variance using the F-max test. If group variances appeared homogeneous a parametric ANOVA was used and pairwise comparisons made via Student's t-test using Fisher's F-protected LSD. If variances were heterogeneous log or square root transformations were used in an attempt to stabilise the variances. If variances were still heterogeneous a non-parametric test such as a Kruskal-Wallis ANOVA was used.

Organ weights were also analysed conditional on body weight (i.e. analysis of covariance).

Histological data were analysed using Fisher's Exact Probability test.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were 4 premature decedents (one Intermediate dose male, one Low dose female and 2 Intermediate dose females). Three of these animals were killed due to eye damage which occurred during the Week 13 orbital sinus bleed and one animal died during the bleed. None of the deaths were due to treatment with AMPA.

B. CLINICAL OBSERVATIONS

There were no notable clinical signs in either sex.

C. BODY WEIGHT

There were no notable intergroup differences in male animals.

A slight reduction was noted in the overall group mean body weight gain (16 %) of Low dose females (see table below). Comparison with Controls did not reveal any statistical significance at any time interval. Due to the lack of any effects observed in the Intermediate or High dose group females, this reduction was considered not to be related to treatment with AMPA.

Table 5.8.1-29: 13 Week Toxicity Study in Rats with Administration by Gavage ([redacted], 1993): Intergroup comparison of group mean body weights and body weight gain

	Mean body weight or body weight gain [g]		
Time point	**Initial body weight**	**Final body weight**	**Total weight gain Time 0 – Week 13**
Dose [mg/kg bw/day]	**Males**		
0	159	512	353
10	157	495	338 (96 % of Control)
100	153	488	335 (95 % of Control)
1000	155	500	345 (98 % of Control)
	Females		
0	115	308	193
10	118	281	163 (84 % of Control)
100	118	297	179 (93 % of Control)
1000	116	298	182 (94 % of Control)

D. FOOD CONSUMPTION

There were no notable intergroup differences for food consumption in either sex.

E. WATER CONSUMPTION

There were no notable intergroup differences for water consumption in either sex.

E. OPHTHALMOSCOPY

All animals examined were considered normal.

G. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

In males, MCH was slightly increased in the Intermediate dose group (5 %, $p<0.05$). Due to the small magnitude of difference and absence of any effects observed in the High dose group, this change was considered not to be treatment-related.

In females, MCV was slightly reduced in the Low and Intermediate dose groups (both 3 %, $p<0.05$). However, due to the small magnitude of difference and absence of effects observed in the High dose group or in males, these changes were considered not to be related to treatment. In addition, Neutrophils were

increased (66 %, p<0.01) in Low dose group females, however, due the absence of any effects observed in the Intermediate or High dose groups this change was considered not to be related to treatment.

Table 5.8.1-30: 13 Week Toxicity Study in Rats with Administration by Gavage ([redacted], 1993): Intergroup comparison of selected group mean haematology parameters (mean ± SD)

Parameter	Dose Groups [mg AMPA/kg bw/day]							
	Males				Females			
	0	10	100	1000	0	10	100	1000
MCH [pg]	17.5 ± 0.9	17.4 ± 0.4	**18.3* ± 0.6**	17.6 ± 1.5	19.2 ± 0.8	18.4 ± 0.5	18.6 ± 0.5	18.8 ± 0.5
MCV [fL]	49.0 ± 2.5	48.8 ± 1.3	50.4 ± 1.6	49.4 ± 3.7	52.3 ± 1.6	**50.7* ± 1.0**	50.9* ± 1.1	51.9 ± 1.8
Neutrophils [× 10^9/L]	2.23 ± 1.00	2.78 ± 1.50	2.25 ± 0.98	3.17 ± 1.39	0.98 ± 0.38	**1.63** ± 0.42**	1.06 ± 0.49	0.87 ± 0.35

*: Statistically significant from controls, p<0.05; **: Statistically significant from controls, p<0.01

Clinical chemistry

There were no notable intergroup differences in males.

In females, ALT was increased in the Intermediate dose group (45 %, p<0.05). Due to an absence of any effect observed in the High dose group this increase was considered not to be treatment-related.

Table 5.8.1-31: 13 Week Toxicity Study in Rats with Administration by Gavage ([redacted], 1993): Intergroup comparison of selected group mean clinical chemistry parameters (mean ± SD)

Parameter	Dose Groups [mg AMPA/kg bw/day]							
	Males				Females			
	0	10	100	1000	0	10	100	1000
ALT [iu/L]	46 ± 10	50 ± 7	53 ± 27	38 ± 4	31 ± 6	35 ± 7	**45* ± 18**	30 ± 11

*: Statistically significant from controls, p<0.05; **: Statistically significant from controls, p<0.01

H. TERMINAL STUDIES

Organ weights

In males, thyroid weight was decreased in the Low dose group after correction for final body weight (13 %, p<0.01). Due to an absence of effects observed in the Intermediate or High dose groups, this decrease was considered not to be related to treatment.

In females, ovary weight was reduced in Low and Intermediate dose groups when expressed as an absolute value (14 %, p<0.05 and 17 %, p<0.05, respectively) and after correction for final body weight (14 %, p<0.05 and 17 %, p<0.05, respectively). Uterus weight was increased in Low and Intermediate dose groups when expressed as an absolute value (31 %, p<0.05 and 36 %, p<0.05, respectively) and after correction for final body weight (42 %, p<0.05 and 40 %, p<0.01, respectively). Due to an absence of any effect in the High dose group the above findings were considered not to be related to treatment. Thyroid weight was reduced in the Low dose group when expressed as an absolute value (19 %, p<0.05). Since this change was not seen after correction for final body weight and due to the lack of effect in the Intermediate or High dose groups, this reduction is considered to be due to chance. Brain weight was slightly reduced (4 %, p<0.05) and heart weight was increased (18 %, p<0.05) in the High dose group after correction for final body weight. The increase in heart weight was considered to be associated with one animal with myocardial hypertrophy which showed a marked increase in heart weight.

Table 5.8.1-32: 13 Week Toxicity Study in Rats with Administration by Gavage ([redacted], 1993): Intergroup comparison of selected absolute and relative mean organ weights

Organ/Tissue [g]	Dose Groups [mg AMPA/kg bw/day]							
	Males				Females			
	0	10	100	1000	0	10	100	1000
Thyroids (corrected for body weight; ± SE)	0.023 ± 0.001	**0.020** ± 0.001**	0.022 ± 0.001	0.023 ± 0.001	0.020 ± 0.001	0.018 ± 0.001	0.020 ± 0.001	0.020 ± 0.001
Ovaries (absolute, ± SD)	-	-	-	-	0.105 ± 0.013	**0.090*** ± 0.012	**0.087**** ± 0.014	0.101 ± 0.014
Ovaries (corrected for body weight; ± SE)	-	-	-	-	0.105 ± 0.004	**0.090*** ± 0.004	**0.087**** ± 0.005	0.103 ± 0.004
Uterus (absolute; ± SD)	-	-	-	-	0.55 ± 0.14	**0.72*** ± 0.23	**0.75*** ± 0.23	0.57 ± 0.11
Uterus (corrected for body weight; ± SE)	-	-	-	-	0.54 ± 0.05	**0.75*** ± 0.06	**0.75*** ± 0.06	0.56 ± 0.06
Brain (corrected for body weight; ± SE)	2.11 ± 0.02	2.12 ± 0.02	2.10 ± 0.02	2.11 ± 0.02	1.96 ± 0.02	1.97 ± 0.03	1.97 ± 0.03	**1.88* ± 0.03**
Heart (corrected for body weight; ± SE)	1.46 ± 0.04	1.51 ± 0.04	1.61 ± 0.04	1.54 ± 0.04	1.03 ± 0.05	1.04 ± 0.05	1.07 ± 0.06	**1.22**** ± 0.05

*: Statistically significant from controls, p<0.05; **: Statistically significant from controls, p<0.01

Gross pathology
There were no necropsy findings which were considered to be due to treatment with AMPA. All findings were typical of background pathology for this age and strain of rat. For some animals lesions associated with the right eye were noted, however, these were considered to be due to the blood sampling process.

Histopathology
There were no histopathological findings related to treatment. Those findings reported were typical of background pathology for rats of this age and strain.

III. CONCLUSIONS

There were no findings considered to be related to treatment with AMPA.
Brain weight was slightly reduced and heart weight increased in females receiving 1000 mg/kg bw/day. Due to the lack of any correlating findings, these changes were considered not to be reproducible.
In conclusion, dosing of Sprague-Dawley rats orally by gavage for 13 weeks with AMPA produced no effects at 10, 100 or 1000 mg/kg bw/day.

3. Assessment and conclusion

Assessment and conclusion by applicant:

In this study groups of 10 male and 10 female Sprague-Dawley rats were dosed orally for 13 weeks with AMPA at dose levels of 0, 10, 100 or 1000 mg/kg bw/day via gavage. The study was conducted according to OECD 408 (1981) and in compliance with GLP (no attest of the competent authority was provided). Deviations from the current version of OECD 408 (2018) are basically due to the fact that the study was aligned to an older version of the OECD test guideline 408.
However, the study was well conducted, in compliance with GLP and OECD 408 (1981) and thus, can provide useful information for the assessment of the metabolites of glyphosate. The study is therefore considered valid.
There were no findings considered to be related to treatment with AMPA. Brain weight was slightly

reduced and heart weight increased in females receiving 1000 mg/kg bw/day. Due to the lack of any correlating findings, these changes were considered not to be reproducible. In conclusion, dosing of Sprague-Dawley rats orally by gavage for 13 weeks with AMPA produced no effects at 10, 100 or 1000 mg/kg bw/day. Thus, under the conditions of this study the NOAEL can be set at 1000 mg/kg bw/day.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.8.1/017
Report author	[redacted]
Report year	1979
Report title	90-Day Subacute Rat Toxicity Study
Report No	401-050
Document No	M-644184-01-1
Guidelines followed in study	No guideline followed but similar to OECD 408.
Deviations from current test guideline (OECD 408, 2018)	Ophthalmoscopy not performed. Functional observation and/or sensor reactivity assessments were not performed. Blood clotting time/potential not included within haematology parameters. Sodium, potassium, HDL, LDL, urea and creatinine were not evaluated as part of clinical chemistry parameters. T4, T3 and TSH were not measured. Vaginal smears for oestrus cycle determination of females not performed at necropsy. Adrenals, epididymides, prostate + seminal vesicles, uterus, thymus, spleen, thyroid and pituitary were not weighed at necropsy. Aorta, cervix, vagina, epididymides, seminal vesicles, coagulation glands, mammary gland and skin were not preserved / fixed. In addition, only one lymph node (mesenteric) was sampled and fixed. Histological assessment of the testes may not be as detailed as current guideline. Identification of the test chemical was limited.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No formal claim of compliance with GLP or specific guidelines since the study was performed pre-GLP.
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive summary

The test material (CP 50435) was administered in the diet to Charles River CD® weanling rats at dosage levels of 400, 1200 or 4800 mg/kg bw/day for 90 days. Each dosage level was fed to a group of 20 male and 20 female rats. The control group, containing a like number of rats, was fed only the basal laboratory diet. Water and respective diets were available *ad libitum*. The rats were observed twice daily for mortality and signs of overt toxicity. Detailed observations, individual body weights and food consumption were recorded weekly. Haematological and biochemical determinations and urinalyses were conducted at 45 and 88 days of study on 10 rats from each sex and group. Baseline values of the clinical laboratory tests were

obtained from 10 male and 10 female weanling rats sacrificed for this purpose.

No changes considered to be related to the compound were seen in general behaviour, appearance, haematological determinations and urinalyses. A moribund male rat in the control group was sacrificed in week 12. Nine rats from the control and treated groups died following the collection of blood at 45 and 88 days. The male rats at the 4800 mg/kg bw/day dosage level consumed less food and gained less body weight than the control. The difference in mean body weight between the control rats and male and female rats at the 4800 mg/kg bw/day dosage level reached statistical significance.

The lactic dehydrogenase activity (LDH) of the blood from rats at the 4800 mg/kg bw/day dosage level was higher than that of the control rats. For male rats at the 4800 mg/kg bw/day dosage level, the mean LDH activity was 48 and 145 % greater than the control value at 45 and 88 days, respectively. Similarily for the female rats at the 4800 mg/kg bw/day dosage level, the mean LDH activity was 29 and 171 % greater than the control values at 45 and 88 days, respectively.

All rats which died or were sacrificed in extremis during the course of study or which were sacrificed at scheduled termination were necropsied. A full set of tissues as specified in the protocol was examined microscopically from the control and 4800 mg/kg bw/day groups. Liver, kidneys, heart, urinary bladder and gross lesions were examined microscopically in rats from the 400 and 1200 mg/kg bw/day groups. No compound related gross pathologic lesions were observed at necropsy in any rats from the test compound (CP 50435) treated groups. Statistical variations, of undetermined biological significance, occurred in male and female group mean weights of kidneys, gonads, heart and brain at one or more dosage levels.

Microscopic changes which were considered compound related were limited to the urinary tract of rats from the 4800 and 1200 mg/kg bw/day dose groups. Several rats from the 1200 mg/kg bw/day groups and most rats from the 4800 mg/kg bw/day groups had hyperplasia of the urinary bladder epithelium. Very slight to slight hyperplasia of the epithelium of the kidney pelvis in several rats from the 4800 mg/kg bw/day group also was considered to be compound-related. No compound related urinary tract lesions were observed at the 400 mg/kg bw/day level.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	CP 50435 (Aminomethylphosphonic acid)
Description:	Grainy white crystalline powder/material
Lot/Batch #:	XHI 45 and XHI-136
Purity:	99.96 % (Lot No. XHI-136)
Stability of test compound:	Unknown
2. Vehicle and/ or positive control:	Purina® Laboratory Chow® / none
3. Test animals:	
Species:	Rat
Strain:	Charles River CD®
Source:	[redacted]
Age:	Approx. 4 weeks
Sex:	Male and female
Weight at dosing:	♂ 70 – 93 g; ♀ 67 – 90 g

Acclimation period:	7 days	
Diet/Food:	Purina® Laboratory Chow®, *ad libitum* (except during laboratory investigations for Day 45 and 88)	
Water:	Tap water, *ad libitum* (except during laboratory investigations for Day 45 and 88)	
Housing:	Individual housing in hanging wire-mesh cages	
Environmental conditions:	Temperature:	Not reported
	Humidity:	Not reported
	Air changes:	Not reported
	Light cycle:	12 hours light / 12 hours dark

B. STUDY DESIGN AND METHODS

In life dates: 1978-06-27 (start of dosing) to 1978-09-26 (terminal necropsies)

Animal assignment and treatment:
The test material (CP 50435) was fed in the diet at varying concentrations (based upon changes in body weight and food consumption) to provide the dosage levels indicated below:

Table 5.8.1-33: 90-Day Subacute Rat Toxicity Study ([redacted]-78-174) ([redacted], 1979): Treatment Groups

Test group	Dose Group [mg AMPA/kg bw/day]	Number of Rats	
		Males	Females
Control	0	20	20
Low	400	20	20
Intermediate	1200	20	20
High	4800	20	20

The test compound was added to ground Purina® Laboratory Chow® on a weight-to-weight basis. The appropriate amount of the test compound for each dosage level was ground with a portion of the basal diet using a mortar and pestle. This premix was combined with the remaining portion of the basal diet in a twin-shell blender. Initially, as indicated in the protocol, 6000 g of the test diet were prepared weekly and unused diets were discarded at the end of each week. Because of the shortage of test material the total amount of test diets prepared was reduced at week 10. Also, because of the shortage of test material the food consumption periods were adjusted as follows: On day 7 of study week 11, the weight of the food container and remaining food was recorded for each rat and containers and food returned to the animal cage. On day 1 of week 12, the food jars for the male rats were weighed and fresh diets were administered to them. On day 2 of week 12, the food jars for the female rats were weighed and fresh diets administered to them. Because of this, the food consumption data for week 12 was summarised in 2 parts. For the food consumption calculations of the periods ending on day 1 of week 12 for the male treatment groups and on day 2 of week 12 for the female treatment groups the body weights recorded on day 7 of week 11 were used.

Diet Samples
Samples of stored test materials were taken at the beginning of the study and at monthly intervals. Weekly samples of the control feed and each treatment diet was taken on day 1 of feeding. Additional containers of the diets were placed in empty cages at the beginning of each feeding week and sampled at the end of the week. All diet samples were frozen immediately after collection. The samples were shipped to the sponsor at monthly intervals.

Mortality
The animals were observed twice a day 7 days a week for mortality and signs of overt toxicity.

Clinical observations
Detailed observations of each rat were recorded weekly.

Body weight
Individual body weights were recorded weekly.

Food consumption
Individual food consumption was recorded weekly.

Haematology and clinical chemistry and urinalysis
10 male and 10 female rats from each dose level were selected for clinical laboratory investigations at 45 and 88 days of study. The baseline data of the clinical laboratory tests were obtained from 10 male and 10 female weanling rats similarly selected and sacrificed for this purpose. Blood samples were obtained by the orbital sinus technique from fasted rats. The urine was collected while the rats were fasting.

Haematology parameters included: Haematocrit value, haemoglobin concentration, erythrocyte count, leucocyte count (total and differential), platelet count and reticulocyte count.

Biochemistry parameters included: Fasting blood glucose, blood urea nitrogen, total cholesterol, total protein, albumin, globulin (calculated), direct and total bilirubin, serum alkaline phosphatase (SAP), serum lactic dehydrogenase (LDH), serum glutamic oxalacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT).

Urinalysis included description of colour and appearance, determination of volume, specific gravity and pH, qualitative tests for glucose, protein, ketones, bilirubin, urobilinogen and microscopic examination of sediment.

Terminal studies
At completion of the compound feeding period, all surviving rats were sacrificed by carbon dioxide asphyxiation and necropsied. At necropsy, examination was made of the external body surface and orifices and the rat was opened. Contents of the cranium, thorax and abdomen were examined *in situ*, removed, and again examined. Representative tissues of organs from each rat were collected and fixed in buffered neutral 10 % formalin. Rats which died or were sacrificed in extremis during the course of the study were necropsied as above.

The liver, kidneys, testes, heart and brain from each rats sacrificed at termination were weighed at necropsy and ovaries were weighed after fixation.

Haematoxylin and eosin stained paraffin sections of the following tissues from all rats from the control and 4800 mg/kg bw/day groups were prepared by standard histologic methods and examined microscopically: Brain, spinal cord, peripheral nerve (sciatic), eye (optic nerve), pituitary, thyroid (parathyroid), adrenals, trachea, lung/bronchi, heart, spleen, lymph node (mesenteric), thymus, sternum (bone marrow), salivary gland (submandibular), oesophagus, stomach, small intestine (duodenum, jejunum, ileum), large intestine (colon, caecum), pancreas, liver, kidneys, urinary bladder, testes/ovaries, prostate/uterus, skeletal muscle and any tissues with gross lesions.

Haematoxylin and eosin stained paraffin sections of kidneys, liver, heart, urinary bladder and tissues with gross lesions were prepared and examined from all rats from the 400 and 1200 mg/kg bw/day feeding groups.

Statistics

All statistical analyses compared the treatment groups with the control group, by sex.
Body weights (week 13), food consumption (week 13) haematological, biochemical and urinalysis parameters (days 45 and 88) and absolute and relative organ weights (terminal), were compared by analysis of variance (one-way classification), Bartlett's test for homogeneity of variances and the appropriate t-test (for unequal variances) as described by Steel and Torrie using Dunnett's multiple comparison tables to judge significance of differences.

II. RESULTS AND DISCUSSION

A. GENERAL BEHAVIOUR, APPEARANCE AND SURVIVAL

One male and five female rats at the 4800 mg/kg/day dosage level were found dead on day 45 following the collection of blood. Similarly, one female in the control group and two female rats in the 1200 mg/kg bw/day group were found dead after the collection of blood on study day 88. In addition, one male rat in the control group was sacrificed in week 12. Previous observation included soft stools, distended abdomen and morbidity. Survival after 90 days of study was a follows:

Table 5.8.1-5.8.1-34 90-Day Subacute Rat Toxicity Study ([redacted]-78-174) ([redacted], 1979): Intergroup comparison of group survival

Dose Level [mg/kg bw/day]	Surviving Males / No. Initiated	Surviving Females / No. Initiated
0 (control)	19/20	19/20
400	20/20	20/20
1200	20/20	18/20
4800	19/20	15/20

B. BODY WEIGHT

For both male and female rats at the 4800 mg/kg bw/day dosage level and male rats at the 1200 mg/kg bw/day dosage level, the differences in body weight from that of the control were statistically significant at 13 weeks (see table below).

Table 5.8.1-35: 90-Day Subacute Rat Toxicity Study ([redacted]-78-174) ([redacted], 1979): Intergroup comparison of group mean body weights and body weight gain

Dosage Group [mg/kg bw/day]	Mean body weight or body weight gain [g]		
	Initial body weight	Final body weight	Total weight change [%] Time 0 – Week 13
	Males		
0 (control)	82	480	485
400	80	480	500
1200	80	**453***	466
4800	81	**346****	327
	Females		
0 (control)	79	264	234
400	76	266	250
1200	79	274	246
4800	79	**252****	219

*: Statistically significant from controls, p<0.05; **: Statistically significant from controls, p<0.01

C. FOOD CONSUMPTION

Treated male rats consumed less food than the control, although when compared with control this was not shown to be statistically significant.
In increasing dosage levels the compound consumption based on food consumption for the male rats was 406, 1230 and 4989 mg/kg bw/day and for the female rats was 388, 1161 and 4625 mg/kg bw/day, respectively.

Table 5.8.1-36 90-Day Subacute Rat Toxicity Study ([redacted]-78-174) ([redacted], 1979): Intergroup Comparison Group Average Food Consumption

Dosage Group	Average Food Consumption [g/rat/day]	
Dose [mg/kg bw/day]	**Males**	**Females**
0 (control)	25.5	17.9
400	25.1 (-1.6)	17.8 (-0.6)
1200	25.2 (-1.2)	19.2 (7.3)
4800	23.5 (-7.8)	18.3 (2.2)

(): % difference from Control

D. CLINICAL LABORATORY TESTS

Haematology
No changes considered to be related to the compound intake were seen in the haematological determinations. For as few specific determinations there were statistically significant differences between the control and some of the treated groups. Since the individual values for the rats were within the range usually observed for Charles River rats at the test laboratory, the differences were regarded as not biologically significant.

Biochemistry
For the control and treated groups the mean values for the lactic dehydrogenase activity was greater at 45 and 88 days than for the control baseline period and the lactic dehydrogenase activities for the rats at the 4800 mg/kg bw/day dosage level were considerably greater than for the other groups.

At both 45 and 88 days the activity for the male rats at the 4800 mg/kg bw/day dosage level was greater than the range of values usually observed for Charles River rats. The differences between the mean values for these male rats and the control rats were statistically significant and the effect may be regarded as dose related. Similarly, for the male rats at the 1200 mg/kg bw/day dosage level after 88 days the increase in lactic dehydrogenase activity may be dose related. By contrast, for the other groups of rats the activities were within the expected range of values.

For the female rats the assessment is complicated by the 25 % decrease in lactic dehydrogenase activity of the control for the determinations at 45 and 88 days. This brings into question the biological significance of the differences between the female control and the female rats at 400 and 1200 mg/kg bw/day for the 88 days determination, especially since the values were within normal limits. At the 4800 mg/kg bw/day dosage level the values were definitely greater than the usual range values. Although the magnitude of the effect at 88 days appeared exaggerated by the lower control values the increase in lactic dehydrogenase activity was real and may be regarded as dose related.

Five male and four female rats at the 4800 mg/kg bw/day dosage level provided blood samples at both 45 and 88 days. For all but one female rat the values at 88 days were greater than those at 45 days.
For a few other specific determinations there were statistically significant differences between the control and some of the treated rats. Since the individual values for the rats were within the range usually observed for Charles River rats at the testing laboratory the differences were not regarded as biologically significant.

Table 5.8.1-37: 90-Day Subacute Rat Toxicity Study ([redacted]-78-174) ([redacted], 1979): Intergroup comparison of selected group mean biochemistry parameters

Parameter	Group Mean Lactic Dehydrogenase Activity [B-B units/mL]							
	Male [mg/kg bw/day]				Female [mg/kg bw/day]			
	0	400	1200	4800	0	400	1200	4800
LDH (Baseline)	1645	-	-	-	1361	-	-	-
LDH (45 Days)	1847	1779 (-3.6)	1901 (2.9)	**2738** (48.2)**	1897	1926 (1.5)	1622 (-14.5)	2437 (28.5)
LDH (88 Days)	1893	1584 (-16.3)	**2442* (29.0)**	**4637** (145.0)**	1427	**2075** (45.4)**	**1971** (38.1)**	**3866** (170.9)**

*: Statistically significant from controls, p<0.05; **: Statistically significant from controls, p<0.01; (): % difference from Control

Urinalysis
No changes considered to be related to the compound intake were seen in the urinalysis.

E. TERMINAL STUDIES

Organ weights
Statistically significant variances in group mean organ weights of liver, kidneys, testes / ovaries, heart and brain were noted for males and females. In the absence of compound related morphologic parenchymal changes in these organs, the biological significance of these statistical variations was undetermined.

Table 5.8.1-38: 90-Day Subacute Rat Toxicity Study ([redacted]-78-174) ([redacted], 1979): Intergroup comparison of selected absolute and relative group mean organ weights

Organ/Tissue	Dosage Groups [mg/kg bw/day]							
	Males				Females			
	0	400	1200	4800	0	400	1200	4800
Liver (absolute) [g]	21.21	**18.94***	**18.08****	**13.90****	11.13	10.48	10.80	**9.10****
Liver (relative) [%]	4.40	4.18	4.10	**4.04***	4.32	4.12	4.07	4.07
Kidneys (absolute) [g]	4.26	3.94	3.84*	**3.27****	2.24	2.27	2.38	**2.00****
Kidneys (relative) [%]	0.89	0.87	0.87	**0.95***	0.87	0.89	0.90	0.93
Testes (absolute) [g]	3.60	3.60	3.54	**3.35****	-	-	-	-
Testes (relative) [% × 10]	0.76	0.80	0.81	**0.98****	-	-	-	-
Ovaries (absolute) [mg]	-	-	-	-	146	**114****	132	130
Ovaries [% × 10]	-	-	-	-	0.57	**0.45****	**0.50***	0.58
Heart (absolute) [g]	1.67	1.57	1.55	**1.24****	1.05	0.99	1.03	**0.88****
Heart (relative) [%]	0.35	0.35	0.35	0.36	0.41	0.39	0.39	0.39
Brain (absolute) [g]	2.17	2.13	2.12	**2.05****	1.97	1.97	**2.04***	1.93
Brain (relative) [%]	0.45	0.47	0.49	**0.60****	0.77	0.78	0.77	**0.86****

*: Statistically significant from controls, p<0.05; **: Statistically significant from controls, p<0.01

Gross pathology
No gross pathologic lesions which were considered related to test material (CP 50435) feeding were observed at necropsy in any rats from the experimental groups. Gross pathologic lesions which were seen in these rats were those which commonly occur in untreated rats of this strain and age and were not considered significant with respect to the outcome of the study.

Histopathology
Microscopic pathologic lesions which were considered related to feeding of the test material (CP 50435)

were limited to the urinary tract of rats from the 1200 and 4800 mg/kg bw/day feeding groups. Several rats from the 1200 mg/kg bw/day feeding groups and most rats from the 4800 mg/kg bw/day group had hyperplasia of the urinary bladder epithelium. Urinary bladders of rats from the 400 mg/kg bw/day group were comparable to those of control group rats. The compound related urinary bladder hyperplasia was more marked in males than in females and in males it was occasionally accompanied by an acute inflammatory infiltrate in the mucosa and submucosa. The lesion, when present, was fairly uniform over most of the circumference of the urinary bladder and did not show papillarity.

Epithelial hyperplasia was noted in the pelvis of the kidney of a few rats in the 4800 mg/kg bw/day group. The hyperplasia had a slight papillarity and was not uniform over the entire epithelial surface. Some hyperplastic epithelial cells contained a hyaline like cytoplasmic inclusion. This lesion was considered compound related. Several other kidneys had areas of pelvic epithelium which appeared to be hyperplastic, but in these the tissue was cut such that the possibility of a tangential section of epithelium could not be eliminated.

Other microscopic findings in these rats were lesions principally of an inflammatory nature, which are commonly seen in untreated rats of this age and strain. Other microscopic lesions in the kidneys from control and experimental group animals were those of an inflammatory nature and were considered early lesions of chronic nephritis, a common spontaneous disease of laboratory rats. These inflammatory lesions were much more common in males than in females. Chronic nephritis had a greater incidence and severity in males. The fresh haemorrhage noted in the lungs of many rats from the control and 4800 mg/kg bw/day group was considered agonal due to death by carbon dioxide asphyxiation. Other lung lesions were those of a minor inflammatory or degenerative nature which are commonly seen in rats and were not considered significant with respect to treatment. Ample areas of bronchi were present for examination in sections from all rats in the control and 4800 mg/kg bw/day groups. Myocarditis noted in the heart of numerous rats from the control and treated groups was characterised by small focal accumulations of mononuclear inflammatory cells scattered throughout the myocardium. This is a common finding in rats. Changes noted in the hearts of these rats were generally quite minimal and considered typical of the degree of myocarditis ordinarily noted in rats of this age. Prostate glands of a number of rats from the control and experimental groups had slight to moderate inflammatory changes in the interstitium and within prostate follicles. This lesion is a common finding in male rats. Small focal haemorrhages observed in the thymus of many control and experimental group rats were fresh with intact red blood cells present and were considered agonal. Mild subacute inflammatory changes in the parenchyma and portal areas were seen in the livers of numerous rats from control and experimental groups. These lesions are common findings in rats. A number of rats in the control and experimental groups also had very slight extramedullary haematopoiesis in their livers. The hepatocellular vacuolation noted in livers, primarily of male rats, in the experimental and control groups were characterised by the presence of a single large vacuole which was usually not spherical but had a smooth irregular outline. Many of these vacuoles appeared to contain aluminous fluid. Their incidence was somewhat greater in the control males. They were not characteristic of the vacuoles of fatty degeneration and were not considered biologically significant in this study.

Table 5.8.1-39: 90-Day Subacute Rat Toxicity Study ([redacted]-78-174) ([redacted], 1979): Intergroup comparison of selected necropsy observations (unscheduled and terminal sacrifice)

Organ		Dosage Groups [mg/kg bw/day]							
		Males				Females			
		0	400	1200	4800	0	400	1200	4800
Kidneys	hydronephrosis	2	-	-	1	-	-	-	3
	red area / cortico-medullary margin dark red	-	-	-	-	1	1	-	-
Urinary bladder	distended with red fluid	1	-	-	-	-	-	-	-

Table 5.8.1-39: 90-Day Subacute Rat Toxicity Study ([redacted]-78-174) ([redacted], 1979): Intergroup comparison of selected necropsy observations (unscheduled and terminal sacrifice)

Organ		Dosage Groups [mg/kg bw/day]							
		Males				Females			
		0	400	1200	4800	0	400	1200	4800
	calculi	-	-	-	1	-	-	-	-
	thickened	-	-	-	1	-	-	-	-

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study was not conducted according to any guideline or in compliance with GLP (pre-GLP), but following a study design similar to OECD 408. Therefore, the deviations listed above are from the current version of OECD 408 (2018).
However, the study was well conducted and thus, can provide useful information for the assessment of the metabolites of glyphosate. The study is therefore considered valid.
No changes considered to be related to the compound were seen in general behaviour, appearance, haematological determinations or urinalysis.
Male rats at the 4800 mg/kg bw/day group consumed less food and gained less body weight than the control. At week 13, the difference in mean body weight gain between control rats and male and female rats at the 4800 mg/kg bw/day dosage level was statistically significant ($p<0.01$).
Lactic dehydrogenase activity (LDH) for rats at 4800 mg/kg bw/day was greater than controls. In males at 4800 mg/kg bw/day mean LDH activity was 48 and 145 % greater than controls at 45 and 88 days, respectively. In females at 4800 mg/kg bw/day mean LDH activity was 29 and 171 % greater than control values at 45 and 88 days, respectively.
No compound related gross pathologic lesions were observed at necropsy in any rats from the test compound (CP 50435) treated groups.
Statistical variations of undetermined biological significance occurred in group mean weights of kidneys, gonads, heart and brain at several dosage levels in both sexes. Most statistically significant alterations in organ weights occurred in the high dose groups, though.
Microscopic changes which were considered compound related were limited to the urinary tract of rats from the 4800 and 1200 mg/kg bw/day dose groups where hyperplasia of the urinary bladder epithelium was observed. Additionally, very slight epithelial hyperplasia was noted in the bladders of several rats from the 1200 mg/kg bw/day. Very slight to slight hyperplasia of the epithelium of the kidney pelvis in several rats from the 4800 mg/kg bw/day dose group was also considered compound related.
No compound related urinary tract lesions were observed at the 400 mg/kg bw/day level in either sex.
Based on the incidence of microscopic changes in the mid and high dose groups (urothelial hyperplasia of the urinary bladder), the NOAEL equalled 400 mg/kg bw/day under the conditions of this 90-days oral study with AMPA (CP 50435).

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.8.1/018
Report author	[redacted]
Report year	1991

Report title	90-Day Oral (Capsule) Toxicity Study in Dogs with AMPA
Report No	[redacted]-50173
Document No	M-645465-01-1
Guidelines followed in study	US EPA Pesticide Assessment Guidelines, Subdivision F, FIFRA, Hazard Evaluation: Human and Domestic Animals, Section 82-1 and also the OECD Toxicity Test Guidelines (ISBN 92-64-12221-4, Section 409; 1981)
Deviations from current test guideline (OECD 409, 1998)	Gall bladder, uterus, thymus, spleen and heart not weighed at necropsy.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive summary

The toxicological potential of AMPA was evaluated in this 90-day sub-chronic toxicity study in outbred beagles. Dosage levels of 10, 30, 100 and 300 mg/kg bw/day were selected for the study.

The test material was administered in capsule form as a single daily dose for 91 or 92 consecutive days. A concurrent control group received empty capsules on a comparable regimen. Each dose group consisted of five males and five females. All animals were observed twice daily for mortality and moribundity. Clinical examinations were performed daily prior to dosing and one hour following dosing. Individual body weights were recorded weekly. Food consumption was recorded daily and reported weekly. Clinical pathologic evaluations were conducted once prior to study initiation and during the 6th and 13th weeks of dosing (weeks 5 and 12). Ocular examinations were conducted prior to study initiation and during the 13th week of dosing. Complete necropsies were performed on all dogs at study termination. Selected organs were weighed and selected organs were examined microscopically.

Test article administration had no adverse effects at any dose level on survival, general clinical condition or behaviour, body weight, food consumption, clinical pathology parameters or organ weight values.
No treatment related lesions were observed neither at the ocular examinations nor following gross and microscopic examinations of selected tissues.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	AMPA (Aminomethylphosphonic acid)
Description:	White solid (powder)
Lot/Batch #:	PIT-9008-2407-T
Purity:	87.8 %
Stability of test compound:	August 1993 (Estimated Expiry). Confirmatory analysis performed by Sponsor from samples of test material at the beginning and end of the study.

2. Vehicle and/ or positive control:	Empty gelatine capsule / none
3. Test animals:	
Species:	Dog
Strain:	Outbred Beagle
Source:	[redacted]
Age:	Approx. 6 months
Sex:	Male and female
Weight at dosing:	♂ 8123 – 11929 g; ♀ 6753 – 10788 g
Acclimation period:	2 weeks
Diet/Food:	Purina® Certified Canine Chow® #5007 (offered 1 – 2 hours each day)
Water:	Tap water, *ad libitum*
Housing:	Individual housing in stainless steel cages
Environmental conditions:	Temperature: 19 – 24 °C (67 – 75 °F) Humidity: 30 – 76 % Air changes: 10 – 15 / hour 12 hours light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 1990-09-05 (start of dosing) to 1990-12-06 (terminal necropsies)

Animal Assignment and Treatment:
In a 90-day oral (capsule) toxicity study groups of 5 outbred beagle dogs per sex received daily doses of 0, 10, 30, 100 or 300 mg/kg bw/day, at approximately the same time each day for 91 or 92 consecutive days. Capsules were administered following the 1 – 2 hours feeding period.

Table 5.8.1-5.8.1-40 90-Day Oral (Capsule) Toxicity Study in Dogs with AMPA ([redacted], 1991): Study design

Group Number	Dose level [mg AMPA/kg bw/day]	Dose level corrected for purity [mg/kg bw/day]	Number of Animals	
			Males	Females
1	0	0	5	5
2	10	8.8	5	5
3	30	26.3	5	5
4	100	87.8	5	5
5	300	263	5	5

The appropriate amount of test material was weighed into tared size gelatine capsules. A sufficient number of capsules were weighed for each entire week of dosing and stored. Individual dosages were adjusted weekly, based on the most recent body weight. One empty gelatine capsule was dispensed daily for each control animal.
Analysis of the test material was conducted by the Sponsor prior to the beginning and at the conclusion of the study. It was demonstrated that AMPA was stable throughout the course of the study. Results were provided by the Sponsor and presented in a separate report (Monsanto Study ML-90-369).

Mortality
All animals were observed twice daily, once each morning and once in the afternoon for mortality and moribundity.

Clinical Observations
All animals were observed daily prior to dosing and one hour following dosing for overt signs of toxicity. Only significant findings were recorded at these observations.

Body Weight
Body weights were recorded weekly for study weeks -1 – 13. Terminal body weights were recorded for each animal on the day of study termination.

Food Consumption
Individual food consumption was recorded daily and the weekly averages reported for the corresponding body weight intervals. Food intake was calculated as mg/kg bw/day.

Ophthalmoscopic Examination
Ocular examinations were conducted prior to study initiation and during the 13th week of dosing. All examinations were conducted using a hand-held slit lamp and an indirect ophthalmoscope, preceded by mydriasis with 1 % topical tropicamide hydrochloride.

Clinical Pathology
Clinical pathology parameters (haematology, serum chemistry and urinalysis) were measured on all dogs once prior to study initiation and during the 6th and 13th week of dosing (study weeks 5 and 12, respectively). Collection of blood and urine samples was conducted prior to feeding and test item administration. Blood samples were taken from the jugular vein. Urine was collected using a catheter except for the following instances where urine for re-evaluation was collected using metabolism cages (15 – 16 hours collection) for male numbers 775 and 793 in the 300 mg/kg bw/day group (weeks 5 and 12, respectively) and female number 817 in the 10 mg/kg bw/day group (week 5). Values for these animals were reported but excluded from group mean calculations.

Haematology
The following parameters were evaluated: Total leukocyte count (white cell), erythrocyte count (red blood cells), haemoglobin, haematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count (Platelet), prothrombin time, activated partial thromboplastin time (APTT), differential white (WBC) count (percent and absolute) for neutrophils (segmented and unsegmented), lymphocytes, monocytes, eosinophils and basophils.

Serum Chemistry
The following parameters were evaluated: Glucose, blood urea nitrogen, creatinine, sodium, potassium, serum aspartate aminotransferase (AST), chloride, calcium, globulin, albumin/globulin ratio, serum alanine aminotransferase (ALT), serum alkaline phosphatase (ALK), total bilirubin (Total Bili), total cholesterol (Cholesterol), total protein, albumin and gamma glutamyltransferase (GGT).

Urinalysis
The following parameters were evaluated: Volume, colour, appearance, specific gravity, pH, protein, glucose, ketones, bilirubin, occult blood, nitrites, leukocytes and microscopy of sediment.

Terminal Studies
Macroscopic and Microscopic Examination
At study termination, all dogs were anaesthetised by intravenous injection of sodium phenobarbital followed by exsanguination. A complete necropsy was conducted on all animals. Necropsy included, but was not limited to, examination of the external surface, all orifices, the cranial cavity, the external and cut surfaces of the brain and spinal cord, and the thoracic, abdominal and pelvic cavities including viscera.

Bone marrow smears were taken at necropsy.
The following organs and tissues were collected and placed in 10 % neutral buffered formalin: Adrenals, aorta, bone with marrow (sternum), brain (forebrain, mid-brain, hindbrain), eyes with optic nerve, femur (with joint), gall bladder, gastrointestinal tract (oesophagus, stomach, duodenum, jejunum, ileum, caecum, colon, rectum), heart, kidneys, liver (sections of 2 lobes), all gross lesions, lungs (including bronchi), lymph node (mesenteric and suprapharyngeal), ovaries with mesovarium, pancreas, peripheral nerve (sciatic), pituitary, prostate, salivary gland (submaxillary), skeletal muscle (*vastus medialis*), skin with mammary gland, spinal cord (mid-thoracic, cervical and lumbar), spleen, testes with epididymides, thymus, thyroid gland (both lobes with parathyroid gland), trachea, urinary bladder, uterus with vagina.
After fixation and following staining with haematoxylin and eosin, the above tissues were examined microscopically.

Organ Weights
The following organs were weighed from all animals: Adrenals, brain, kidneys, liver, ovaries, testes with epididymides and thyroid gland (with parathyroids).
Statistics
All means were presented with standard deviations, and the number of sampling units used to calculate the means. All statistical tests were performed by a DEC (Digital Equipment Corporation) computer with appropriate programming. Analysis of body weights, body weight changes, food consumption, clinical laboratory values and absolute and relative organ weights were analysed by a one-way analysis of variance followed by Dunnett's Test. All analyses were conducted using two-tailed tests for minimum significance levels of 5 % and 1 % comparing the treatment groups with vehicle control group by sex.

II. RESULTS AND DISCUSSION

A. MORTALITY
All animals survived to scheduled necropsy.

B. CLINICAL OBSERVATIONS
There were no clinical signs in either sex which could be positively attributed to treatment with the test material.
Numerous clinical signs were observed in both sexes at all dose levels, including controls. These consisted primarily of lacrimation, scabbed, reddened and/or swollen ears, soft stool, diarrhoea, salivation and emesis at the daily examinations prior to dosing. In general, these findings occurred at a similar frequency in the control and treated groups and/or in a non-dose-related manner, although occasional disproportionate frequencies occurred.

C. BODY WEIGHT
No treatment related effects were apparent on mean body weights or body weight changes in males and females at dose levels of 10, 30, 100 or 300 mg/kg bw/day.
A mean body weight loss in the 300 mg/kg bw/day female group during weeks 5 – 6 was greater than the loss observed in the control group. However, the severity of loss in females at 300 mg/kg bw/day was due to one female (number 801) and this loss was followed by a substantial body weight gain during weeks 6 – 7. It should be noted that during this time period, red material was found in the faeces of this animal and food intake was notably reduced, for which it was concluded that these effects were transitory and not related to treatment.
The only statistically significant difference in body weight between control and treated groups was an increase in mean body weight gain ($p<0.05$) in females at 300 mg/kg bw/day during weeks 8 – 9 (see **Error! Reference source not found.**e below). No other remarkable differences were observed in body weight data between the control and the 10, 30, 100 and 300 mg/kg bw/day dose groups.

Table 5.8.1-5.8.1-41: 90-Day Oral (Capsule) Toxicity Study in Dogs with AMPA ([redacted], 1991): Intergroup comparison of selected group mean body weights and body weight gain

Dose Group [mg/kg bw/day]	Mean body weight or body weight gain [g]				
	Body weight (Week 0)	Body weight (Week 13)	Body weight gain (week 5 – 6)	Body weight gain (week 6 – 7)	Body weight gain (week 8 – 9)
	Males				
0	9781 ± 1147.8	11849 ± 1200.9	12 ± 214.3	431 ± 95.2	144 ± 232.4
10	10064 ± 1167.1	12724 ± 1187.8	-145 ± 133.3	493 ± 128.5	160 ± 199.5
300	9704 ± 830.2	11910 ± 1908.7	62 ± 250.6	280 ± 120.9	378 ± 89.1
100	9861 ± 1242.9	12226 ± 1375.3	59 ± 160.5	381 ± 154.9	253 ± 91.2
300	9818 ± 1095.7	12297 ± 1573.4	-7 ± 198.9	444 ± 79.5	135 ± 183.9
	Females				
0	8606 ± 1084.2	10084 ± 1396.9	-217 ± 98.0	354 ± 162.1	55 ± 184.4
10	8683 ± 1365.5	10108 ± 1067.0	-138 ± 126.1	287 ± 44.9	118 ± 145.5
30	8214 ± 726.9	9889 ± 491.0	-235 ± 77.3	438 ± 175.6	121 ± 54.7
100	8443 ± 1126.7	9507 ± 1126.5	-93 ± 195.6	378 ± 170.0	51 ± 88.8
300	8360 ± 1190.8	9759 ± 1401.6	-461 ± 507.7	554 ± 100.8	**291* ± 102.5**

*: Statistically significant from controls, p<0.05

D. FOOD CONSUMPTION

Food consumption was unaffected by treatment at any dose level.

Food consumption values (grams/animal/day and g/kg bw/day) in females at 300 mg/kg bw/day were slightly lower, but not statistically significant, than the control group during weeks 5 – 6. The decreases were due to one female (number 801) in the 300 mg/kg bw/day group, and food consumption in this group during weeks 6 – 7 was comparable to that of the control group. Therefore, the brief reduction in food consumption at this dose level was not considered to be a treatment related effect. A statistically significantly increased (p<0.05) food consumption (g/kg bw/day) was noted in females at 300 mg/kg bw/day during weeks 8 – 9. This was the only statistically significant difference in food consumption between the control and treated groups. All other values in the 10, 30, 100 and 300 mg/kg bw/day groups were comparable with the control group.

Table 5.8.1-5.8.1-42: 90-Day Oral (Capsule) Toxicity Study in Dogs with AMPA ([redacted], 1991): Intergroup comparison of selected food consumption values (means ± SD)

Dose Group [mg/kg bw/day]	Group Mean Food Consumption				
	Food Consumption Week 5 – 6 [mg/dog/day]	Food Consumption Week 5 – 6 [g/kg bw/day]	Food Consumption Week 6 – 7 [mg/dog/day]	Food Consumption Week 6 – 7 [g/kg bw/day]	Food Consumption Week 8 – 9 [g/kg bw/day]
	Males				
0	302 ± 60.2	29 ± 7.0	343 ± 60.2	32 ± 5.7	29 ± 5.7
10	288 ± 52.2	25 ± 2.4	353 ± 30.0	31 ± 1.2	28 ± 1.7
300	329 ± 48.7	32 ± 6.2	344 ± 53.8	32 ± 5.2	33 ± 5.8
100	316 ± 58.9	29 ± 5.0	353 ± 53.7	32 ± 3.3	30 ± 3.3
300	315 ± 15.0	30 ± 3.2	357 ± 14.5	33 ± 4.1	30 ± 4.7
	Females				
0	219 ± 35.5	24 ± 3.2	275 ± 51.9	29 ± 3.4	26 ± 2.9
10	228 ± 29.8	25 ± 4.0	283 ± 23.8	31 ± 4.0	29 ± 2.7
30	227 ± 12.1	26 ± 2.5	285 ± 31.0	32 ± 3.3	30 ± 2.0
100	234 ± 18.9	27 ± 4.3	292 ± 29.8	33 ± 4.4	28 ± 4.2

300	178 ± 80.9	21 ± 8.2	289 ± 54.3	33 ± 3.0	**33* ± 4.8**

*: Statistically significant from controls, p<0.05

E. OPHTHALMOSCOPY

No oculopathical lesions indicative of a toxic effect were observed at any dose level.

F. CLINICAL PATHOLOGIC EVALUATION

Haematology

Haematology parameters were unaffected by test article administration in the 10, 30, 100 and 300 mg/kg bw/day group males and females.

Values in the treated groups were generally comparable to those in the control group. The following statistically significant differences were observed.

The mean MCHC value in males at 10 mg/kg bw/day was significantly reduced (p<0.01) at the week 5 evaluation when compared to the control group. Similar decreases were not observed at higher dose levels or in females at any dose level. The mean MCV value in the 10 mg/kg bw/day females was significantly reduced (p<0.05) in week 12 when compared to the control group. However, similar decreases were not observed in females at higher dose levels or in males at any dose level.

At the week 12 evaluation, significant reductions (p<0.05 or p<0.01) were observed in mean red cell count, haemoglobin and haematocrit values for males in the 30 mg/kg bw/day group, in mean haemoglobin and MCH values in the 100 mg/kg bw/day group, and in the mean MCV value in the 10 mg/kg bw/day group females. Similar findings were not observed in male or females at 300 mg/kg bw/day and no relationship to treatment was evident.

Table 5.8.1-5.8.1-43: 90-Day Oral (Capsule) Toxicity Study in Dogs with AMPA ([redacted], 1991): Intergroup comparison of selected group mean haematology parameters (means ± SD)

Dose Group [mg/kg bw/day]	Group Mean Haematology Parameters						
	MCHC Week 5 [g/dL]	MCV Week 5 [µm³]	MCV Week 12 [µm³]	MCH Week 12 [pg]	Red Cell Count Week 12 [10⁶/L]	Haemoglobin Week 12 [g/dL]	Haematocrit Week 12 [%]
	Males						
0	33.8 ± 0.52	71.6 ± 1.58	72.4 ± 1.55	24.4 ± 0.35	7.19 ± 0.281	17.5 ± 0.62	52.0 ± 2.03
10	**32.8** ± 0.48**	70.5 ± 1.83	71.8 ± 1.91	23.8 ± 0.40	7.40 ± 0.181	17.6 ± 0.46	53.2 ± 1.93
300	33.8 ± 0.42	69.9 ± 0.66	71.0 ± 1.48	23.9 ± 0.42	**6.50* 0.258**	**15.6** ± 0.58**	**46.1** ± 1.66**
100	33.7 ± 0.37	69.1 ± 2.66	70.2 ± 2.02	**23.4* ± 0.62**	6.89 ± 0.692	**16.1* ± 1.40**	48.3 ± 4.50
300	33.7 ± 0.40	69.3 ± 1.69	70.5 ± 1.77	23.6 ± 0.75	7.00 ± 0.205	16.5 ± 0.39	49.3 ± 1.18
	Females						
0	33.5 ± 0.35	71.6 ± 1.35	72.6 ± 0.91	24.3 ± 1.25	6.87 ± 0.979	16.6 ± 1.49	49.9 ± 6.84
10	33.4 ± 1.38	**68.9* ± 1.45**	**70.2* ± 1.79**	23.8 ± 0.79	7.03 ± 0.376	16.7 ± 0.70	49.3 ± 2.76
30	33.4 ± 0.66	69.7 ± 1.05	71.0 ± 1.42	23.6 ± 0.46	7.11 ± 0.373	16.8 ± 0.76	50.4 ± 2.96
100	33.9 ± 0.59	69.4 ± 1.98	71.3 ± 1.16	23.8 ± 0.35	7.37 ± 0.182	17.6 ± 0.59	52.5 ± 1.53
300	33.8 ± 0.68	69.6 ± 1.66	71.4 ± 1.18	23.9 ± 0.51	7.23 ± 0.487	17.3 ± 0.99	51.5 ± 3.01

*: Statistically significant from controls, p<0.05; **: Statistically significant from controls, p<0.01

Serum chemistry
No treatment related effects were apparent on serum chemistry parameters at any dose level.
Mean glucose values were significantly higher ($p<0.05$) in the 30 and 100 mg/kg bw/day group males than in the control group males at week 12. However, this was attributed to a slightly lower control mean (91 mg/dL) at week 12 than in week 5; the means for the 30 and 100 mg/kg bw/day group males were each 101 mg/dL and identical to the week 5 control mean of 101 mg/dL.
All other serum chemistry values in the treated groups were comparable to those in the control group and week 5 and week 12.

Table 5.8.1-5.8.1-44: 90-Day Oral (Capsule) Toxicity Study in Dogs with AMPA ([redacted], 1991): Intergroup comparison of selected group mean serum chemistry parameters

Group Mean Parameter	Dose Groups [mg AMPA/kg bw/day]									
	Males					Females				
	0	10	30	100	300	0	10	30	100	300
Glucose Week 5 [mg/dL]	101 ± 7.7	106 ± 7.2	100 ± 10.0	100 ± 10.5	103 ± 5.8	100 ± 9.0	102 ± 2.7	105 ± 8.5	101 ± 10.9	106 ± 5.0
Glucose Week 12 [mg/dL]	91 ± 4.8	95 ± 4.5	**101* ± 6.1**	**101* ± 6.5**	100 ± 5.2	91 ± 6.0	98 ± 6.8	89 ± 8.0	86 ± 4.2	85 ± 7.9

*: Statistically significant from controls, $p<0.05$; **: Statistically significant from controls, $p<0.01$

Urinalysis
No adverse effects on urinalysis parameters were observed at any dose level. None of the differences between the control and treated groups were statistically significant.

G. PATHOLOGY

Macroscopic Examination
At study termination, no treatment related lesions were noted in the 10, 30, 100 or 300 mg/kg bw/day group males and females.
The lesions observed in the treated groups were commonly observed lesions and either occurred at a similar frequency in the control or occurred infrequently (generally in a single animal).

Microscopic Examination
No microscopic lesions indicative of treatment-related effect were apparent at any dose level.
The lesions observed occurred similarly in the control and treated groups or were limited, in general, to single occurrences.

Organ weights
Test article administration did not adversely affect mean absolute organ weights and organ weights relative to final body weight in males and females at dose levels of 10, 30, 100 or 300 mg/kg bw/day.
The only statistically significant differences between the control and treated groups were low ($p<0.05$ or $p<0.01$) mean absolute and relative liver weights in the 100 mg/kg bw/day group females. Decreased liver weights were not similarly observed in females at the higher dose level of 300 mg/kg bw/day and no relationship to treatment was evident. No other remarkable difference in organ weight data were observed between the control group and the 10, 30, 100 and 300 mg/kg bw/day groups.

Table 5.8.1-5.8.1-45: 90-Day Oral (Capsule) Toxicity Study in Dogs with AMPA ([redacted], 1991): Intergroup comparison of selected absolute and relative (to body weight) mean organ weights

Organ / Tissue	Dose Groups [mg/kg bw/day]									
	Males					Females				
	0	10	30	100	300	0	10	30	100	300
Absolute Liver weight [g]	313.74 ± 47.483	320.22 ± 48.634	314.85 ± 48.712	302.04 ± 24.138	324.36 ± 44.436	294.97 ± 55.697	269.93 ± 26.698	264.46 ± 14.855	**229.85 * ± 27.905**	261.66 ± 34.830
Relative Liver weight [g/100 g]	2.658 ± 0.3631	2.553 ± 0.1949	2.657 ± 0.4170	2.470 ± 0.0920	2.653 ± 0.2288	2.921 ± 0.2044	2.728 ± 0.1532	2.688 ± 0.1518	**2.441* * ± 0.1140**	2.696 ± 0.2353

*: Statistically significant from controls, p<0.05; **: Statistically significant from controls, p<0.01

III. CONCLUSIONS

Administration of the test material AMPA had no adverse effects at any dose level on the survival, the general clinical condition or behaviour of animals, body weight data, food consumption data, haematology parameters, serum chemistry parameters, urinalysis parameters or organ weight values.
No treatment related lesions were observed at the ophthalmologic examination or at the gross and microscopic examinations of selected tissues.

Based on the results of this study, the highest dose level of 300 mg/kg bw/day administered orally to dogs for 91 or 92 consecutive days was considered to be the NOAEL (No Observed Adverse Effect Level).

3. Assessment and conclusion

Assessment and conclusion by applicant:

The test material AMPA was administered orally via capsule to Beagle dogs at dose levels of nominally 0, 10, 30, 100 and 300 mg/kg bw/day. After adjustment for purity (87.8 %) the dose levels were 0, 8.8, 26.3, 87.8 and 263 mg/kg bw/day.
The study was conducted according to OECD 408 (1981) and in compliance with GLP (no attest of the competent authority was provided). Deviations from the current version of OECD 409 (1998) are basically due to the fact that the study was aligned to an older version of the OECD test guideline 409. However, the study was well conducted, in compliance with GLP and OECD 409 (1981) and thus, can provide useful information for the assessment of the metabolites of glyphosate. The study is therefore considered valid.
Administration of the test material AMPA had no adverse effects at any dose level on the survival, the general clinical condition or behaviour of animals, body weight data, food consumption data, haematology parameters, serum chemistry parameters, urinalysis parameters or organ weight values.
No treatment related lesions were observed at the ophthalmologic examination or at the gross and microscopic examinations of selected tissues.
Based on the results of this study, the highest dose level of nominally 300 mg/kg bw/day (263 mg/kg bw/day after adjustment for purity) administered orally to dogs for 91 or 92 consecutive days was considered to be the NOAEL (No Observed Adverse Effect Level).

Assessment and conclusion by RMS:

Genotoxicity - *in vitro*

1. Information on the study

Data point	CA 5.8.1/019
Report author	[redacted]
Report year	1996
Report title	AMPA: Reverse Mutation Test
Report No	IET 96-0076
Document No	NA
Guidelines followed in study	OECD 471 (1983), OECD 472 (1983), U.S. EPA FIFRA Guidelines, Subdivision F, Japanese MAFF (1985)
Deviations from current test guideline (OECD 471, 1997)	Information on historical control data was not reported. 2-Aminoanthracene was used as sole positive control in the presence of metabolic activation. In the repeat-experiment, no parameter was changed. Both experiments were conducted under the same conditions using the pre-incubation method.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive summary

S. typhimurium strains TA 98, TA 100, TA 1535 and TA 1537, and *E. coli* strain WP2 uvrA were exposed to AMPA, metabolite of glyphosate (batch A-960719, purity: 99.33 %) in the presence and absence of metabolic activation (phenobarbital and 5,6 benzoflavone-induced rat liver S9 fraction). Two independent experiments were performed according to the pre-incubation method.

Based on the results of a preliminary range-finding study, in which no cytotoxicity was observed up to 5000 µg/plate, test item concentrations for the reverse mutation assay were selected in the range of 313 – 5000 µg/plate. Solvent (water) and appropriate positive controls were included in all experiments. The bacteria strains were exposed for 48 hours at 37 °C, followed by an inspection of the bacterial background lawn and counting of the revertant colonies for each plate.

Precipitation of the test substance was not reported. In addition, there was no cytotoxicity observed in any test strain up to the highest AMPA concentration, neither in the presence nor absence of S9 mix.

There was no relevant increase in the number of his^+ and trp^+ revertants (exceeding a factor of 2 when compared to solvent controls) observed in any experiment in any strain, neither with nor without metabolic activation.

The number of revertants induced by the solvent control was within expected range for each strain, thus demonstrating an acceptable experimental performance. The positive control compounds induced a marked increase in the number of revertant colonies in all strains, demonstrating the validity of the test system and the functionality of the S9 mix.

Based on the results of the present study and under the conditions of the test, AMPA, metabolite of glyphosate is not mutagenic in the Ames pre-incubation test with and without metabolic activation.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: AMPA

Identification: Not specified

Description: White powder

Lot/Batch #: A-960719

Purity: 99.33 %

Stability of test compound: The test item was stable for 1 year at room temperature. The stability of the test item in solvent was not specified.

Solvent used: Sterile water

2. Control materials:

Negative control: A negative control was not employed in this study.

Solvent control: Sterile water

Solvent/final concentration: 100 µL/plate

Positive controls: Please refer to the table below.

Strain	Metabolic activation	Mutagen	Solvent	Conc. [µg/plate]
S. typhimurium strains				
TA 100	-S9	2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2)	DMSO	0.01
	+S9	2-Aminoanthracene (2-AA)	DMSO	1.0
TA 1535	-S9	Sodium azide (NaN_3)	Water	0.5
	+S9	2-Aminoanthracene (2-AA)	DMSO	2.0
TA 98	-S9	2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2)	DMSO	0.1
	+S9	2-Aminoanthracene (2-AA)	DMSO	0.5
TA 1537	-S9	9-Aminoacridine (9-AA)	Water	80.0
	+S9	2-Aminoanthracene (2-AA)	DMSO	2.0
E. coli strain(s)				
WP2 uvrA	-S9	2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2)	DMSO	0.01
	+S9	2-Aminoanthracene (2-AA)	DMSO	10.0

3. Metabolic activation:

S9 mix was purchased from [redacted] ([redacted]; Lot no. RAA-350). The homogenate was produced from the livers of male Sprague-Dawley rats. The animals were about 7 weeks old, weighing 203 - 254 g and received intraperitoneal injections of phenobarbital (30 mg/kg bw on Day 1, each 60 mg/kg bw on Days 2, 3 and 4) and 5,6 benzoflavone (80 mg/kg bw on Day 3). The S9 mix was prepared immediately before the experiment by mixing S9 fraction and co-factors.

S9 mix component	Concentration	Unit
Sodium phosphate buffer (pH 7.4)	100	mM
KCl	33	mM
NADPH-generating system		
Glucose 6-phosphate	5	mM
NADH	4	mM
NADP	4	mM
$MgCl_2$	8	mM
S9	10	% (v/v)

4. Test organisms:

Tester strains				Bacteria batch checked for	
S. typhimurium		*E.coli*			
TA 98	✓	WP2 uvrA	✓	deep rough character (rfa)	✓
TA 100	✓	WP2 uvrA (pKM101)		ampicillin resistance (R factor plasmid)	✓
TA 1535	✓			UV-light sensitivity (absence of uvrB and uvrA genes in *S. typhimurium* and *E. coli* strains, respectively)	✓
TA 1537	✓				
TA 102					
TA 1538				Histidine and tryptophan auxotrophy (automatically via the spontaneous rate)	✓

5. Test concentrations:

3. **Preliminary cytotoxicity assay:**

Pre-incubation assay ± S9 mix:	
Concentrations:	*200, 500, 1000, 2000 and 5000 µg/plate*
Tester strains:	TA 1535, TA 1537, TA 98, TA 100 and WP2 uvrA
Replicates:	*A single plate was used per condition.*

(c) Mutation assays:

Pre-incubation assay ± S9 mix:	
Concentrations:	*313, 625, 1250, 2500 and 5000 µg/plate*
Tester strains:	TA 1535, TA 1537, TA 98, TA 100 and WP2 uvrA
Replicates:	*Triplicates in two independent experiments*

B. STUDY DESIGN AND METHODS

7. Dates of experimental work: 09 Sep – 11 Oct 1996
Finalisation date: 09 Dec 1996

8. Pre-incubation test (PIT):

0.1 mL of test solution, solvent or positive control, 0.1 mL pre-cultured bacterial suspension and 0.5 mL of S9 mix (in tests with metabolic activation) or 0.5 mL of 100 mM sodium phosphate buffer (in tests without metabolic activation) were pre-incubated with shaking for 20 minutes at 37 °C. Afterwards, 2 mL of molten soft agar (supplemented with 0.5 mM L-histidine and 0.5 mM D-biotin or 0.5 mM L-tryptophan) was added to each test tube. The contents were mixed uniformly and overlaid on a minimal glucose agar plate. Each concentration and the controls were tested in triplicates. After an incubation period of 48 h at 37 °C, bacterial background lawn was inspected and the number of bacterial colonies (his^+ or trp^+ revertants) were counted.

9. Cytotoxicity:

Cytotoxicity was investigated in a preliminary dose-range finding study. Toxicity was detected by a

- reduction in the number of revertants
- clearing or diminution of the background lawn (= reduced his^- or trp^- background growth)

and recorded for all test groups both with and without S9 mix in all experiments.

10. Statistics:

Results were judged without statistical analysis.

11. Acceptance criteria:

The test was valid if

- The culture of the tester strains, the solution of the test substance and S9 mix were free from contamination or other bacteria.
- A normal number of spontaneous revertant colonies was observed for the solvent control.
- An at least 3-fold increase above the solvent control in the mean number of revertants was observed in the positive control.

12. Evaluation criteria:

Results were judged positive without statistical analysis when the following criteria were met:

- A two-fold or greater increase above solvent control in the mean number of revertants was observed.
- This increase in the number of revertants was accompanied by a dose-response relationship.
- This increase in the number of revertants was reproducible.

Reproducibility of results was confirmed by two independent experiments.

II. RESULTS AND DISCUSSION

E. ANALYTICAL DETERMINATIONS

Analytical determinations were not performed in the study.

F. CYTOTOXICITY

In the preliminary cytotoxicity test, as well as in the main gene mutation assay, the test substance did not show any cytotoxicity in any strain up to the highest dose of 5000 µg/plate, neither in the presence, nor in the absence of metabolic activation.

G. SOLUBILITY

Precipitation of the test substance was not reported.

H. MUTATION ASSAY

A relevant increase (≥ 2-fold when compared to corresponding solvent controls) in the number of his^+ or trp^+ revertants was not observed in any experiment at any tested concentration neither in the presence nor absence of metabolic activation.

The number of revertants induced by the solvent control was within the expected range for each strain, thus demonstrating an acceptable experimental performance.

The positive control compounds induced a marked increase in the number of revertant colonies in all strains, demonstrating the validity of the test system and the functionality of the S9 mix.

Table 5.8.1-46: AMPA, metabolite of glyphosate - mutagenicity results (Ames test) with and without metabolic activation ([redacted], 1996), first experiment

Experiment 1: Pre-incubation test (PIT)										
Strain	**TA 100**		**TA 1535**		**WP2 uvrA**		**TA 98**		**TA 1537**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Vehicle control										
Water mean	101	105	11	10	17	19	18	30	4	10
± *SD*	± *17*	± *11*	± *1*	± *4*	± *4*	± *1*	± *3*	± *5*	± *3*	± *1*
Test item [µg/plate]										
313 mean	84	105	10	12	17	16	14	28	5	9
± *SD*	± *7*	± *5*	± *5*	± *2*	± *6*	± *4*	± *2*	± *5*	± *3*	± *3*
625 mean	91	92	8	6	17	16	16	28	4	13
± *SD*	± *14*	± *6*	± *6*	± *1*	± *7*	± *1*	± *4*	± *7*	± *2*	± *2*
1250 mean	97	90	8	6	14	16	13	25	4	11
± *SD*	± *17*	± *3*	± *3*	± *1*	± *2*	± *2*	± *2*	± *7*	± *2*	± *3*
2500 mean	91	83	9	9	15	20	15	25	6	10
± *SD*	± *7*	± *9*	± *1*	± *4*	± *8*	± *4*	± *2*	± *8*	± *2*	± *3*
5000 mean	100	93	7	10	16	24	16	32	3	7
± *SD*	± *4*	± *10*	± *4*	± *4*	± *4*	± *6*	± *5*	± *10*	± *1*	± *1*
Positive control										
§ mean	619	529	619	184	160	384	667	407	710	94
± *SD*	± *57*	± *33*	± *45*	± *5*	± *22*	± *20*	± *60*	± *11*	± *73*	± *2*

§ = information on respective positive control is reported in Material and Method section A.2

Table 5.8.1-47: AMPA, metabolite of glyphosate - mutagenicity results (Ames test) with and without metabolic activation ([redacted], 1996), second experiment

Experiment 2: Pre-incubation test (PIT)										
Strain	**TA 100**		**TA 1535**		**WP2 uvrA**		**TA 98**		**TA 1537**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Vehicle control										
Water mean	120	95	9	8	15	17	18	28	3	7
± *SD*	± *3*	± *3*	± *3*	± *2*	± *3*	± *3*	± *4*	± *5*	± *2*	± *2*
Test item [µg/plate]										
313 mean	136	112	4	8	18	17	14	21	4	10
± *SD*	± *9*	± *14*	± *1*	± *3*	± *3*	± *4*	± *4*	± *6*	± *3*	± *5*
625 mean	124	84	5	7	16	16	13	21	3	7
± *SD*	± *16*	± *5*	± *2*	± *4*	± *3*	± *5*	± *3*	± *5*	± *2*	± *3*

Table 5.8.1-47: AMPA, metabolite of glyphosate - mutagenicity results (Ames test) with and without metabolic activation ([redacted], 1996), second experiment

Experiment 2: Pre-incubation test (PIT)										
Strain	**TA 100**		**TA 1535**		**WP2 uvrA**		**TA 98**		**TA 1537**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
1250 mean	107	106	6	7	12	17	15	28	3	7
± *SD*	± *11*	± *8*	± *4*	± *2*	± *4*	± *4*	± *2*	± *9*	± *3*	± *1*
2500 mean	95	97	9	11	12	16	16	21	4	6
± *SD*	± *6*	± *4*	± *4*	± *3*	± *3*	± *2*	± *6*	± *1*	± *0*	± *1*
5000 mean	117	115	7	9	20	22	13	22	3	6
± *SD*	± *2*	± *12*	± *3*	± *5*	± *5*	± *2*	± *2*	± *3*	± *2*	± *5*
Positive control										
§ mean	668	584	696	169	182	461	650	334	698	82
± *SD*	± *27*	± *56*	± *20*	± *28*	± *16*	± *8*	± *8*	± *14*	± *53*	± *4*

§ = information on respective positive control is reported in Material and Method section A.2

Study conclusion:
According to the results of the present study, the test item is not mutagenic in the Ames pre-incubation test with and without metabolic activation under the experimental conditions of the test.

3. Assessment and conclusion

Assessment and conclusion by applicant:

In the present study, AMPA was negative for gene mutation in bacteria (*S. typhimurium* TA 100, TA 98, TA 1535 and TA 1537 and *E. coli* WP2 uvrA) in the Ames pre-incubation test with and without metabolic activation.
The study was conducted in accordance with OECD guideline 471 and 472 (1983) and in compliance with GLP. When compared with the current OECD guideline 471 (1997), deviations of minor degree were observed. The study is considered supplementary because two tests were performed under identical conditions (using the pre-incubation method only and a plate-incorporation test was apparently not performed).

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.8.1/020
Report author	[redacted]
Report year	1993
Report title	Mutagenicity test: Ames Salmonella Test with AMPA, batch 286-JRJ-73-4
Report No	13269
Document No	145-GLY
Guidelines followed in	OECD 471 (1983), US EPA FIFRA 84-2

study	
Deviations from current test guideline (OECD 471, 1997)	The test was conducted in 4 valid strains only. Strains like *S. typhimurium* TA 102 or *E. coli* WP2 enabling the detection of cross-linking mutagens were not included. In addition, 2-aminoanthracene was used as sole positive control substance in the presence of S9 mix. Historical control data were not provided. Acceptance criteria were not defined and evaluation criteria did not consider historical control data.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive summary

AMPA (batch: 286-JRJ-73-4, purity: 99.2 %), metabolite of glyphosate was investigated for its potential to cause gene mutation in bacteria (Ames test). *S. typhimurium* strains TA 98, TA 100, TA 1535 and TA 1537 were exposed to the test item, solvent (distilled water) and appropriate positive controls in the presence and absence of metabolic activation (Aroclor 1254-induced rat liver S9 fraction). Dose levels were selected based on the results of a preliminary toxicity test in strain TA 98, in which no cytotoxicity was observed up to the highest tested concentration of 5000 µg/plate.

In the main experiment, two independent experiments were performed, using the plate incorporation method (standard plate test, first experiment) and the pre-incubation method (second experiment) and test item concentrations in the range of 310 – 5000 µg/plate.

Precipitation of the test item was not reported. In both experiments, there was no cytotoxicity, evident as a reduction in the number of revertant colonies or a diminution of the bacterial background lawn observed up to the highest concentration of 5000 µg/plate, neither in the presence, nor in the absence of S9 mix.

In addition, there was no statistically significant and biologically relevant increase in the number of revertant colonies observed in any experiment for any strain at any concentration, neither in the presence nor in the absence of metabolic activation. A single statistically significant increase in the number of revertant colonies was observed for strain TA 1535 at 630 µg/plate in the absence of S9 mix, but as the increase was only of marginal magnitude and without any dose response relationship, the effect was considered to be incidental.

The number of revertant colonies induced by the negative and the positive controls were within the expected range, demonstrating the functionality of the metabolic activation system and the sensitivity of the test.

Under the experimental conditions chosen, AMPA, metabolite of glyphosate, is not mutagenic in the Ames standard plate and pre-incubation test with and without metabolic activation.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	Aminomethyl phosphonic acid (AMPA, metabolite of glyphosate)
Identification:	AMPA
Description:	White powder
Lot/Batch number:	286-JRJ-73-4
Purity:	99.2 %

Stability of test compound: The stability of the test item at storage conditions (at room temperature in the dark) was guaranteed for at least 3 years. The stability of the test item in solvent was not specified.

Solvent (vehicle) used: Distilled water

2. Control materials:

Negative control: A negative control was not employed in this study.

Solvent (vehicle) control: Distilled water

Solvent (vehicle)/final concentration: 0.1 mL per plate.

Positive controls: Please refer to table below.

Strain	Metabolic activation	Mutagen	Conc. [µg/plate]
S. typhimurium strains			
TA 100	-S9	Sodium azide	1.0
	+S9	2-Aminoanthracene	1.25
TA 1535	-S9	Sodium azide	1.0
	+S9	2-Aminoanthracene	1.25
TA 98	-S9	2-Nitrofluorene	1.0
	+S9	2-Aminoanthracene	1.25
TA 1537	-S9	2-Nitrofluorene	1.0
	+S9	2-Aminoanthracene	1.25

3. Metabolic activation:

S9 mix was obtained from the livers of SPF Wistar rats (Mol:WIST) weighing approx. 200 g. The animals received a single intraperitoneal injection of Aroclor 1254 at a dose of 500 mg/kg bw. The livers were prepared 5 days after treatment. The S9 mix was thawed prior to each experiment and co-factors were immediately added to prepare S9-mix containing the following components:

S9 mix component	Concentration	Unit
Phosphate buffer (pH 7.4)	100	mM
KCl	33	mM
NADPH-generating system		
Glucose 6-phosphate	5	mM
NADP	4	mM
$MgCl_2$	8	mM
S9	5	% (v/v)

4. Test organisms:

Tester strains				Bacteria batch checked for	
S. typhimurium		***E.coli***			
TA 98	✓	WP2 uvrA		deep rough character (rfa)	✓
TA 100	✓	WP2 uvrA (pKM101)		ampicillin resistance (R factor plasmid)	✓
TA 1535	✓			UV-light sensitivity (absence of uvrB and uvrA genes in *S. typhimurium* and *E. coli* strains, respectively)	✓
TA 1537	✓				
TA 1538				Histidine auxotrophy (automatically via the spontaneous rate)	✓

5. Test concentrations:

(c) Preliminary cytotoxicity assay:

Plate incorporation test ± S9 mix:	
Concentrations:	*62 - 5000 µg/plate*
Tester strains:	TA98
Replicates:	*Not specified*

(d) Mutation assays:

Plate incorporation test ± S9 mix:	
Concentrations:	*310, 630, 1300, 2500 and 5000 µg/plate*
Tester strains:	TA 1535, TA 1537, TA 98 and TA 100
Replicates:	*Triplicates*
Pre-incubation test ± S9 mix:	
Concentrations:	*310, 630, 1300, 2500 and 5000 µg/plate*
Tester strains:	TA 1535, TA 1537, TA 98 and TA 100
Replicates:	*Triplicates*

B. STUDY DESIGN AND METHODS

7. **Dates of experimental work:** 20 Oct – 06 Nov 1992
   Finalisation date: 18 Feb 1993

8. **Standard plate test (plate-incorporation test, SPT):**
   An aliquot of 0.1 mL test solution, vehicle or positive control, an aliquot of 0.1 mL fresh bacterial culture and 0.5 mL S9 mix (in tests with metabolic activation only) were added to 2 mL of molten top agar (supplemented with 0.05 mM histidine and 0.05 mM biotin). After whirl mixing, the mixture was spread on a Vogel-Bonner agar plate and incubated for 48 – 72 hours at 37 °C. Each concentration and the controls were tested in triplicates. Following incubation, the bacterial background lawn was examined and the number of his^+ revertants colonies were counted.

9. **Pre-incubation test (PIT):**
   0.1 mL of test solution, vehicle or positive control, 0.1 mL bacterial suspension and 0.5 mL of S9 mix (in tests with metabolic activation only) were mixed and pre-incubated in a test tube for 30 minutes at 37 °C under gentle shaking. After pre-incubation, 2.0 mL of top agar was added, the mixture was whirl mixed and spread on a Vogel-Bonner agar plate. After an incubation period of 48 – 72 hours at 37 °C the bacterial background lawn was examined and the number of his^+ revertants colonies were counted.

10. **Cytotoxicity**
    Toxicity was detected by a
    - reduction in the number of spontaneous revertants
    - clearing or diminution of the background lawn (= reduced his^- or trp^- background

growth)
and recorded for all test groups both with and without S9 mix in all experiments.

11. Statistics

Statistical analysis of the negative control versus test data was performed using the Analysis of Variance method.

12. Acceptance criteria

Acceptance criteria were not specified in the study report.

13. Evaluation criteria

A test item was considered positive (mutagenic) in the assay if the following criteria were met:

- There was a statistically significant and dose related increase in the level of revertants on the test plates as compared to the control plates.
- The number of revertants at the dose level were the highest effect was found should be more than twice the concurrent spontaneous level.

Sporadic occurring statistically significant increases in revertants which were not dose related (i.e. occurring at the lower dose level when there was no increase at higher non-toxic doses) were considered incidental and not relevant for the evaluation.

II. RESULTS AND DISCUSSION

E. ANALYTICAL DETERMINATIONS

Analytical determinations were not performed in the study.

F. CYTOTOXICITY

In the preliminary toxicity test in strain TA 98, cytotoxicity measured as a colony count reduction was not observed at any dose level, neither in the presence nor absence of S9 mix.
In the main mutagenicity assay there was no cytotoxicity observed for any tester strain up to the highest tested concentration of 5000 µg/plate, neither with nor without S9 mix. In addition, there was no depression of the background growth observed in any tester strain at any concentration, neither in the presence nor absence of S9 mix.

G. SOLUBILITY

Precipitation was not reported in the study report.

H. MUTATION ASSAY

There was no statistically significant and biologically relevant increase in the number of his^+ revertant colonies observed in any experiment for any strain at any concentration, neither in the presence nor in the absence of S9 mix. A single statistically significant increase in the number of revertant colonies was observed for TA 1535 at 630 µg/plate in the absence of S9 mix, but as the increase was only of marginal magnitude and without any dose response relationship, the observation was considered to be incidental.
The number of revertant colonies induced by the negative and the positive controls were within the expected range, demonstrating the functionality of the metabolic activation system and the sensitivity of the test.

Table 5.8.1-48: AMPA, metabolite of glyphosate - mutagenicity results (Ames test) with and without metabolic activation ([redacted] 1993), first experiment

Experiment 1: Standard plate test (SPT)								
Strain	**TA 100**		**TA 98**		**TA 1537**		**TA 1535**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Vehicle control (distilled water)								
mean	130.7	126.7	28.7	33.0	8.3	13.0	16.3	14.3
± *SD*	± *26.5*	± *6.8*	± *17.0*	± *6.0*	± *5.1*	± *4.4*	± *4.0*	± *3.1*
Test item [µg/plate]								
310 mean	119.0	127.3	20.0	28.3	6.7	10.7	16.7	13.7
± *SD*	± *15.9*	± *7.6*	± *5.0*	± *2.5*	± *3.8*	± *3.1*	± *4.2*	± *2.3*
630 mean	116.3	117.0	23.0	34.3	6.3	11.3	22.7*	15.7
± *SD*	± *14.6*	± *18.5*	± *4.6*	± *4.7*	± *5.1*	± *2.9*	± *2.1*	± *2.5*
1300 mean	115.7	118.3	23.3	34.7	6.7	10.3	15.3	11.0
± *SD*	± *12.7*	± *19.6*	± *4.2*	± *6.5*	± *3.2*	± *3.5*	± *4.6*	± *3.5*
2500 mean	118.3	116.7	23.3	35.7	6.0	10.7	19.0	18.0
± *SD*	± *9.8*	± *2.5*	± *1.5*	± *3.8*	± *1.0*	± *5.5*	± *1.7*	± *7.0*
5000 mean	101.0	113.7	29.3	34.7	8.0	10.0	13.7	15.7
± *SD*	± *1.7*	± *2.5*	± *15.5*	± *7.8*	± *1.7*	± *4.4*	± *3.2*	± *3.8*
Positive control								
§ mean	660.0**	1706.7**	320.0**	1203.3**	143.3**	150.0**	473.3**	340.0**
± *SD*	± *165.2*	± *194.3*	± *20.0*	± *119.3*	± *15.3*	± *17.3*	± *68.1*	± *45.8*

§ = information on respective positive control is reported in Material and Method section A.2

**: statistically significant at 1 % level (analysis of variance)

Table 5.8.1-49: AMPA, metabolite of glyphosate - mutagenicity results (Ames test) with and without metabolic activation ([redacted] 1993), second experiment

Experiment 2: Pre-incubation test (PIT)								
Strain	**TA100**		**TA 98**		**TA 1537**		**TA 1535**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Vehicle control (distilled water)								
mean	143.0	143.0	22.7	26.3	15.0	11.0	14.0	20.7
± *SD*	± *21.3*	± *13.1*	± *4.5*	± *2.1*	± *1.0*	± *4.6*	± *1.0*	± *4.9*
Test item [µg/plate]								
310 mean	138.3	134.7	22.0	28.3	14.3	14.7	12.7	13.3
± *SD*	± *16.2*	± *15.0*	± *4.6*	± *2.3*	± *3.2*	± *2.5*	± *3.1*	± *3.8*
630 mean	129.0	125.3	21.7	26.3	17.7	11.3	15.7	14.7
± *SD*	± *15.0*	± *2.5*	± *5.5*	± *4.0*	± *1.5*	± *2.1*	± *2.1*	± *2.3*
1300	134.0	133.3	21.3	32.7	15.3	16.0	13.3	12.7

Table 5.8.1-49: AMPA, metabolite of glyphosate - mutagenicity results (Ames test) with and without metabolic activation ([redacted] 1993), second experiment

Experiment 2: Pre-incubation test (PIT)								
Strain	**TA100**		**TA 98**		**TA 1537**		**TA 1535**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
mean								
± *SD*	± *26.0*	± *14.2*	± *2.3*	± *3.1*	± *4.0*	± *1.7*	± *0.6*	± *1.5*
2500 mean	125.0	122.7	26.7	29.3	16.7	15.7	14.3	16.0
± *SD*	± *13.0*	± *7.0*	± *1.2*	± *4.5*	± *3.5*	± *4.0*	± *2.5*	± *5.6*
5000 mean	109.0	140.7	24.3	26.7	10.3	11.3	13.7	16.0
± *SD*	± *4.4*	± *12.5*	± *0.6*	± *6.0*	± *4.0*	± *2.1*	± *3.5*	± *4.4*
Positive control								
§ mean	700.0**	910.0**	266.7**	893.3**	123.3**	310.0**	500.0**	210.0**
± *SD*	± *219.3*	± *157.2*	± *20.8*	± *136.5*	± *5.8*	± *45.8*	± *45.8*	± *17.3*

§ = information on respective positive control is reported in Material and Method section I.A.2

**: statistically significant at 1 % level (analysis of variance)

III. CONCLUSIONS

Based on the experimental findings, AMPA, metabolite of glyphosate was negative for gene mutation in bacteria in the Ames standard plate test and the pre-incubation test in the presence and absence of metabolic activation.

3. Assessment and conclusion

Assessment and conclusion by applicant:

In this Ames test, AMPA was negative for gene mutation in bacteria (*S. typhimurium* TA 100, TA 98, TA 1535 and TA 1537) with and without metabolic activation.
The study was conducted in accordance with OECD guideline 471 (1983) and compliant with GLP. It is considered to provide supporting information, as both experiments were conducted using only 4 valid tester strains. Strains like *S. typhimurium* TA 102 or *E. coli* WP2 enabling the detection of cross-linking mutagens were not included. Further deviations from the currently valid OECD guideline 471 (1997) were of minor degree and considered to not compromise the validity of the study.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.8.1/021
Report author	[redacted]
Report year	1988
Report title	Aminomethyl Phosphonic Acid: An Evaluation of Mutagenic Potential Using *S. typhimurium* and *E. coli*
Report No	CTL/P/2206

Document No	YV2281
Guidelines followed in study	OECD 471 (1983); OECD 472 (1983)
Deviations from current test guideline (OECD 471 , 1997)	2-Aminoanthracene was used as sole positive control substance in the presence of S9 mix for all strains. Historical control data were not provided. Evaluation of cytotoxicity and precipitation were not reported, but concentrations were tested up to limit doses. No confirmation in the report of bacterial cell density at the time of treatment. Acceptance and evaluation criteria specified in the study report were not fully in accordance with those specified in OECD guideline 471 (1997).
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive summary

S. typhimurium strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538, and *E. coli* strain WP2 uvrA pKM101 were exposed to aminomethyl phosphonic acid (batch: 48F-3893, purity: > 99 %) in the presence and absence of metabolic activation (Aroclor 1254 - induced rat liver S9 fraction). For each strain, two independent experiments were conducted according to the standard plate test (plate-incorporation method) at test item concentrations in the range of 1.8 – 5000 µg/plate. Solvent (deionized water) and appropriate positive controls were included in each experiment. After 64 – 68 hours of incubation, the bacterial background lawn was inspected and the number of revertant colonies was examined.

Evaluation of precipitation and cytotoxicity was not provided in the study report. Cytotoxicity was evaluated retrospectively by comparing the presented mean numbers of revertant colonies induced by the test item to the mean number of spontaneous revertants observed in the corresponding solvent control. Cytotoxicity was observed in the first experiment for strain TA 1538 at 1000 µg/plate in the absence of S9 mix and in the second experiment for strain TA 1537 at 5000 µg/plate in the presence of S9 mix.

There was no statistically significant, reproducible increase in in the number of his^+ and trp^+ revertant colony numbers in any of the six tester strains at any dose level, neither in the presence nor absence of metabolic activation. Statistical significance in the mean number of revertant colonies was observed for all strains at single test item concentrations in the presence or absence of metabolic activation. However, none of the observations was consistent in independent experiments or showed a dose-response relationship. For strain TA 1537 in the first experiment, the increase in the mean number of revertant colonies was up to 2-fold over control conditions (200 µg/plate) in the absence of S9 mix and up to 1.9-fold over control conditions (8 and 40 µg/plate) in the presence of S9 mix. In both cases, the responses were only of limited dose-response relationship and of limited statistical significance ($p > 0.01$). Besides, the effects were not reproducible in two further experiments with this strain. Considering all available data of the present study, there was no consistent, statistically significant or biologically relevant increase in the mean number of his^+ or trp^+ revertant colonies observed for any tester strain up to the highest concentration of 5000 µg/plate.

The positive control compounds showed the expected results of markedly increased numbers of revertant colonies, indicating the functionality of the S9 mix and demonstrating the validity of the test system.

Based on the results and under the experimental conditions of the present study, there is no indication for mutagenicity in the Ames standard plate test in the presence and absence of metabolic activation.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Aminomethyl phosphonic acid

Identification: Y06384/001/001

Description: White solid

Lot/Batch #: 48F-3893

Purity: > 99 %

Stability of test compound: Not specified.

Solvent (vehicle) used: Sterile deionised water

2. Control materials:

Negative control: Not specified.

Solvent (vehicle) control: Sterile deionised water

Solvent (vehicle)/ final concentration: 100 µL per plate

Positive controls: Please refer to the table below.

Strain	Metabolic activation	Mutagen	Solvent	Conc. [µg/plate]
S. typhimurium strains				
TA 100	-S9	N-Methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	1.0, 2.0 and 5.0
	+S9	2-Aminoanthracene (2AA)	DMSO	0.2, 0.5 and 1.0
TA 1535	-S9	N-Methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	1.0, 2.0 and 5.0
	+S9	2-Aminoanthracene (2AA)	DMSO	0.5, 1.0 and 2.0
TA 98	-S9	Daunomycin Hydrochloride (DR)	DMSO	0.2, 0.5 and 1.0
	+S9	2-Aminoanthracene (2AA)	DMSO	0.2, 0.5 and 1.0
TA 1537	-S9	Acridine Mutagen ICR191	DMSO	0.5, 1.0 and 2.0
	+S9	2-Aminoanthracene (2AA)	DMSO	0.5, 1.0 and 2.0
TA 1538	-S9	4-Nitro-o-phenylenediamine (4NOPD)	DMSO	1.0, 2.0 and 5.0
	+S9	2-Aminoanthracene (2AA)	DMSO	0.2, 0.5 and 1.0
E. coli strains				
WP2 uvrA	-S9	N-Methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	0.5, 1.0 and 2.0
	+S9	2-Aminoanthracene (2AA)	DMSO	1.0, 2.0 and 5.0

3. Metabolic activation:

S9 mix was obtained from the livers of male Sprague-Dawley rats, that received a single dose of 500 mg/kg bw Aroclor 1254 by intraperitoneal injection. The treated animals were kept on a normal diet for three days, starved of food but not water on the fourth day and the livers were prepared on the fifth day. A 25 % (w/v) homogenate fraction was prepared and aliquots were frozen. The S9 mix was thawed prior to each experiment and co-factor was immediately added to prepare S9 mix containing the following components:

S9 mix component	Concentration	Unit
Na_2HPO_4 buffer	150	mM
KCl	33	mM
NADPH-generating system		
Glucose 6-phosphate	5	mM
NADP, Na salt	4	mM
$MgCl_2$	8	mM
S9	25	% (v/v)

4. Test organisms:

Tester strains				Bacteria batch checked for	
S. typhimurium		***E.coli***			
TA 98	✓	WP2 uvrA pKM101	✓	deep rough character (rfa)	✓
TA 100	✓	WP2 P		ampicillin resistance (R factor plasmid)	✓
TA 1535	✓			UV-light sensitivity (absence of uvrB and uvrA genes in *S. typhimurium* and *E. coli* strains, respectively)	✓
TA 1537	✓				
TA 102					
TA 1538	✓			Histidine and tryptophan auxotrophy (automatically via the spontaneous rate)	✓

5. Test concentrations:

Plate incorporation test ± S9 mix:	
Concentrations:	*1.6, 8.0, 40.0, 200, 1000 and 5000 µg/plate*
Tester strains:	TA 1535, TA 1537, TA 1538, TA 98, TA 100 and WP2 uvrA
Replicates:	*Triplicates*

B. STUDY DESIGN AND METHODS

8. Dates of experimental work: 10 – 23 May 1988
Finalisation date: 21 Sep 1988

9. Standard plate test (plate-incorporation test, SPT):

An aliquot of 0.1 mL test solution or solvent/ positive control, an aliquot of 0.1 mL bacterial overnight culture and 0.5 mL S9 mix (in tests with metabolic activation) or 0.5 mL of sucrose-tris-EDTA buffer (in tests without metabolic activation) were added to 2 mL of molten top agar (supplemented with 0.5 mM histidine/ 0.5 mM biotin stock solution (10 mL solution : 100 mL agar) or 10 mL 0.5 mM tryptophan solution per 100 mL agar). The resulting mixture was poured rapidly onto Vogel Bonner agar plates. The plates were allowed to gel and incubated inverted at 37 °C for 64 - 68 h in the dark. Afterwards, the plates were checked for microbial contamination, the background bacterial lawn was examined and the number of the bacterial colonies (his^+ or trp^+ revertants) was counted.
All strains were tested in two independent experiments and a third experiment was performed for tester strain TA1537. In each experiment, all test item concentrations and the controls were tested in triplicates.

10. Cytotoxicity

Evaluation of cytotoxicity was not further specified in the study report. Thus, retrospectively, cytotoxicity was evaluated by comparing the presented mean numbers of revertant colonies induced by the test item to the mean number of spontaneous revertants observed in the corresponding solvent control. Cytotoxicity was considered evident when the mean number of revertant colonies induced by the test item concentration was ≤ 0.5 fold of the mean number of spontaneous revertant colonies induced by the solvent control.

11. Statistics

An assessment of statistical significance was carried out using a one-tailed Student's t-test. The corresponding probability for each dose level was derived by computer using the appropriate degrees of freedom. Values of $p < 0.01$ were treated as significant with values of $0.01 \leq p < 0.05$ being indicative

of a possible effect.

12. Acceptance criteria

The test was valid if;

- The concurrent solvent control data were acceptable.
- The positive control data showed unequivocal positive responses.
- At least the lowest test item dose showed no evidence of toxicity, and at least three test item doses showed no significant overt toxicity.

Failure of one or more tester strain / S9 combinations did not invalidate the data for the remainder of a concurrent experiment.

13. Evaluation criteria

A positive response in an individual experiment was achieved when one or both of the following criteria were met:

- A statistically significant dose-related increase in the mean number of revertant colonies was obtained.
- A two-fold or greater increase of statistical significance in the mean number of revertant colonies was observed at one or more concentrations.

For a positive response in an individual experiment to be considered indicative of an unequivocal positive, i.e. mutagenic, result for that strain / S9 combination, then the observed effects must be consistently reproducible.

A negative response in an individual experiment was achieved when

- There was no statistically significant dose-related increase in the mean number of revertant colonies per plate observed for the test substance.
- In the absence of any such dose response, no increase in colony numbers was observed at any test concentration, which exceeded 2x the concurrent solvent control.

II. RESULTS AND DISCUSSION

E. ANALYTICAL DETERMINATIONS

Analytical determinations were not performed in the study.

F. CYTOTOXICITY

Cytotoxicity, evident as a reduced number of revertant colonies was observed in the first experiment for strain TA 1538 at 1000 µg/plate in the absence of S9 mix and in the second experiment for strain TA 1537 at 5000 µg/plate in the presence of S9 mix.

G. SOLUBILITY

There was no precipitation of the test substance reported.

H. MUTATION ASSAY

Although a statistically significant increase in the mean number of revertant colonies was observed in the first experiment in strains TA 1535, TA 1537 and WP2 uvrA in the absence of S9 mix and in strains TA 1535, TA 1537, TA 1538, TA 98 and WP2 uvrA in the presence of S9 mix, as well as in the second experiment in strain TA1535 in the absence of S9 mix and in strains TA 100 and WP2 uvrA in the presence of S9 mix, none of these observations showed a dose-response relationship. In addition, the observations were not reproducible in independent experiments. For strain TA 1537 in the first experiment, the increase in the mean number of revertant colonies was up to 2-fold over control conditions (200 µg/plate) in the absence of S9 mix and up to 1.9-fold over control conditions (8 and 40 µg/plate) in the presence of S9 mix. In both cases, the responses were only of limited dose-response relationship and of limited statistical significance ($p > 0.01$). Therefore, for strain TA 1537 a third experiment was conducted under the same experimental conditions. In the latter two experiments, no significant increases in colony numbers were observed for TA 1537 either with or without S9 mix. Considering all available data of the present study, there was no consistent, statistically significant or biologically relevant increase in the mean number of his^+ or trp^+ revertant colonies observed for any tester strain up to the highest concentration of 5000 µg/plate.
The positive control compounds showed the expected results of markedly increased numbers of revertant colonies, indicating the functionality of the S9 mix and demonstrating the validity of the test system.

Table 5.8.1-50: AMPA, metabolite of glyphosate - mutagenicity results (Ames test) with and without metabolic activation ([redacted], 1988), first experiment, plate-incorporation test

Experiment 1: Standard plate test (SPT, plate-incorporation method)												
Strain	**TA 1535**		**TA 1537**		**TA 1538**		**TA 98**		**TA 100**		**WP2 uvrA**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Vehicle control												
Water mean	13.0	10.2	7.8	5.8	13.0	14.4	22.8	17.8	101.0	95.3	126.8	105.4
± *SD*	*± 4.0*	*± 2.3*	*± 5.7*	*± 2.8*	*± 2.0*	*± 4.2*	*± 5.3*	*± 6.0*	*± 8.5*	*± 9.9*	*± 24.0*	*± 12.3*
Test item [µg/plate]												
1.6 mean	20.0*	15.3	10.3	9.7	13.3	18.7	20.7	26.0	94.0	98.3	157.0*	111.3
± *SD*	*± 2.0*	*± 6.1*	*± 5.1*	*± 4.2*	*± 1.2*	*± 1.2*	*± 1.5*	*± 6.0*	*± 8.7*	*± 6.8*	*± 12.8*	*± 7.4*
8.0 mean	20.7*	17.0*	14.7	11.3	14.3	24.3*	22.3	20.7	101.3	98.3	128.7	107.7
± *SD*	*± 1.2*	*± 4.6*	*± 4.9*	*± 6.4*	*± 2.5*	*± 6.7*	*± 10.8*	*± 5.0*	*± 6.7*	*± 10.6*	*± 7.5*	*± 10.4*
40 mean	14.3	13.3*	10.3	11.0*	9.0	18.0	22.7	25.3*	95.7	89.0	161.0*	99.3
± *SD*	*± 4.2*	*± 0.6*	*± 2.1*	*± 1.0*	*± 3.6*	*± 1.4*	*± 6.7*	*± 1.2*	*7.2*	*± 11.1*	*± 13.5*	*± 10.6*
200 mean	18.7*	13.7*	15.3*	10.3*	9.3	16.0	25.3	25.7*	94.3	102.0	138.0	119.7
± *SD*	*± 0.6*	*± 2.5*	*± 2.3*	*± 2.3*	*± 4.7*	*± 2.6*	*± 10.0*	*± 2.1*	*± 6.7*	*± 12.5*	*± 27.9*	*± 11.2*
1000 mean	18.3*	15.7*	12.3	6.7	6.7	14.7	22.0	20.7	92.0	97.0	149.3	135.7*
± SD	± 0.6	± 5.5	± 3.5	± 2.9	± 4.2	± 2.3	± 8.7	± 3.8	± 3.6	± 5.3	± 16.3	± 14.5
5000 mean	15.3	11.3	12.3	9.3*	10.0	15.3	21.3	23.0	89.7	96.0	150.0	104.0
± *SD*	*± 4.2*	*± 1.5*	*± 2.9*	*± 1.5*	*± 6.1*	*± 2.1*	*± 3.2*	*± 4.6*	*± 6.5*	*± 3.6*	*± 19.1*	*± 19.1*
Positive control												
§ 1. mean	34.0**	38.5**	82.0**	20.5**	80.0**	34.0**	119.0**	31.0*	179.5**	143.0**	217.0**	127.5*
± *SD*	*± 5.7*	*± 4.9*	*± 7.1*	*± 0.7*	*± 11.3*	*± 2.8*	*± 28.3*	*± 2.8*	*± 14.8*	*± 5.7*	*± 8.5*	*± 3.5*
§ 2. mean	2747.5**	66.0**	115.5**	28.0**	153.0**	161.0**	518.5**	111.5**	1003.5**	327.0**	944.5**	139.5*
± *SD*	*± 204.4*	*± 8.5*	*± 2.1*	*± 12.7*	*± 8.5*	*± 15.6*	*± 17.7*	*± 4.9*	*± 219.9*	*± 1.4*	*± 50.2*	*± 31.8*
§ 3. mean	9171.5**	106.0**	359.5**	101**	282.0**	493.5**	1219.5**	417.0**	7704.5**	1027.0**	1512.0**	1767.0**
± *SD*	*± 587.6*	*± 11.3*	*± 4.9*	*± 28.3*	*± 1.4*	*± 58.7*	*± 50.2*	*± 106.1*	*± 297.7*	*± 0.0*	*± 19.8*	*± 38.2*

§ = information on respective positive control is reported in Material and Method section A.2. 3 Concentrations were used per plate. 1 = low, 2 = middle, 3 = high.
* = 0.01 ≤ P < 0.05; ** = P < 0.01 (One sided t-test assumes Test > Control)

Table 5.8.1-51: AMPA, metabolite of glyphosate - mutagenicity results (Ames test) with and without metabolic activation ([redacted], 1988), second experiment, plate-incorporation test

Experiment 2: Standard plate test (SPT, plate-incorporation method)												
Strain	**TA 1535**		**TA 1537**		**TA 1538**		**TA 98**		**TA 100**		**WP2 uvrA**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Vehicle control												
Water mean	13.2	14.4	11.4	10.6	11.0	18.2	22.2	23.4	101.8	102.2	197.4	196.8
± *SD*	*± 3.0*	*± 5.6*	*± 2.3*	*± 2.6*	*± 5.1*	*± 1.9*	*± 4.6*	*± 5.7*	*± 13.0*	*± 13.2*	*± 4.1*	*± 15.4*
Test item												
1.6 mean	17.7	11.0	6.3	6.7	12.0	17.7	15.0	18.7	90.7	109.0	174.0	215.0
± *SD*	*± 8.0*	*± 2.0*	*± 1.5*	*± 3.8*	*± 5.3*	*± 12.5*	*± 2.6*	*± 5.0*	*± 2.5*	*± 7.8*	*± 20.0*	*± 19.3*
8.0 mean	19.0*	20.3	12.7	8.0	11.7	14.0	20.3	23.7	104.3	112.7	170.0	218.0*
± *SD*	*± 2.0*	*± 12.3*	*± 3.5*	*± 7.0*	*± 2.1*	*± 4.4*	*± 7.0*	*± 8.4*	*± 14.4*	*± 18.8*	*± 13.1*	*± 7.5*
40 mean	18.0*	11.7	11.7	14.3	15.3	20.7	28.0	18.7	96.3	116.3	201.7	229.7*
± *SD*	*± 3.5*	*± 4.6*	*± 2.1*	*± 6.7*	*± 7.8*	*± 5.7*	*± 6.1*	*± 6.8*	*± 6.5*	*± 8.3*	*± 5.0*	*± 7.4*
200 mean	16.7	6.3	12.3	11.7	9.7	18.3	19.3	17.7	102.3	109.3	185.3	211.0
± *SD*	*± 3.2*	*± 2.5*	*± 3.2*	*± 2.1*	*± 1.5*	*± 3.1*	*± 5.8*	*± 1.5*	*± 22.0*	*± 13.7*	*± 6.0*	*± 19.5*
1000 mean	17.7	11.3	12.7	7.0	12.0	11.7	22.7	14.7	107.7	121.7*	187.3	230.0**
± *SD*	*± 4.5*	*± 4.2*	*± 6.4*	*± 4.6*	*± 4.6*	*± 1.5*	*± 5.0*	*± 2.9*	*± 17.9*	*± 11.8*	*± 7.2*	*± 5.6*
5000 mean	19.3**	10.3	10.3	5.3	9.3	12.3	19.7	19.0	110.7	95.0	184.7	197.3
± *SD*	*± 0.6*	*± 1.5*	*± 5.9*	*± 0.6*	*± 6.4*	*± 1.5*	*± 5.6*	*± 3.0*	*± 10.4*	*± 7.2*	*± 11.0*	*± 19.6*
Positive control												
§ 1. mean	20.0*	49.5**	58.5**	23.5**	90.0**	49.5**	129.0**	52.0**	200.5**	167.5**	290.0*	231.5*
± *SD*	*± 5.7*	*± 4.9*	*± 7.8*	*± 3.5*	*± 18.4*	*± 2.1*	*± 2.8*	*± 0.0*	*± 10.6*	*± 16.3*	*± 82.0*	*± 31.8*
§ 2. mean	450.5**	84.0**	114.0**	37.0**	161.0**	195.0**	495.5**	217.0**	2913.5**	455.0**	996.5**	953.5**
± *SD*	*± 275.1*	*± 7.1*	*± 4.2*	*± 1.4*	*± 33.9*	*± 43.8*	*± 40.3*	*± 2.8*	*± 494.3*	*± 35.4*	*± 194.5*	*± 98.3*
§ 3. mean	8244.5**	142.5**	217.0**	101.0**	506.0**	495.5**	1043.0**	650.5**	7668.0**	1180.0**	1843.5**	1709.5**
± *SD*	*± 507.0*	*± 4.9*	*± 21.2*	*± 0.0*	*± 35.4*	*± 171.8*	*± 58.0*	*± 37.5*	*± 302.6*	*± 96.2*	*± 65.8*	*± 88.4*

§ = information on respective positive control is reported in Material and Method section I.A.2. 3 Concentrations were used per plate, 1 = low, 2 = middle, 3 = high.

* = 0.01 ≤ P < 0.05; ** = P < 0.01 (One sided t-test assumes Test > Control)

Table 5.8.1-52: AMPA, metabolite of glyphosate - mutagenicity results (Ames test) with and without metabolic activation ([redacted], 1988), third experiment, plate-incorporation test

Experiment 3: Standard plate test (SPT, plate-incorporation method)		
Strain	**TA 1537**	
Metabolic activation	**- S9**	**+ S9**
Vehicle control		
Water mean	5.6	8.2
± *SD*	± *1.8*	± *2.2*
Test item		
1.6 mean	11.7	8.7
± *SD*	± *9.1*	± *3.2*
8.0 mean	6.7	7.0
± *SD*	± *3.8*	± *3.5*
40 mean	9.3	9.0
± *SD*	± *6.7*	± *3.6*
200 mean	4.0	6.3
± *SD*	± *1.0*	± *1.5*
1000 mean	5.7	9.3
± *SD*	± *0.6*	± *0.6*
5000 mean	7.0	7.3
± *SD*	± *5.3*	± *2.1*
Positive control		
§ 1. mean	17.5**	68.0**
± *SD*	± *0.7*	± *8.5*
§ 2. mean	33.5**	137.0**
± *SD*	± *4.9*	± *9.9*
§ 3. mean	75.0**	403.0**
± *SD*	± *2.8*	± *35.4*

§ = information on respective positive control is reported in Material and Method section I.A.2. 3 Concentrations were used per plate, 1 = low, 2 = middle, 3 = high.
** = P < 0.01 (One sided t-test assumes Test > Control)

III. CONCLUSIONS

In conclusion, under the experimental conditions reported, the test item is not mutagenic in the Ames test (standard plate test / plate-incorporation method) with and without metabolic activation.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Although statistical significance in the mean number of revertant colonies was observed for all strains at single test item concentrations in the presence or absence of metabolic activation, none of the observations was consistent in independent experiments or showed a dose-response relationship. For strain TA 1537 in the first experiment, the increase in the mean number of revertant colonies was up to 2-fold over control conditions (200 µg/plate) in the absence of S9 mix and up to 1.9-fold over control

conditions (8 and 40 µg/plate) in the presence of S9 mix. In both cases, the responses were only of limited dose-response relationship and of limited statistical significance ($p > 0.01$). Besides, the effects were not reproducible in two further experiments with this strain. Considering all available data of the present study, there was no consistent, statistically significant or biologically relevant increase in the mean number of his^+ or trp^+ revertant colonies observed for any tester strain up to the highest concentration of 5000 µg/plate. Thus, the test item was considered negative for gene mutation in bacteria (*S. typhimurium* TA 100, TA 98, TA 1535, TA 1537 and TA1538 and *E. coli* WP2 uvrA pKM101) with and without metabolic activation.
The study was performed in compliance with GLP and according to OECD guideline 471 (1983). There were only minor deviations when compared to the currently valid OECD guideline 471 (1997), which were considered to not compromise the outcome and the validity of the study. The study is therefore considered valid and acceptable.

Assessment and conclusion by RMS:

1. Information on the study

Data point:	CA 5.8.1/022
Report author	[redacted]
Report year	1980
Report title	CP50435: Microbial Mutagenicity Study
Report No	ET-80-402
Document No	NA
Guidelines followed in study	No guideline followed, the study was conducted similarly to OECD 471 (1983)
GLP	No, not conducted under GLP/ Officially recognised testing facilities. When the study was conducted, GLP was not compulsory.
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	Aminomethylphosphonic acid (CP50435, batch: not reported, purity: 99 %), metabolite of glyphosate was investigated for gene mutation in bacteria in an Ames test. *S. typhimurium* strains TA 98, TA 100, TA 1535, TA1537 and TA 1538, and *E. coli* strain WP2 hcr were exposed to the test item, solvent (water) and appropriate positive controls in the presence and absence of metabolic activation (Aroclor-induced rat liver S9 fraction). A single experiment (plate-incorporation method) was performed, with duplicate cultures, using test item concentrations in the range of 10 – 5000 µg/plate. After incubation at 37 °C for two days, the number of bacterial colonies (his^+ or trp^+ revertants) were counted.
Short description of results:	Evaluation of precipitation and cytotoxicity were not included in the study report. However, when comparing the number of revertant colonies of test item treated plates to vehicle controls, there was no evidence for cytotoxicity. The test item did not induce a statistically significant increase in the number of his^+ or trp^+ revertants in any of the tester strains at any concentration when compared to solvent controls, neither in the presence nor in the absence of metabolic activation. The positive controls markedly increased the number of revertant colonies in all strains, demonstrating the functionality of the S9 fraction and the sensitivity of the test system. Based on the experimental results and under the conditions of the test, aminomethylphosphonic acid, metabolite of glyphosate, is negative for gene mutation in bacteria (Ames test) with and without metabolic activation.

Reasons for why the study is not considered relevant/reliable or not considered as key study:	The study was considered not acceptable due to a number of guideline deviations when compared to OECD guideline 471 (1997). Only a single experiment was performed without giving a justification for the missing confirmatory experiment. Instead of *E. coli* strain WP2 uvrA, strain WP2 hcr was used. In addition, 2-aminoanthracene was used as sole positive control substance in the presence of S9 mix. Historical control data were not provided and evaluation of cytotoxicity and precipitation were not reported. Acceptance and evaluation criteria were not specified in the study report. The study is therefore considered not valid.
Reasons why the study report is not available for submission	
Category study in AIR5 dossier (L-docs)	Category 3b

1. Information on the study

Data point	CA 5.8.1/023
Report author	[redacted]
Report year	1993
Report title	Mutagenicity test: In vitro Mammalian Cell Gene Mutation Test performed with Mouse Lymphoma Cells (L5178Y). Test compound: AMPA, batch 286-JRJ-73-4.
Report No	13270
Document No	NA
Guidelines followed in study	OECD 476 (1983), US CFR part 700 (F) § 798.5300 (1987)
Deviations from current test guideline (OECD 490, 2016)	The newly introduced cytotoxicity parameters RTG (relative total growth), SG (suspension growth) and RSG (relative suspension growth) in OECD TG 490 (2016) could not be re-calculated, since no data on suspension growth were generated. In the current study, cytotoxicity was evaluated based on cloning efficiency after treatment (CE_1, survival) and after selection (CE_2, viability), in accordance with the previous guideline version. Although the growth rates of the cultures were monitored, the data were not provided within the study report. Further, the number of cells treated was below 6 x 10^6 cells, the number of cells recommended in OECD 490 (2016). In several instances, vehicle control cultures exceeded spontaneous levels recommended by OECD guideline 490 (2016). pH and osmolality changes were not assessed. Acceptance criteria specified in the current guideline were not applied and evaluation criteria were based solely on statistics but not on GEF. Although GEF was applied retrospectively, no linear trend was assessed. It was indicated in the report that statistical analysis were conducted, however, they were not clearly reported. In addition, no historical control data were provided for the negative and the positive controls.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive summary

AMPA (batch: 286-JRJ-73-4, purity: 99.2 %), metabolite of glyphosate, was tested in a Mouse Lymphoma assay for its ability to induce forward mutations in mammalian cells *in vitro*. In two independent experiments, duplicate cultures of Mouse Lymphoma L5178Y $TK^{+/-}$ cells were exposed to the test item, medium or appropriate positive controls (75 and 100 µg/mL ethylnitrosourea (ENU) without S9 mix and 5 and 10 µg/mL dimethylbenzanthracene (DMBA) with S9 mix). The assay was conducted in the presence and absence of metabolic activation (Aroclor 1254-induced rat liver S9 fraction).
After 3 hours of exposure with S9 mix or 4 hours of exposure without S9 mix, the cells were incubated for 2 - 3 days to allow expression of the mutant phenotype. The expression period was followed by a selection period, in which the cells were cultivated in selection medium containing trifluorothymidine (TFT) for a period of 10 days. Cell survival and cell viability were assessed as cloning efficiency 1 and cloning efficiency 2 at the end of the exposure period and after the expression period, respectively.
Precipitation of the test item in the medium was not reported and there was no cytotoxicity observed up to the highest tested concentration, neither in the presence nor in the absence of S9 mix. Upon treatment with AMPA there was no statistically significant increase in the number of mutant colonies observed in any of the experiments at any concentration, neither in the presence nor in the absence of S9 mix. Mutant frequencies of the medium control cultures were in the expected range. The positive controls ethylnitrosourea and dimethylbenzanthracene markedly increased the mutation frequency, thus demonstrating functionality of the metabolic activation system and the sensitivity of the test.
Based on the results of the present study, AMPA, metabolite of glyphosate, did not induce mutant frequencies in L5178Y $TK^{+/-}$ cells in the presence or absence of S9-mix and is therefore considered non-mutagenic for mammalian cells *in vitro*.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

	Aminomethyl phosphonic acid (AMPA, metabolite of glyphosate)
Identification:	AMPA
Description:	White powder
Lot/Batch number:	286-JRJ-73-4
Purity:	99.2 %
Stability of test compound:	The stability of the test item at storage conditions (at room temperature in the dark) was guaranteed for at least 3 years. The stability of the test item in solvent was not specified.
Solvent (vehicle) used:	Culture medium

2. Control material:

Negative control:	Untreated cell cultures which were cultivated in cultivation medium only were included in each experiment.
Solvent (vehicle) control:	As culture medium was used as solvent for the test item, the solvent control represents actually the negative control.
Positive control:	- S9 mix: Ethylnitrosourea (ENU), 75 and 150 µg/mL + S9 mix N-nitrosodimethylamine (DMBA), 5 and 10 µg/mL

3. Metabolic activation:

S9 mix was obtained from the livers of Wistar rats weighing approximately 200 g. The rats received a single intraperitoneal injection of Aroclor-1254 at a dose of 500 mg/kg bw. The animals were sacrificed for preparation of liver homogenates 5 days after treatment following a 16 hour period of fasting. The

S9 mix was prepared immediately before the experiment by mixing S9 fraction and co-factors.

S9 mix component	Concentration	Unit
Hepes buffer, 1M (pH 7.2)	20	mM
KCl	33	mM
NADPH-generating system		
Glucose 6-phosphate	3	mM
NADP	4	mM
$MgCl_2$	5	mM
S9	12	% (v/v)

4. Test organism:

L5178Y $TK^{+/-}$ mouse lymphoma cells were used. Stocks were maintained in liquid nitrogen and thawed immediately before use. Each batch used for mutagenicity testing was checked for general morphology, growth characteristics and absence of mycoplasma.

5. Cell culture media:

Cultivation medium: RPMI 1640 medium, supplemented with 10 % horse serum, 200 µg/mL sodium pyruvate and 50 µg/mL gentamicin

Pre-treatment medium A ("THMG medium"): Cultivation medium, supplemented with 9 µg/mL hypoxanthine, 15 µg/mL methotrexate and 22.5 µg/mL glycine

Pre-treatment medium B / treatment medium ("THG medium"): Cultivation medium supplemented with 50 % conditioned medium, 9 µg/mL hypoxanthine and 22.5 µg/mL glycine

Selection medium: Cultivation medium, supplemented with 10 % horse serum and 4 µg/mL trifluorothymidine (TFT)

Incubation: *At 37 °C and 5 % CO_2*

6. Locus examined: Thymidine kinase (TK)

7. Test concentrations and number of replicates:

The dose-range was selected on the basis of a preliminary toxicity test (data not provided in study report) in which the cell-growth was measured for a period of 2-3 days after exposure. If possible the maximum dose was chosen so that the survival (cloning efficiency) was approximately 20 % and so that the cells grew at a normal rate before the end of the 3 day period.

First experiment			
Metabolic activation	**Duration of exposure**	**Concentrations**	**Replicates**
-S9 mix	*4 h*	*0.31, 0.63, 1.3, 2.5 and 5.0*	*Duplicate*

		mg/mL		
+S9 mix	3 h	*0.31, 0.63, 1.3, 2.5 and 5.0 mg/mL*		Duplicate
Second experiment				
Metabolic activation	**Duration of exposure**	***Concentrations***		**Replicates**
-S9 mix	4 h	*0.63, 1.3, 2.5 and 5.0 mg/mL*		Duplicate
+S9 mix	3 h	*0.63, 1.3, 2.5 and 5.0 mg/mL*		Duplicate

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 14 Dec 1992 – 21 Jan 1993

Finalisation date: 18 Feb 1993

2. Mutation assay:

Pre-treatment of cells:

Thawed cells were maintained at a density of 2 x 10^5 - 1.5 x 10^6 in sterile NUNC plastic flasks and incubated at 37 °C and 5 % CO_2. Prior to treatment, spontaneous TK deficient mutants ($TK^{-/+}$ cells) were eliminated from the stock cultures by incubating the cells for 1 - 2 days in THMG medium (pre-treatment medium A), followed by a recovery period of 1 - 3 days in THG medium (pre-treatment medium B).

Treatment:

Two independent experiments using duplicate cultures per condition were performed in the presence and absence of metabolic activation.

For treatment of cells in the absence of S9 mix, cell suspensions of 15 mL containing 3 x 10^5 cells/mL were mixed with 1 mL test item solution, resulting in final concentrations in the range of 0.31 – 5.0 mg/mL (first experiment) and 0.63 – 5.0 mg/mL (second experiment). Afterwards the cultures were incubated for 4 hours at 37 °C under gentle shaking.

For treatment of cells in the presence of S9 mix, cell suspensions of 2 mL containing 2.25 x 10^6 cells were mixed with 0.5 mL S9 mix and 0.5 mL of test item solutions, resulting in final concentrations in the range of 0.31 – 5.0 mg/mL (first experiment) and 0.63 – 5.0 mg/mL (second experiment). The cultures were incubated for 3 hours at 37 °C under gentle shaking.

At the end of the exposure period, the cells were centrifuged, re-suspended in 15 mL fresh medium and a small sample of cells from each culture was diluted and seeded in a microtiter plate at a density of 2 cells/well for determination of relative cell survival (cloning efficiency 1).

Expression period:

After the exposure period, the cells were incubated for a 2 - 3 days expression period, in which each culture was counted daily, diluted to 3 x 10^5 cells/mL and the growth rate was recorded. After the expression period, each culture was divided. One aliquot of each culture was plated to determine the cell viability (cloning efficiency 2) of the cultures, the other one was used for the selection of mutants.

Selection period:
For the selection of mutants, two microtiter plates were prepared from each post-expression culture, seeding 2000 cells per well in medium supplemented with 4 µg/mL trifluorothymidine (TFT). After an incubation period of 10 days, the number of cell clones was counted. The clones were differentiated into large clones and small dense clones. Small colonies were considered to be associated with clastogenic effects, large colonies were considered to be associated with gene mutation effects.

3. Cytotoxicity:

Cloning efficiency (CE_1 survival)
At the end of the exposure period, a sample of each cell culture was collected to assess cell survival. A full 96-well microtiter plate was seeded at a density of 2 cells/well for each culture. After 10 days of incubation, the number of colonies was counted.

CE_2 (viability)
After the expression period, 2 - 3 days after end of exposure, a sample of each cell culture was collected to assess cell viability. For each culture, a full 96-well microtiter plate was seeded at a density of 2 cells/well. After 10 days of incubation, the number of colonies was counted.

4. Evaluation:

Cytotoxicity (cloning efficiency CE)
The number of colonies divided by the number of cells plated was calculated for each sample. The absolute cloning efficiency was determined for each test group, as well as the relative cloning efficiency in comparison to the solvent control group.
CE_1 (survival)
The cytotoxicity of the test substance after the exposure period was determined for each test group and is indicated as absolute and relative cloning efficiency (CE_1 and RCE_1, respectively).

CE_2 (viability)
The cytotoxicity of the test substance at the end of the expression period was determined for each test group and is given as absolute and relative cloning efficiency (CE_2 and RCE_2, respectively).
The cloning efficiency (CE, %) was calculated for each test group as follows:

$$CE_x = -\frac{1}{2}\ln\frac{\text{Number of empty wells}}{\text{Number of wells seeded}}$$

$$RCE_x = \frac{CE_x \text{ of the test group}}{CE_x \text{ of the negative or vehicle control}} \times 100$$

Mutant frequency (MF)
The number of empty wells and the number of wells containing colonies were scored and reported. The colonies are classified into large colonies (indication of gene mutation) and small colonies (indication of chromosome breakage).

Uncorrected mutant frequency:
The uncorrected mutant frequency per 10^6 cells (MF_{uncorr}) was calculated for each test group as follows:

$$MF_{uncorr.} = -\frac{1}{2000}\ln\frac{\text{Number of empty wells}}{\text{Number of wells seeded}}$$

Corrected mutant frequency
The corrected mutation frequency (MF_{corr}) was calculated regarding the values of CE_2:

$$MF_{corr.} = \frac{MF_{uncorr.}}{CE_2} \times 100$$

Determination of borderline mutant frequency based on GEF
The GEF (global evaluation factor) method requires that the MF exceeds a value based on the global distribution of the background MF of the test method. This value is defined as the mean of the negative/vehicle MF distribution plus one standard deviation.
Based on a large data base (n = 493 experiments) from six laboratories a GEF of 126 mutant colonies per 10^6 cells [mean $MF_{corr} = 99 \times 10^{-6}$ colonies; standard deviation = 27×10^{-6} colonies] was calculated for the microwell method. To be judged positive, the mutation frequency has to exceed a threshold of 126 colonies per 10^6 cells (GEF) above the concurrent negative/vehicle control value. The borderline mutant frequency was calculated for each experiment separately as follows:
Borderline MF = $MF_{vehicle\ control\ corr}$ + GEF (126×10^{-6}). The borderline MF was not evaluated as part of the present study, but was determined retrospectively for this evaluation.

5. Statistics:
Statistical analysis was conducted using the Analysis of Variance method on the corresponding test and control cultures.

6. Acceptance criteria:
Acceptance criteria were not defined in the study report.

7. Evaluation criteria:
A substance was considered to be mutagenic if the following criteria were met:

- There was a statistically significant and reproducible increase in the mutation frequency as compared to the negative control cultures.
- A dose-response was evident.
- The mutation frequency at the dose level where the highest effect was found was more than twice the concurrent spontaneous mutant frequency.

Sporadic occurring statistically significant increases in revertants which were not dose related (i.e. occurring at the lower dose level when there was no increase at higher non-toxic doses) were considered incidental and not relevant for the evaluation.

II. RESULTS AND DISCUSSION

1. ANALYTICAL DETERMINATIONS
Analytical determinations were not performed in the present study.

2. CYTOTOXICITY
There was no significant toxicity observed upon treatment with AMPA up to the highest tested concentration of 5.0 mg/mL.

Table 5.8.1-53: Results of the MLA - gene mutation assay in mammalian cells with AMPA, metabolite of glyphosate ([redacted] 1993), first experiment

Test group	Mutagenicity data[$]			Toxicity data[$]			
	Corrected Mutant Frequency per 10^6 cells			Cloning efficiency (CE_1 -survival)		Cloning efficiency (CE_2 -viability)	
	total	small	large	absolute	relative (RCE_1)	absolute	relative (RCE_2)
Without metabolic activation; 4-hour exposure period							

Table 5.8.1-53: Results of the MLA - gene mutation assay in mammalian cells with AMPA, metabolite of glyphosate ([redacted] 1993), first experiment

Test group	Mutagenicity data[$]			Toxicity data[$]			
	Corrected Mutant Frequency per 10^6 cells			Cloning efficiency (CE_1 -survival)		Cloning efficiency (CE_2 -viability)	
	total	small	large	absolute	relative (RCE_1)	absolute	relative (RCE_2)
Medium control	109	45	57	59.0	100.0	1.3	100.0
MF threshold[§]	235	171	183				
Test item [mg/mL]							
0.31	82	43	35	82.0	139.0	131.5	100.4
0.63	138	65	61	65.0	110.2	135.0	103.1
1.30	132	63	58	82.5	139.8	119.5	91.2
2.50	162	72	77	74.5	126.3	103.0	78.6
5.00	172	89	69	66.0	111.9	98.5	75.2
ENU 75 µg/mL	747	306	251	48.0	81.4	71.0	54.2
IMF	*638*	*262*	*194*				
ENU 150 µg/mL	1177	402	381	36.0	61.0	62.0	47.3
IMF	*1095*	*360*	*346*				
With metabolic activation; 3-hour exposure period							
Medium control	178	87	79	59.5	100.0	82.0	100.0
MF threshold[§]	304	213	205				
Test item [mg/mL]							
0.31	118	46	67	77.0	129.4	85.5	104.3
0.63	165	76	77	52.5	88.2	103.5	126.2
1.30	202	82	105	85.5	143.7	78.0	95.1
2.50	170	72	87	65.5	110.1	77.0	93.9
5.00	255	100	139	56.5	95.0	55.0	67.1
DMBA 5 µg/mL	2311	756	859	36.0	60.5	28.0	34.1
IMF	*2433*	*669*	*780*				
DMBA 10 µg/mL	1537	537	518	28.0	47.1	44.0	53.7
IMF	*1420*	*491*	*451*				

IMF: Induced Mutant Frequency, an increase above vehicle MF, IMF should be ≥ 300 x 10^{-6} for total MF or ≥ 150 x 10^{-6} for small colonies
MF: Mutant frequency; § = $MF_{vehicle\ control\ corr}$ + GEF (126 x 10^{-6}), rounded
$ = Mutant frequency values and toxicity data for 10^6 cells. Values differ from those mentioned in study report, where the MF and CE values were given for 10^4 cells.
ENU: Ethylnitrosourea, DMBA: Dimethylbenzanthracene

Table 5.8.1-54: Results of the MLA - gene mutation assay in mammalian cells with AMPA, metabolite of glyphosate ([redacted] 1993), second experiment

Test group	Mutagenicity data[$]			Toxicity data[$]			
	Corrected Mutant Frequency per 10^6 cells			Cloning efficiency (CE_1 -survival)		Cloning efficiency (CE_2 -viability)	
	total	small	large	absolute	relative (RCE_1)	absolute	relative (RCE_2)
Without metabolic activation; 4-hour exposure period							
Medium control	189	88	87	45.0	100.0	81.0	100.0
MF threshold[§]	315	214	213				
Test item [mg/mL]							
0.61	200	96	87	61.0	135.6	89.0	109.9
1.30	198	91	90	58.5	130.0	86.0	106.2
2.50	225	112	94	47.0	104.4	85.5	105.6
5.00	173	86	75	45.0	100.0	82.0	101.2
ENU 75 µg/mL	1484	396	338	45.0	100.0	84.0	103.7
IMF	*1484*	*396*	*338*				
ENU 150 µg/mL	1337	338	384	28.0	47.5	81.0	61.8
IMF	*1337*	*338*	*384*				
With metabolic activation; 3-hour exposure period							
Medium control	164	78	75	56.0	100.0	84.0	100.0
MF threshold[§]	290	204	201				
Test item [mg/mL]							
0.63	87	82	184	60.0	107.1	87.0	103.6
1.30	105	111	236	52.5	93.8	78.5	93.5
2.50	104	100	223	31.0	55.4	79.5	94.6
5.00	91	94	201	38.0	67.9	79.0	94.0
DMBA 5 µg/mL	3356	786	811	22.0	39.3	39.0	46.4
IMF	*3356*	*786*	*811*				
DMBA 10 µg/mL	3479	832	886	18.0	30.3	36.0	43.9
IMF	*3479*	*832*	*886*				

IMF: Induced Mutant Frequency, an increase above vehicle MF, IMF should be ≥ 300 x 10^{-6} for total MF or ≥ 150 x 10^{-6} for small colonies

MF: Mutant frequency; § = $MF_{vehicle\ control}$ corr + GEF (126 x 10^{-6}), rounded

$ = Mutant frequency values and toxicity data for 10^6 cells. Values differ from those mentioned in study report, where the MF and CE values were given for 10^4 cells.

ENU: Ethylnitrosourea, DMBA: Dimethylbenzanthracene

3. SOLUBILITY

Precipitation of the test item was not reported.

4. MUTANT FREQUENCY

There was no statistically significant increase in mutation frequency observed upon treatment with AMPA in both experiments at any of the tested concentrations, neither in the presence, nor in the absence of metabolic activation. Mutant frequencies of the medium control cultures were in the expected range. The positive controls ethylnitrosourea and dimethylbenzanthracene markedly increased the mutation frequency, thus demonstrating functionality of the metabolic activation system and the sensitivity of the test.

III. CONCLUSIONS

Based on the experimental findings, AMPA, metabolite of glyphosate, did not induce mutant frequencies in L5178Y $TK^{+/-}$ cells in the presence or absence of S9-mix. Under the conditions of the test, the test item is negative for mutagenicity in mammalian cells *in vitro*.

3. Assessment and conclusion

Assessment and conclusion by applicant:

In the present study, AMPA was negative for mutagenicity in L5178Y $TK^{+/-}$ cells with and without metabolic activation.
The study was conducted according to OECD guideline 476 (1983) and in compliance with GLP. When compared to the currently valid OECD test guideline 490 (2016), there were a number of deviations of minor degree. Cytotoxicity was evaluated based on cloning efficiencies, in accordance with the previous guideline version. Mutant frequency and toxicity data given in the study report included data from 10^4 cells. For this evaluation, data were calculated retrospectively for 10^6 cells. In addition, the borderline mutant frequency based on GEF was determined retrospectively. The deviations were considered to not compromise the validity of the study. Therefore, the study was considered valid.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.8.1/024
Report author	[redacted]
Report year	2002
Report title	Measurement of unscheduled DNA synthesis (UDS) in rat hepatocytes using an *in vitro* procedure with AMPA (Aminomethylphosphonic acid)
Report No	IPL-R 020625
Document No	NA
Guidelines followed in study	OECD 482 (1986)
Deviations from current test guideline	Not applicable. OECD 482 was deleted in 2014. When compared to the previous OECD 482 (1986), no deviations were observed.
Previous evaluation	Yes, accepted in the RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive

Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary
Executive summary

AMPA, metabolite of glyphosate (batch: A015478701; purity: 99.9 %) was investigated for induction of unscheduled DNA synthesis (UDS) in primary rat hepatocytes *in vitro*. Two independent experiments were performed. Hepatocytes were isolated from the livers of young male Fischer rats and exposed to test item concentrations in the range of 0.625 – 10 mM in medium supplemented with ^{3}H-thymidine. Solvent (medium) and positive controls (2-acetamidofluorene, 6.25 µM) were included in each experiment. After 17 – 20 hours of incubation at 37 °C, slides were prepared for autoradiography. In parallel, cell viability was assessed by MTT assay in separate cultures. A total of 150 cells per condition were scored for nuclear and cytoplasmatic grains and the mean number of net nuclear grains (NNG) per cell was determined to evaluate unscheduled DNA synthesis for each condition. In addition, the percentage of cells in repair and the percentage of cells in S-phase were determined.
Precipitation of AMPA in culture medium was not reported. In addition, there were no changes on osmolality. At 10 mM there was a slight change in pH value with 6.70 vs. 7.26 when compared with the solvent control. Cytotoxicity was observed in the first experiment at 10 mM and in the second experiment at 5 and 10 mM, respectively.
In both experiments, there was no statistically significant or dose-related increase in the mean number of net nuclear grain counts up to the highest tested concentration and no statistically significant increase in the percentage of cells in repair when compared with solvent controls at any tested concentration. Moreover, the frequency of cells in S-phase was low for all conditions.
Values obtained for the solvent and the positive control were within the range of the laboratories historical control data, demonstrating the validity and the sensitivity of the test. Based on the experimental findings and under the conditions of the test, AMPA, metabolite of glyphosate did not induce unscheduled DNA synthesis in primary rat hepatocytes *in vitro*.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Aminomethylphosphonic acid (AMPA)

Identification:	020404
Description:	White crystalline powder
Lot/Batch number:	A015478701
Purity:	99.9 %
Stability of test compound:	The stability of the test item at storage conditions or in solvent were not specified.

1. **Control material:**

Negative control:	The negative control is actually the solvent control.
Solvent (vehicle) control:	Culture Medium (William's E medium)
Positive controls:	2-Acetamidofluorene, 6.25 µM in DMSO

3. Hepatocyte isolation:
Primary rat hepatocytes were isolated by *in situ* collagenase perfusion from the livers of young male Fischer rats. As two independent experiments were performed, one rat liver perfusion was used for each experiment. The animals were anesthetised with pentobarbital and a V-shaped incision was made in the abdominal wall of the animals. The liver was perfused via the portal vein at 37 °C with Hepes buffer for 5 minutes, followed by 5 minutes perfusion with collagenase buffer consisting of Hepes buffer with 4 mM $CaCl^{2+}$ and 0.025 % collagenase. Finally, the perfused liver was dissected and the isolated hepatocytes were washed in a centrifugation step. Cell viability was determined using the trypan blue

technique.

4. Cell culture:

Cell culture establishment:	Freshly isolated hepatocytes were seeded in plating medium at a density of 4.5 x 10^5 cells on coverslips in conventional 6-well plates. The cells were incubated for approx. 90 minutes and allowed to attach.
Plating medium:	William's medium E
Treatment medium:	*William's medium E, supplemented with 10 µCi/mL tritiated thymidine*
Incubation:	*At 37 °C in an atmosphere of 5 % CO_2*

5. Test concentrations:

Experiment	Concentrations	Replicates
First experiment:	0.625, 1.25, 2.5, 5 and 10 mM	Triplicates
Second experiment:	0.625, 1.25, 2.5, 5 and 10 mM	Triplicates

B. STUDY DESIGN AND METHODS

(a) Dates of experimental work: 29 Apr – 02 Jul 2002
Finalisation date: 09 Oct 2002

(b) Cytotoxicity:
Cytotoxicity was determined using the MTT assay. 4.5 x 10^5 cells/well were seeded in two separate wells of a conventional 6-well plate. The cells were exposed to the test item, solvent (medium) or positive control (6.25 µM 2-acetamidofluorene) under conditions which were identical to those used for determination of unscheduled DNA synthesis. Test item concentrations of 0.625 – 10 mM were used. After 17 – 20 hours of incubation at 37 °C, the medium was removed and replaced by 2 mL medium containing 0.5 mg/mL MTT, followed by incubation for 2 - 3 hours. Thereafter, the cells were washed with phosphate buffered saline (PBS). The insoluble formozan was solubilised using HCl : isopropoanol (1 : 23) and the absorbance was read at 550 nm for each concentration.

(c) Unscheduled DNA synthesis:

Cell treatment:
After cell culture establishment, the medium was aspirated from the cells, the hepatocytes were washed with culture medium and finally exposed to the test item, solvent (medium) or positive control (6.25 µM 2-acetamidofluorene) in ^{3}H-thymidine-enriched medium. Test item concentrations in the range of 0.625 – 10 mM were used. After 17 – 20 hours of incubation at 37 °C, the cells were prepared for autoradiography.

Cell harvest and slide preparation:
After treatment, the slides were coated in Kodak NTB-2 liquid emulsion. After gelling, the slides

were incubated in a light-protected box and kept in the refrigerator for 14 days. Finally, the cells were stained with Harris hemalum, dehydrated in ethanol, cleared in xylene and mounted with coverslips for microscopic examination.

Evaluation:
To assess unscheduled DNA synthesis in hepatocytes, grain counting was performed using an image analysis system. 150 cells per condition (3 slides with 50 cells/slide) were scored for nuclear and cytoplasmic grain counts. Only cells with normal morphology were scored. Isolated nuclei with no surrounding cytoplasm, cells with unusual staining artefacts and heavily labelled cells in S-phase were not scored. Net nuclear grains per cell were determined by subtracting the number of cytoplasmatic grain counts from the number of nuclear grain counts.

4. Statistics:
Performance of statistical analysis was not specified in the study report.

5. Acceptance criteria:
The assay was considered valid if the following criteria were met:

- The negative control slides had a group mean net nuclear grain (NNG) value which was in the laboratories historical control range.
- The positive control had a group mean NNG value of not less than 5 NNG counts with 50 % or more cells having NNG counts of 5 or more and the values were statistically significant relative to the solvent control.

6. Evaluation criteria:
The test item was considered positive for unscheduled DNA synthesis (UDS) if the following criteria were met:

- At any concentration tested, group mean net nuclear grain (NNG) values were > 0 and 20 % or more of the cells responded (NNG ≥ 5).
- An increase was seen in both NNG and the percentage of cells in repair.
- A dose related increase was seen in both NNG and the percentage of cells in repair.
- Any induction of UDS was reproduced in an independent experiment.

If a test item failed to induce UDS at any dose in either experiment, it was considered clearly negative in this system.

H. RESULTS AND DISCUSSION

5. ANALYTICAL DETERMINATIONS
Analytical determinations were not performed in the study.

6. CYTOTOXICITY
Cytotoxicity was observed in the first experiment at 10 mM (84.42 % of control) and in the second experiment at 5 and 10 mM (85.9 and 81.80 % of control), respectively.
Osmolality measurements revealed no changes upon addition of AMPA to the culture medium. There was a slight decrease of the pH at 10 mM with 6.70 vs. 7.26 in the solvent control; however, the value was within the biologically acceptable range of pH (6.5 – 8). Based on these findings, 10 mM was chosen as highest concentration for the unscheduled DNA synthesis assay.

7. SOLUBILITY
Precipitation of the test item in culture medium was not reported.

8. UNSCHEDULED DNA SYNTHESIS
In two experiments, AMPA did not induce a statistically significant or dose-related increase in the mean number of net nuclear grain counts when compared to solvent controls up to the highest tested concentration

of 10 mM. In addition, there was no statistically significant increase in the percentage of cells in repair when compared with solvent controls at any tested concentration. Moreover, the frequency of cells in S-phase was low for all conditions.
Values obtained for the solvent and the positive control were within the range of the laboratories historical control data, demonstrating the validity and the sensitivity of the test.

Table 5.8.1-55: Results of the UDS assay with AMPA, metabolite of glyphosate, first experiment ([redacted], 2002)

	Viability (%)	NNG	NNG of cells in repair	% of cells in repair NNG > 5	% of cells in S-phase
Medium control	100.00	-2.38 ± 5.20	5.63 ± 0.51	4.21 ± 1.53	0.0
HCD# mean	/	-2.62 ± 2.04	7.22 ± 1.21	7.42	/
range	/	-5.57 - -0.13	5.96 - 9.5	2 - 12	/
Test item [mM]					
0.625	102.66	-3.81 ± 5.29	6.61 ± 1.56	3.89 ± 1.53	0.0
1.250	104.49	-3.61 ± 5.23	6.99 ± 0.56	4.02 ± 1.53	0.0
2.500	104.03	-3.93 ± 5.24	6.83 ± 0.91	5.18 ± 1.53	0.0
5.000	106.78	-3.35 ± 5.10	6.28 ± 1.66	3.45 ± 1.00	0.0
10.000	84.42	-2.04 ± 4.82	6.91 ± 0.74	5.43 ± 1.53	0.3
Positive control 2-AAF					
6.25 µM	128.69	30.81 ± 18.22	31.55 ± 4.84	96.61 ± 2.00	13.9
HCD# mean	/	20.29 ± 5.72	20.6 ± 5.60	95.25	/
range	/	11.56 - 30.87	12.87 - 31.72	88 - 100	/

HCD#: Historical control data, generated in the testing laboratory in 1999 - 2000 (5 assays)
NNG: Net nuclear grain count

Table 5.8.1-56: Results of the UDS assay with AMPA, metabolite of glyphosate, second experiment ([redacted], 2002)

	Viability (%)	NNG	NNG of cells in repair	% of cells in repair NNG > 5	% of cells in S-phase
Medium control	100.00	-4.612 ± 5.81	5.37 ± 0.21	3.74 ± 2.08	0.3
HCD# mean	/	-2.62 ± 2.04	7.22 ± 1.21	7.42	/
range	/	-5.57 - -0.13	5.96 - 9.5	2 - 12	/
Test item [mM]					
0.625	96.89	-4.77 ± 5.46	6.90 ± 0.60	1.78 ± 0.00	0.7
1.250	94.80	-4.04 ± 5.35	6.87 ± 1.00	4.49 ± 1.53	0.5
2.500	91.34	-3.81 ± 5.82	7.07 ± 1.61	6.18 ± 2.08	0.2
5.000	85.90	-3.47 ± 5.83	7.83 ± 0.29	7.73 ± 1.73	0.6
10.000	81.78	-5.58 ± 6.06	6.97 ± 1.29	3.76 ± 1.53	0.0
Positive control 2-AAF					
6.25 µM	88.89	17.57 ± 11.18	17.73 ± 3.29	94.94 ± 9.45	0.0
HCD# mean	/	20.29 ± 5.72	20.6 ± 5.60	95.25	/
range	/	11.56 - 30.87	12.87 - 31.72	88 - 100	/

HCD#: Historical control data, generated in the testing laboratory in 1999 - 2000 (5 assays)

Table 5.8.1-56: Results of the UDS assay with AMPA, metabolite of glyphosate, second experiment ([redacted], 2002)

	Viability (%)	NNG	NNG of cells in repair	% of cells in repair NNG > 5	% of cells in S-phase

NNG: Net nuclear grain count

III. CONCLUSIONS

Under the conditions of the test, AMPA, metabolite of glyphosate did not induce unscheduled DNA synthesis in primary rat hepatocytes *in vitro*.

3. Assessment and conclusion

Assessment and conclusion by applicant:

In the present study, AMPA was negative for unscheduled DNA synthesis in primary rat hepatocytes *in vitro*.
The study was performed under GLP conditions and in accordance with OECD guideline 482 (1986), which was deleted in 2014. As the Unscheduled DNA Synthesis (UDS) assay is no longer a standard method described by current guidelines, the study was considered to provide supporting information.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.8.1/025
Report author	[redacted]
Report year	1991
Report title	Evaluation of the potential of AMPA to induce unscheduled DNA synthesis in the *in vitro* hepatocyte DNA repair assay using the male F-344 rat.
Report No	SR-91-234
Document No	Not reported
Guidelines followed in study	Similar to OECD 482 (1986)
Deviations from current test guideline	Not applicable. OECD 482 was deleted in 2014. There were no deviations when compared to the previous OECD 482 (1986).
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive summary

AMPA, metabolite of glyphosate (batch: HET-9001-1463-T; purity: 94.38 %) was tested for induction of DNA repair (unscheduled DNA synthesis, UDS) in primary rat hepatocytes *in vitro*. Two independent experiments were performed. Hepatocytes were isolated from the livers of adult male F344 rats and the cells were exposed to test item concentrations in the range of 5 to 5000 µg/mL in medium supplemented with radiolabeled tritiated thymidine (^{3}H-TdR). In the first experiment, 8 concentration steps were used whereas 10 concentration steps were used in the second experiment. Solvent (medium) and positive controls (3 µg/mL 2-acetylaminofluorene) were included in each experiment. After 19 hours of incubation at 37 °C, the cells were visually inspected for cytotoxicity, processed for slide preparation and 90 cells per condition (3 per slide) were evaluated for UDS. UDS was quantified by determining the net increase in nuclear grain counts and the number of cells in repair.
Precipitation of the test item in solvent was not observed. Cytotoxicity was noted at 5000 µg/mL in the first experiment and at 3800 and 5000 µg/mL in the second experiment.
In both experiments, AMPA did not induce significant increases in the mean number of net nuclear grain counts in rat hepatocytes when compared to the solvent control at any of the tested concentrations. In addition, the percentage of cells in repair was ≤ 6 %. Values obtained for the solvent and the positive control were in the expected range, demonstrating the validity and the sensitivity of the test system.
Under the conditions of the test and based on the results of the present study, AMPA, metabolite of glyphosate, did not induce unscheduled DNA synthesis in primary rat hepatocytes *in vitro*.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: AMPA

Identification:	Not specified
Description:	White solid
Lot/Batch number:	HET-9001-1463-T
Purity:	94.38 %
Stability of test compound:	The stability of the test item at storage conditions was guaranteed until the expiry date Jan 1993.

2. Control material:

Negative control:	Untreated cell cultures which were cultivated in cultivation medium only were included in each experiment.
Solvent (vehicle) control:	As culture medium was used as solvent for the test item, the solvent control represents actually the negative control.
Positive controls:	2-Acetylaminofluorene (2-AAF), 3 µg/mL in DMSO

3. Hepatocyte isolation:

Primary rat hepatocytes were isolated by *in situ* collagenase perfusion from the livers of adult male F344 rats. A total of two rats were used in the study, using one rat for each experiment. The rats were approximately 15 – 19 weeks old and weighed 315.4 and 345.6 g. The animals were anesthetised with 60 mg/kg bw pentobarbital, perfused with a collagenase solution and the liver lobes were combined to isolate hepatocytes from the perfused livers. Finally, the cell viabilities were determined prior to plating.

4. Cell culture:

Cell culture establishment:	Freshly isolated hepatocytes were seeded in plating medium at a density of 3 x 10^5 cells. The cells were inoculated into numbered six-well culture dishes containing coverslips. The cells were allowed to attach for 1.5 - 2 hours and afterwards washed with culture medium.

Plating medium: William's medium E, supplemented with 10 % fetal bovine serum, 2 mM L-glutamine and 50 µg/mL gentamycin

Treatment medium: *William's medium E, supplemented with 2 mM L-glutamine, 50 µg/mL gentamicin and 10 µCi/mL tritiated thymidine (^{3}H-TdR, 80 Ci/mmol)*

Incubation: *At 37 °C in a humidified atmosphere containing 5 % CO_2.*

4. Test concentrations:

Experiment	Concentrations	Replicates
First experiment:	5, 10, 50, 100, 500, 1000, 2500 and 5000 µg/mL	Triplicate
Second experiment:	5, 10, 50, 100, 250, 500, 1000, 2500, 3800 and 5000 µg/mL	Triplicate

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 20 Jun – 18 Sep 1991

Finalisation date: 04 Dec 1991

2. Cytotoxicity

Cytotoxicity of the test substance was assessed via visual inspection by light microscopy. Cultures were characterised as "good" (normal cell morphology, sufficient attachment of cells to easily permit scoring of cells), "sparse" (most cells showed normal morphology but attachment of cells was < 25 % of control attachment), "pyknotic nuclei" (a large proportion of cells had small, darkly stained nuclei) or "cytotoxic" (few or no cells present on coverslips, or virtually all cells had pyknotic nuclei or other obvious morphologic effects such as damaged cell membranes or absence of cytoplasm).

6. Unscheduled DNA synthesis

Cell treatment:
Two independent experiments using hepatocytes of different rats were performed. The purpose of the first experiment was to determine cytotoxicity and to interpret any UDS response. On the basis of the cytotoxicity results from the first experiment, a second experiment was performed to confirm the results of the first. After cell culture establishment, triplicate cultures of the hepatocytes were cultivated in ^{3}H-TdR enriched treatment medium. In the first experiments, hepatocytes were exposed for 19 hours at 37 °C to eight test item concentrations in the range of 5 to 5000 µg/mL. In the second experiment, the cells were treated under identical conditions to ten test item concentrations in the range of 5 to 5000 µg/mL. In both experiments, solvent and positive controls (3 µg/mL 2-acetylaminofluorene) were included. After exposure, all cultures were washed with culture medium and processed for slide preparation.

Cell harvest and slide preparation:
Immediately following treatment, the cells were swelled in 1 % sodium citrate solution, fixed in 1:3

glacial acetic acid / ethanol and washed with deionised water. The coverslips were mounted on slides and dipped in Kodak NTB-2 photographic emulsion for 7 days at -20 °C before development. Subsequently, cells were stained with 1 % methyl-green pyronin Y, dried, coverslipped and evaluated.

Evaluation:
Primary hepatocyte DNA repair was determined by quantitative audioradiographic grain counting. The net increase in nuclear grains was quantified for at least 30 morphologically unaltered cells on a randomly selected area of each slide. Three slides per condition were scored. The higher count from two nuclear sized areas over the most heavily labeled cytoplasmic areas adjacent to the nucleus was subtracted from the nuclear count to give the net grains per nucleus. The percentage of cells in repair (cells showing at least 5 nuclear grains) was calculated for each concentration.

4. Statistics:
No statistical methods were used for the evaluation of data.

5. Acceptance criteria:
Data were considered acceptable if the mean number of net grains per nucleus and the percentage of cells in repair values of the solvent control were within the normal range of the laboratories historical control data and if the positive control produced an unscheduled DNA response of greater than 5.0 net grains per nucleus.

3. Evaluation criteria:
A substance was considered unequivocally positive for unscheduled DNA synthesis if the mean net grain count for any dose was greater than 5.0 nuclear grains per cell.
A substance was considered unequivocally negative if the mean net grain count was less than 0 nuclear grains per cell and the percentage of cells in repair was less than 10 % for all groups.
When results fell within 0 to 5 nuclear grains per nucleus or when the percentage of cells in repair exceeded 10 %, the presence of a dose response, the frequency distribution of cellular responses, increases in percentage of cells in repair and reproducibility data among concentrations were considered.

H. RESULTS AND DISCUSSION

1. ANALYTICAL DETERMINATIONS
Analytical determinations have not been performed in the study. Solubility tests with AMPA in culture medium, DMSO, acetone and ethanol were performed prior to the study. Culture medium allowed the highest possible test concentration of 5000 µg/mL and was selected as solvent for the unscheduled DNA synthesis assay.

2. CYTOTOXICITY
Cytotoxicity was observed at 5000 µg/mL in the first experiment and at 3800 and 5000 µg/mL in the second experiment.

3. SOLUBILITY
Precipitation of the test item in culture medium was not reported.

4. UNSCHEDULED DNA SYNTHESIS
In both experiments, AMPA did not induce significant increases in the mean number of net nuclear grain counts in rat hepatocytes when compared to those of the solvent control at any tested concentration. In triplicate cultures per condition, the values for nuclear grains per nucleus were negative and the percentage of cells in repair was ≤ 6 % up to the highest concentration tested.
Values obtained for the solvent and the positive control were as expected, demonstrating the validity and the sensitivity of the test system.

Table 5.8.1-57: Results of the UDS assay with AMPA, first experiment ([redacted], 1991)

First experiment			
	Net grains / nucleus Mean ± SE	**Median**	**% of cells in repair**
Medium control	-15.3 ± 0.7	-14.9	3
Test item [µg/mL]			
5.00	-17.3 ± 1.4	-17.6	0
10.00	-14.1 ± 2.5	-16.2	6
50.00	-13.9 ± 3.1	-12.2	1
100.00	-17.8 ± 3.1	-17.6	2
500.00	-18.6 ± 2.7	-18.9	0
1000.00	-13.1 ± 2.2	-13.5	0
2500.00	-12.9 ± 1.8	-12.2	1
5000.00	Toxic[#]	-	-
Positive control 2-AAF [3 µg/mL]	9.4 ± 3.7	10.8	63

2-AAF: 2-acetylaminofluorene
SE: Standard errors, represent slide-to-slide variation
[#]: Slide unscorable

Table 5.8.1-58: Results of the UDS assay with AMPA, second experiment ([redacted], 1991)

Second experiment			
	Net grains / nucleus Mean ± SE	**Median**	**% of cells in repair**
Medium control[#]	-12.6 ± 0.5	-13.5	2
Test item [µg/mL]			
5.00	-11.1 ± 0.9	-10.8	1
10.00	-12.7 ± 2.2	-12.8	2
50.00	-13.0 ± 1.9	-12.2	1
100.00	-12.3 ± 1.5	-12.2	1
250.00	-11.5 ± 1.3	-12.2	2
500.00	-11.8 ± 1.1	-12.2	2
1000.00	-11.6 ± 1.2	-11.5	2
2500.00	-8.8 ± 1.5	-8.8	1
3800.00	Toxic[#]	-	-
5000.00	Toxic[#]	-	-
Positive control 2-AAF [3 µg/mL]	19.7 ± 3.0	20.3	85

2-AAF: 2-acetylaminofluorene
SE: Standard errors, represent slide-to-slide variation
[#]: Slide unscorable

III. CONCLUSIONS

Based on the results of the present study, AMPA, metabolite of glyphosate did not induce unscheduled DNA synthesis in primary rat hepatocytes *in vitro*.

3. Assessment and conclusion

Assessment and conclusion by applicant:

In the present study, AMPA was negative for unscheduled DNA synthesis in primary rat hepatocytes *in vitro*.
The study was conducted in accordance with GLP and similar to OECD guideline 482 (1986), which was deleted in 2014. The study was considered to provide supporting information only because the Unscheduled DNA Synthesis (UDS) assay is no longer a standard method described by current guidelines. There were no deviations when compared to the previous OECD guideline 482 (1986).

Assessment and conclusion by RMS:

Genotoxicity – *in vivo*

1. Information on the study

Data point	CA 5.8.1/026
Report author	[redacted]
Report year	1993
Report title	Mutagenicity test: Micronucleus test with AMPA, batch 286-JRJ-73-4
Report No	13268
Document No	146-GLY
Guidelines followed in study	OECD 474 (1983), US EPA 40 CFR part 700 (F) § 798.5395 (1987)
Deviations from current test guideline (OECD 474, 2016)	According to the current guideline OECD 474 (2016), at least 4000 polychromatic erythrocytes per animal should be evaluated for the presence of micronuclei. However, in the present study only 1000 polychromatic erythrocytes were evaluated. The percentage of polychromatic erythrocytes among total erythrocytes was determined for 200 erythrocytes instead of 500 erythrocytes. Only a single dose was tested. Although bone marrow toxicity was observed, no lower dose levels were additionally tested. In addition, no 24-hour control animals were included (first sampling time point). No data on proficiency and/or historical control data were provided and acceptance and evaluation criteria were not specified in the study report.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (4L docs)	Category 2a

2. Full summary

Executive summary

AMPA, metabolite of glyphosate (batch: 286-JRJ-73-4, purity: 99.2 %) was tested for its clastogenic potential in NMRI mice in a micronucleus test. Based on the results of an initial toxicity study in which no signs of toxicity became evident, the micronucleus test was performed at a dose level of 5000 mg/kg bw.

The test item was dissolved in 0.9 % NaCl with 1 % carboxymethyl cellulose and administered as a single dose to groups of 5 mice/sex at a constant dosage volume of 20 mL/kg bw. Similar constituted groups of 5 mice/sex received the positive (cyclophosphamide, 30 mg/kg bw) or vehicle control (0.9 % NaCl with 1 % carboxymethyl cellulose).
Bone marrow sampling of the test item treated groups was performed 24, 48 and 72 hours after treatment. Positive and vehicle control animals were sacrificed 24 and 48 hours after dosing, respectively. Smears were prepared from the femoral bone marrow of each animal and 1000 immature erythrocytes (PCE) per animal were scored for the presence of micronuclei. The percentage of PCEs in 200 erythrocytes was calculated. In addition, the number of micronucleated normochromatic erythrocytes (NCEs) was determined.
Oral administration of 5000 mg/kg bw AMPA was associated with a clear depression of erythropoiesis, as the ratio of PCE to NCE was significantly decreased when compared to solvent control animals.
Upon treatment with AMPA there was no statistically significant increase in the frequency of micronucleated PCEs at any sampling time point when compared to those of control animals. The incidence of micronucleated PCEs in the solvent and positive control groups were in accordance with the laboratory's historical control data, confirming the sensitivity of the test and demonstrating the capability of the test animals to respond to mutagenic substances.
Based on the experimental findings, AMPA, metabolite of glyphosate is negative for cytogenetic effects in bone marrow in mice *in vivo*.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	AMPA, metabolite of glyphosate
Description:	White powder
Lot/Batch #:	286-JRJ-73-4
Purity:	99.2 %
Stability of test compound:	The stability of the test item at storage conditions (at room temperature in the dark) was guaranteed for at least 3 years. The stability of the test item in solvent was not specified.
Solvent (vehicle) used:	1 % carboxymethyl cellulose (CMC) in 0.9 % NaCl

2. Control materials

Solvent (vehicle) control:	1 % carboxymethyl cellulose (CMC) in 0.9 % NaCl
Positive control:	Cyclophosphamide, 30 mg/kg bw

3. Test animals:

Species:	Mouse
Strain:	Bom:NMRI
Sex:	Male and female
Source:	[redacted]
Age at study initiation:	8 weeks
Weight at dosing:	25 - 37 g
Acclimation period:	5 days
Diet/Food:	Altromin 1314 (Chr. Petersen Ltd., Ringsted, Denmark), *ad libitum*

Water:	Tap water acidified with hydrochloric acid to pH 2.5, *ad libitum*
Housing:	In groups of 5/sex in Macrolon type III cages measuring 420 x 260 x 150 mm with pinewood softwood sawdust bedding.

6. Environmental conditions:

Temperature:	21 ± 3 °C
Humidity:	55 ± 15 %
Air changes:	Approximately 10/hour
Photoperiod:	12-hour light and dark cycle

7. Test concentrations and treatment groups:

c) Preliminary toxicity study

Dose levels:	5000 mg/kg bw
Concentrations:	250 mg/mL
Dose volume:	20 mL/kg bw
Number of animals:	3/sex
Route of administration:	Oral gavage

d) Main micronucleus test

Dose levels:	5000 mg/kg bw
Concentrations:	250 mg/mL
Dose volume:	20 mL/kg bw
Number of animals:	5/sex/group
Route of administration:	Oral gavage

B. STUDY DESIGN AND METHODS

3. Dates of experimental work: 26 Oct - 12 Nov 1992
Finalisation date: 18 Feb 1993

4. Animal assignment and treatment:

Preliminary toxicity study:
AMPA, metabolite of glyphosate was expected to be of low toxicity. To determine the maximum tolerated dose, a single dose of 5000 mg/kg bw was administered by oral gavage to 3 mice/sex. Each one male and one female mouse were sacrificed 24, 48 and 72 hours after dosing and bone marrow smears were prepared. For each animal, the percentage of polychromatic erythrocytes among the total of erythrocytes was determined for 200 erythrocytes.
Based on the results of the preliminary toxicity study, 5000 mg/kg bw was selected as dose level for the main micronucleus test.

Main micronucleus test:
Groups of 5 mice/sex received a single dose of 5000 mg/kg bw by oral gavage at a constant dosage volume of 20 mL/kg bw. Similar constituted groups of 5 mice/sex received the vehicle (0.9 % NaCl with 1 % carboxymethyl cellulose) or the positive control (30 mg/kg bw cyclophosphamide, administered via intraperitoneal injection).
At 24, 48 and 72 hours after treatment, each one group of animals was sacrificed by cervical dislocation and bone marrow smears were prepared. Positive control animals were sacrificed 24 hours after treatment, whereas the vehicle control group was sacrificed 48 hours after dosing.

5. Slide preparation:

Immediately after sacrifice, the right femoral bone was dissected, the proximal end of the femur was cut and marrow cells were flushed out with foetal calf serum. After whirl-mixing, the bone marrow cells were centrifuged and smears were prepared. The specimens were fixed in methanol and stained with May-Grünwald/Giemsa.

6. Slide evaluation:

Slides were randomly coded and examined by microscopical analysis. About 1000 polychromatic erythrocytes (PCE) per animal were scored for the presence of micronuclei. The percentage of PCEs in 200 erythrocytes was calculated. In addition, the number of micronucleated normochromatic erythrocytes (NCEs) was determined during the counting of 1000 PCEs.

7. Statistics:

The number of micronucleated PCEs in the test group were compared to the number found in the vehicle group. Statistical analysis was performed using the one-way analysis of variance performed on the values transformed to normal scores according to Blom's method[33].

8. Acceptance criteria:

Acceptance criteria were not specified in the study report.

9. Evaluation criteria:

Evaluation criteria were not specified in the study report.

II. RESULTS AND DISCUSSION

D. ANALYTICAL DETERMINATIONS

Analytical determinations of the test substance in vehicle were not performed.

E. PRELIMINARY TOXICITY STUDY

Clinical signs of systemic toxicity were not described in the study report. Bone marrow toxicity, evident as a reduced ratio of polychromatic (PCE) to normochromatic erythrocytes (NCE) was observed to a minor degree. Between 32 – 46 % of PCE were found in the animals, indicating that erythropoiesis was not or only slightly affected.

Based on the results of the preliminary toxicity study, 5000 mg/kg bw was selected as dose level for the main micronucleus test.

F. MAIN MICRONUCLEUS TEST

Systemic toxicity:

Mortality:

No mortality occurred.

Clinical signs of toxicity:

Clinical signs of toxicity have not been described in the study report.

Evaluation of bone marrow slides:

The percentage of polychromatic erythrocytes (PCEs) in AMPA-treated animals was statistically significantly lower than in control animals, indicating a clear depression of erythropoiesis and demonstrating that bone marrow toxicity was evident.

In addition, there was no statistically significant increase in the frequency of micronucleated PCEs (mPCEs) of test item-treated animals at any sampling time point when compared to those of control

[33] Blom, 1958: Statistical Estimates and Transformed Beta Variables, New York: John Wiley and Sons, Inc.

animals. Although there was a slight trend towards an increase in mPCE in test item-treated males with later sampling time points, the values remained within those of control animals, were not significant and standard deviations were comparably low. The incidence of micronuclei in the solvent and positive control groups were in accordance with the laboratory's historical control data, confirming the sensitivity of the test and demonstrating the capability of the test animals to respond to mutagenic substances.

Table 5.8.1-59: Summary of genotoxicity data for AMPA obtained in the micronucleus test in mice ([redacted] 1993)

Treatment	Dose (mg/kg bw)	Sampling time	Males		Females	
			mPCE ± SD# /1000 PCE	mean %# PCE ± SD / 200 erythrocytes	mPCE ± SD# /1000 PCE	mean %# PCE ± SD / 200 erythrocytes
CMC	20 mL/kg bw	48 h	0.80 ± 0.45	36.40 ± 2.61	0.20 ± 0.45	34.40 ± 2.97
Test item	5000	24 h	0.20 ± 0.45	28.20 ± 5.63	0.20 ± 0.45	25.20 ± 1.79
Test item	5000	48 h	0.60 ± 0.55	28.80 ± 4.92	0.20 ± 0.45	28.40 ± 3.21
Test item	5000	72 h	0.80 ± 0.45	26.60 ± 5.03	0.40 ± 0.55	24.20 ± 1.64
CPA	30	24 h	12.40 ± 2.07	35.00 ± 2.55	13.08 ± 3.03	33.40 ± 4.16

mPCE: micronucleated polychromatic erythrocytes
CMC: carboxymethyl cellulose, solvent control; CPA: cyclophosphamide, positive control
#: all values wre re-calculated based on raw data of individual animals provided in the study report.

III. CONCLUSIONS

Based on the experimental findings, AMPA, metabolite of glyphosate did not induce micronuclei in the bone marrow of mice *in vivo*.

3. Assessment and conclusion

Assessment and conclusion by applicant:

In the present study, AMPA was negative for clastogenic effects in the bone marrow of male and female NMRI mice *in vivo*.
The study was conducted under GLP and in accordance with OECD guideline 474 (1983). There were a number of deviations when compared to the current OECD guideline 474 (2016). Only 1000 polychromatic erythrocytes (PCE) were investigated per animal. However, the number of micronucleated PCE did not exceed those of control animals, standard deviations were comparably low and the values were statistically not significant when compared to control conditions. Thus, the test result was considered to be clearly negative. In addition, the percentage of polychromatic erythrocytes among total erythrocytes was determined for 200 erythrocytes instead of 500 erythrocytes and control animals were not included for the first sampling time point. These and further deviations were considered to be of minor degree and to not compromise the validity of the study. Therefore, the study is considered valid and acceptable.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.8.1/027
Report author	[redacted]
Report year	1993
Report title	Mouse Micronucleus Study of AMPA
Report No	[redacted]-13243
Document No	EHL-90170 / ML-90-404
Guidelines followed in study	The study was performed similarly to OECD 474 (2016)
Deviations from current test guideline (OECD 474, 2016)	According to the current guideline OECD 474 (2016), at least 4000 polychromatic erythrocytes per animal should be evaluated for the presence of micronuclei. However, in the present study only 1000 polychromatic erythrocytes were evaluated. The animals were dosed via intraperitoneal injection, which is not representative for a human route of exposure. A justification for the route of administration was not given. Although there was no concurrent proof for bone marrow exposure, signs of systemic toxicity were observed and it can be assumed that bone marrow exposure was achieved by the intraperitoneal injection. Historical control data for positive control animals were not included. Acceptance criteria were not specified and evaluation criteria mentioned were inconsistent with those recommended by the guideline.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive summary

AMPA, metabolite of glyphosate (batch: HET-9001-1463T, purity: 94.38 %) was tested for its clastogenic potential in CD-1 mice in a micronucleus test. In two initially performed toxicity studies, in which clinical signs of toxicity and mortality were observed at ≥ 606 mg/kg bw, a LD_{50} of 1357.7 mg/kg bw was determined. 1000 mg/kg bw (representing approx. 74 % of the LD_{50}) was selected as top dose level for the main micronucleus study.

The test item was dissolved in corn oil and administered as a single intraperitoneal injection to groups of 5 mice/sex at dose levels of 100, 500 and 1000 mg/kg bw. Similarly constituted groups of 5 mice/sex received the solvent or the positive control (cyclophosphamide, 40 mg/kg bw).

Bone marrow sampling of the test item and solvent control treated groups was performed 24, 48 and 72 hours after treatment. Positive control animals were sacrificed 24 hours after dosing. Smears were prepared from the femoral bone marrow of each animal and 1000 polychromatic erythrocytes (PCE) per animal were scored for the presence of micronuclei. In addition, the percentage of PCEs among a total of 1000 erythrocytes was determined.

Intraperitoneal injection of AMPA induced signs of systemic toxicity, evident as listlessness, in 6/15 males and 1/15 females at 500 mg/kg bw and in 13/15 males and 3/15 females at 1000 mg/kg bw. The symptoms were observed until study termination. In addition, body weight development was statistically significantly impaired in mid and high dose males of the 48-hour sampling time point and in high dose

group females of the 72-hour sampling time point. The findings were attributed to treatment and considered toxicologically relevant.
Although bone marrow exposure can be assumed, due to the observations on systemic toxicity, there was no bone marrow toxicity in terms of a decreased PCE/total erythrocyte ratio observed in any AMPA treated group and for any sampling time point.
A statistically significant increase in the frequency of micronucleated PCEs when compared to solvent controls was observed for females at 100 mg/kg bw at the 72 hour sampling time point. The values were within the range of the laboratories historical control data and showed no dose-response relationship, therefore the finding was not attributed to treatment. There was no statistically significant increase in the mean number of micronucleated PCEs observed for any other male or female dose group at any other sampling time point.
The incidence of micronucleated PCEs in the solvent controls remained within the range of historical control data, whereas those of the positive controls showed a marked increase of statistical significance. The results obtained for the solvent and positive controls thus confirmed the sensitivity of the test and demonstrated the capability of the test animals to respond to mutagenic substances.
Based on the experimental findings, AMPA, metabolite of glyphosate is negative for cytogenetic effects in bone marrow in mice *in vivo*.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	Aminomethylphosphonic acid (AMPA, metabolite of glyphosate)
Identification:	AMPA
Description:	White solid
Lot/Batch #:	HET-9001-1463T (Test sample T900031)
Purity:	94.38 %
Stability of test compound:	The stability of the test item at storage conditions (at room temperature in the dark) was guaranteed until the expiry date Jan 1993. The stability of the test item in solvent was not specified.
Solvent (vehicle) used:	Corn oil
2. Control materials	
Solvent (vehicle) control:	Corn oil
Positive control:	Cyclophosphamide monohydrate, 40 mg/kg bw in Hank's balanced salt solution (HBSS)
3. Test animals:	
Species:	Mouse
Strain:	CD-1
Sex:	Male and female
Source:	[redacted]
Age at study initiation:	7 – 10 weeks
Weight at dosing:	27.9 – 38.7 (males) and 21.5 – 33.2 (females)
Acclimation period:	Minimum 7 days
Diet/Food:	Purina Certified Laboratory Rodent Chow® No. 5002 (Purina Mills Inc., St. Louis, Missouri), *ad libitum*
Water:	Tap water from the public water supply, *ad libitum*

Housing:	Individually in stainless steel cages with stainless steel mesh bottoms.

4. Environmental conditions:

Temperature:	64 - 79 °F (18 – 26 °C)
Humidity:	40 - 70 %
Air changes:	Not specified
Photoperiod:	12-hour light and dark cycle

5. Test concentrations and treatment groups:

e) First preliminary toxicity study

Dose levels:	1000 and 5000 mg/kg bw
Concentrations:	Not specified
Dose volume:	10 mL/kg bw
Number of animals:	2/sex/dose level
Route of administration:	Intraperitoneal injection

f) Second preliminary toxicity study

Dose levels:	303, 606, 1250, and 2500 mg/kg bw
Concentrations:	Not specified
Dose volume:	10 mL/kg bw
Number of animals:	3/sex/dose level
Route of administration:	Intraperitoneal injection

g) Main micronucleus test

Dose levels:	100, 500 and 1000 mg/kg bw
Not specified	Not specified
Dose volume:	10 mL/kg bw
Number of animals:	5/sex/group
Route of administration:	Intraperitoneal injection

B. STUDY DESIGN AND METHODS

1. **Dates of experimental work:** 20 Aug – 24 Oct 1990
   Finalisation date: 08 Dec 1993

2. **Animal assignment and treatment:**

Preliminary toxicity studies:

An initial range-finding experiment was conducted using 2 mice/sex/dose level. A single dose of 1000 or 5000 mg/kg bw was administered via intraperitoneal injection at a constant dosage volume of 10 mL/kg bw. Afterwards, the animals were observed for mortality and clinical signs of toxicity during a 4-days observation period.

Based on the results of the first preliminary toxicity study, a second toxicity study was performed prior to the main micronucleus assay in order to estimate the maximum tolerated dose (MTD). In the second preliminary study, groups of 3 mice/sex were injected with test item concentrations in the range of 303 - 2500 mg/kg bw. After dosing, the animals were also observed for clinical signs of toxicity and mortality. Each 1/3 males and 1/3 females of the 303 and 606 mg/kg bw dose group and 1/3 females of the 1250 mg/kg bw dose group were sacrificed 24 hours after dosing for erythrocyte evaluation (data

not provided in study report). The remaining animals were observed for three further days.
LD_{50} values and the maximum tolerated dose were estimated with the data obtained in both preliminary experiments. Based on the findings of the two initial toxicity studies, the dose levels for the main micronucleus study were selected.

Main micronucleus test:
Groups of 5 mice/sex received a single AMPA dose of 100, 500 or 1000 mg/kg bw via intraperitoneal injection. The test item was administered at a constant dosage volume of 10 mL/kg bw. Similarly constituted groups of 5 mice/sex received the vehicle (corn oil) or the positive control (40 mg/kg bw cyclophosphamide).
Each one group of test item-treated or vehicle control animals was sacrificed by cervical dislocation 24, 48 and 72 h after treatment, followed by preparation of bone marrow smears. Positive control animals were sacrificed 24 hours after treatment.

3. Slide preparation:
After sacrifice, the femora of the animals were removed, cut and marrow cells were flushed out with fetal bovine serum. The cells were centrifuged and smears were prepared. For each animal two slides were prepared. The slides were allowed to air-dry over night and stained with Wright-Giemsa Stain Pak by using a Hema-Tek II slide staining machine.

4. Slide evaluation:
Slides were randomly coded prior to analysis. For each animal, a total of 1000 erythrocytes was evaluated for the amount of polychromatic (PCE) among total erythrocytes and about 1000 PCEs were scored for the presence of micronuclei. PCEs containing more than one micronucleus were scored as single micronucleated PCE (mPCE). In a few cases significant discordance in mPCE frequency were initially observed between two slide scorers (e.g. a difference of 4 or more mPCE). In these cases slides were rescored to determine if the discordance was reproducible and the rescored values were used for reporting and analysis.

5. Statistics:
LD_{50} estimates were calculated using the Probit method on toxicity range-finding data. The individual test animal was used as the individual unit for analysis of micronucleated polychromatic erythrocyte (PCE) frequency, PCE/total erythrocyte ratio and body weight change. Micronucleated PCE frequencies per animal were transformed as the square root prior to analysis (Snedecor and Cochran[34] (1967) and MacGregor[35] et al. (1987)). PCE/total erythrocyte ratios were not transformed. A Dunnett's test (one sided) was used for comparison of treatment group and positive control values with solvent control values.

6. Acceptance criteria:
Acceptance criteria were not specified in the study report.

7. Evaluation criteria:
Evaluation criteria were not specified in the study report. A test item was considered to induce a statistically significant, treatment-related response in micronuclei formation if the following criteria were met:

- There was a dose- and time-dependent effect, which was consistent with a treatment-induced response.
- The degree of the response was in relation to both concurrent and historical negative and positive control data.

II. RESULTS AND DISCUSSION

[34] Snedecor, G.W. and Cochran, W. G. (1967) Statistical Methods, 6th edition, 223-226 and 325-327, Iowa State Press, Ames, Iowa.
[35] MacGregor, J.T., Heddle, J.A., Hite, M., Margolin, B. H., Ramel, C., Salaake, M.F., Tice, R.R. and Wild, D. (1987). Guidelines for the conduct of micronucleus assays in mammalian bone marrow erythrocytes. *Mutation Research 189* 103-112.

A. ANALYTICAL DETERMINATIONS

Analytical determinations were not performed in the present study.

B. PRELIMINARY TOXICITY STUDY

In the first preliminary toxicity study, mortality was observed in 2/2 males and 1/2 females at 1000 mg/kg bw 3 and 4 days following treatment and in 2/2 males and 2/2 females at 5000 mg/kg bw within the first two days after dosing. During the 4-day observation period until death, all animals showed ante-mortem signs of systemic toxicity which included listlessness and unresponsiveness.

In the second preliminary toxicity study, mortality was noted in 1/2 males and 0/2 females at 606 mg/kg bw on study Day 4, in 2/3 males and 0/2 females at 1250 mg/kg bw on study Days 2 and 3 and in 2/3 males and 1/3 females at 2500 mg/kg bw on study Day 2, respectively. Clinical signs of toxicity, evident as listlessness or unresponsiveness were noted in 2/2 males at 606 mg/kg bw on study Days 3 and 4, in 2/3 males and 3/3 females at 1250 mg/kg bw throughout the whole observation period and in 3/3 males and 2/3 females at 2500 mg/kg bw throughout the whole observation period.

The LD_{50} for male and female mice combined was calculated to be 1357.7 mg/kg bw. Based on the results of the two preliminary toxicity studies, 1000 mg/kg bw (representing approx. 74 % of the LD_{50} value) was selected as maximum tolerated dose level for the main micronucleus test.

C. MAIN MICRONUCLEUS TEST

Systemic toxicity:

Mortality:

No mortality occurred.

Clinical signs of toxicity:

Listlessness was observed in 6/15 males and 1/15 females at 500 mg/kg bw and in 13/15 males and 3/15 females at 1000 mg/kg bw. The symptoms were present until study termination up to 72 hours after dosing. No clinical signs of toxicity were observed at the 100 mg/kg bw dose group or in solvent control animals.

Body weight development:

Statistically significant decreases in mean body weight change were observed for the mid (500 mg/kg bw) and high dose (1000 mg/kg bw) test group in males sacrificed 48 hours after dosing and for the mid dose group in females sacrificed 24 and 72 hours after dosing. The impaired body weight development in males of the 48-hour sampling time point exhibited a dose-response pattern. Thus, the observations were attributed to treatment and considered toxicologically relevant.

Table 5.8.1-60: Body weight development after treatment with AMPA, observed in the micronucleus test in mice ([redacted], 1993)

Treatment	Dose (mg/kg bw)	Sampling time	Mean BW change [g] mean ± SD	
			Males	Females
Corn oil	10 mL/kg bw	24 h	-0.8 ± 0.5	-0.6 ± 0.5
		48 h	-0.8 ± 0.6	-0.9 ± 0.4
		72 h	-0.5 ± 1.4	-0.2 ± 0.8
Test item	100	24 h	-0.5 ± 1.3	-1.4 ± 1.0
		48 h	-1.5 ± 0.4	-0.8 ± 0.6
		72 h	-0.3 ± 1.6	-0.6 ± 0.8

Table 5.8.1-60: Body weight development after treatment with AMPA, observed in the micronucleus test in mice ([redacted], 1993)

Treatment	Dose (mg/kg bw)	Sampling time	Mean BW change [g] mean ± SD	
			Males	Females
	500	24 h	-1.9 ± 0.7	-1.6 ± 0.5*
		48 h	-2.2 ± 1.2*	-1.1 ± 1.2
		72 h	-2.1 ± 1.9	-2.0 ± 0.8**
	1000	24 h	-1.4 ± 0.16	-1.4 ± 0.8
		48 h	-3.2 ± 1.0**	-1.6 ± 1.2
		72 h	-1.0 ± 0.9	-0.4 ± 0.7
CPA	40	24 h	-1.2 ± 0.3	-0.5 ± 0.4

CPA: cyclophosphamide, positive control
BW: body weight
* $p \leq 0.05$ and ** $p \leq 0.01$ by one-sided Dunnett's test

Evaluation of bone marrow slides:
There was no statistically significant decrease in the mean polychromatic erythrocytes (PCE)/total erythrocytes ratio for any of the AMPA treated or control groups at any sampling time point, indicating that no bone marrow toxicity was evident. However, as clinical signs of toxicity were observed in AMPA-treated animals of the 500 and 1000 mg/kg bw groups, it can be assumed that bone marrow was exposed.
A statistically significant increase in the frequency of micronucleated PCEs when compared to solvent controls was noted for females at the low dose level (100 mg/kg bw) at the 72 hour sampling time point. The values were within the range of the laboratories historical control data and showed no dose-response relationship, therefore the finding was not considered to reflect a treatment-related effect. There was no statistically significant increase in the mean number of micronucleated PCEs observed for any other male or female dose group at any other sampling time point.
The incidence of micronuleated PCEs in the solvent controls remained within the range of historical control data. The positive control (cyclophosphamide) yielded expected positive responses in micronucleated PCE frequency, indicating the adequacy of the experimental conditions.

Table 5.8.1-61: Summary of genotoxicity data for AMPA obtained in the micronucleus test in mice ([redacted], 1993)

Treatment	Dose (mg/kg bw)	Sampling time	Males		Females	
			mPCE ± SD /1000 PCE	% PCE/ 500 erythrocytes mean ± SD	mPCE ± SD /1000 PCE	% PCE/ 500 erythrocytes mean ± SD
Corn oil	10 mL/kg bw	24 h	0.2 ± 0.4	0.40 ± 0.05	1.0 ± 1.4	0.40 ± 0.10
		48 h	0.6 ± 1.3	0.48 ± 0.05	0.4 ± 0.9	0.50 ± 0.07
		72 h	0.2 ± 0.4	0.56 ± 0.02	0.0 ± 0.0	0.52 ± 0.04
	HCD mean *range*	24 -72 h	0.954 ± 1.303 *0.00 - 2.40*		1.421 ± 2.676 *0.00 - 7.40*	
Test item	100	24 h	0.2 ± 0.4	0.42 ± 0.07	0.8 ± 0.8	0.48 ± 0.06
		48 h	0.0 ± 0.0	0.44 ± 0.07	0.2 ± 0.4	0.42 ± 0.08
		72 h	0.0 ± 0.0	0.51 ± 0.02	1.6 ± 1.1*	0.63 ± 0.09
	500	24 h	0.1 ± 0.3	0.43 ± 0.05	2.0 ± 2.9	0.38 ± 0.12
		48 h	0.6 ± 0.9	0.54 ± 0.09	0.2 ± 0.4	0.49 ± 0.02

Treatment	Dose (mg/kg bw)	Sampling time	Males		Females	
			mPCE ± SD /1000 PCE	% PCE/ 500 erythrocytes mean ± SD	mPCE ± SD /1000 PCE	% PCE/ 500 erythrocytes mean ± SD
		72 h	0.0 ± 0.0	0.50 ± 0.07	0.8 ± 0.8	0.62 ± 0.04
	1000	24 h	0.8 ± 1.3	0.42 ± 0.09	0.8 ± 0.8	0.41 ± 0.06
		48 h	0.2 ± 0.4	0.46 ± 0.07	0.0 ± 0.0	0.45 ± 0.08
		72 h	0.0 ± 0.0	0.51 ± 0.03	0.4 ± 0.9	0.58 ± 0.06
CPA	40	24 h	18.3 ± 10.9**	0.43 ± 0.06	12.0 ± 12.3*	0.48 ± 0.05

mPCE: micronucleated polychromatic erythrocytes

CPA: cyclophosphamide, positive control

HCD: historical control data on mean mPCE/1000 PCEs (24 - 72 h sampling combined data), generated in the testing laboratory (time phrame not specified)

* $p \leq 0.05$ and ** $p \leq 0.01$ by one-sided Dunnett's test. Square root transformed data used for statistical analysis of mPCE

III. CONCLUSIONS

Based on the experimental findings, AMPA, metabolite of glyphosate did not induce micronuclei in the bone marrow of mice *in vivo*.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study was conducted in compliance with GLP and similar to OECD guideline 474 (2016), although some deviations became evident. The number of polychromatic erythrocytes (PCE) evaluated was less than the 2000 PCE which are recommended by the current guideline. Based on the evaluation of 1000 PCE/animal, the test item was considered negative for clastogenic effects in the bone marrow of male and female CD-1 mice *in vivo*. Although there was a statistically significant increase in the frequency of micronucleated polychromatic erythrocytes (PCE) in females at the low dose level (100 mg/kg bw) at the 72 hour sampling time point, the values were within the range of the laboratories historical control data and showed no dose-response relationship. Thus, the finding was not considered to reflect a treatment-related effect. It can be assumed that the negative test result obtained in the study would be the same if more cells were evaluated.

With regard to the positive controls, male animals showed a distinct increase in mPCEs of statistical significance. In 2/5 positive control females no induction of micronuclei in PCEs was observed, while a marked increase in mPCEs was observed for the remaining 3/5 positive control females. This finding was not mentioned or further discussed in the study report. However, a statistically significant increase in mPCE was still observed when combining the data of all 5 animals. Therefore, the results obtained were considered to confirm the sensitivity of the test and to demonstrate the capability of the test animals to respond to mutagenic substances.

Although bone marrow toxicity, indicated by a decreased PCE to normochromatic erythrocyte (NCE) ratio, was not observed, clinical signs of toxicity indicated systemic availability of the test substance. In addition, the test item was administered by intraperitoneal injection, therefore it can be assumed that bone marrow exposure was achieved.

Further deviations were of minor degree and considered to not compromise the validity of the study. The study is therefore considered valid and acceptable.

Assessment and conclusion by RMS:

Reproductive toxicity

Developmental toxicity

1. Information on the study

Data point	CA 5.8.1/028
Report author	[redacted]
Report year	1992
Report title	AMPA: Teratogenicity study in rats
Report No	7891
Document No	124-GLY
Guidelines followed in study	US EPA Pesticide Assessment Guidelines Subdivision F, 83-3
Deviations from current test guideline (OECD 414, 2018)	Duration of treatment was shorter than required. The following endpoints were not assessed: weight and histopathological changes of the thyroid glands, foetal anogenital distance (AGD), indication of incomplete testicular descent/cryptorchidism in male foetuses; blood samples from dams to assess thyroid hormons (T4, T3 and TSH) were not collected.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Executive summary

This developmental study in rats was performed in two successive replicates, the first containing 10 mated females per treatment group, the second a further 15 females per group. For each replicate, mated female Sprague Dawley rats were randomised into four treatment groups. The animals were dosed by gavage once daily over Days 6-16 inclusive of gestation, where Day 0 was the day of detection of mating. Dose levels of test material were as follows: 0, 100, 350 and 1000 mg/kg bw/day. The animals were monitored during gestation for clinical signs of toxicity and for body weight and food consumption performance. They were sacrificed on Day 20 of gestation and the conceptuses were evaluated. The live foetuses were subsequently examined for developmental abnormalities and variants of the viscera and skeleton, including the state of skeletal ossification.

There was no evidence of toxicity in either the dam or embryo-fetal development at any of the dose levels applied.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** AMPA

Description:	white powder
Lot/Batch number:	286-JRJ-73-4
Purity:	99.2 %
CAS#:	1066-51-9
Stability of test compound:	Stable until 09-Jun-1995

2. Vehicle:	0.5 % carboxymethylcellulose (CMC) in distilled water
3. Test animals:	
Species	Rat
Strain	Sprague-Dawley CD
Age/weight on arrival	Approximately 9 weeks / approx. 250 g
Source	[redacted]
Housing	Individually, in polypropylene cages (42 x 27 x 20 cm), with a stainless steel grid bottom, top and food hopper
Acclimatisation period	3-5 days
Diet	Rat and Mouse Breeder Diet No. 3 (Expanded) SQC (Special Diets Services (SOS) Limited), *ad libitum*
Water	Mains water, *ad libitum*
Environmental conditions	Temperature: 20 ± 2°C Humidity: 50 ± 15 % Air changes: 15 - 20 changes / hour Photoperiod: 12 hours light / 12 hours dark

B. STUDY DESIGN AND METHODS

Dates of Experimental work: Not reported

In-life dates: Not reported (QA audits conducted between March 1992 and July 1992)

Mating procedure: Mating was on the basis of two females to each male, female siblings not being paired with the same male. For each female, cohabitation with a male was continuous until mating was detected. A vaginal lavage was examined each morning and the day of detection of sperm in the lavage, or of a copulatory plug in situ, was considered as Day 0 of gestation. A record was kept of the male which inseminated each female.

Animal assignment: On detection of mating, the females were re-housed in an individual cage and allocated to a treatment group using a computer-generated series of randomly sequenced numbers representing the treatment groups.

Dose selection rationale: Dose levels were agreed with the Sponsor after evaluation of existing toxicity data. These data indicated that the material in rats has a low toxic potential, with a lowest effect level (LEL) of 1200 mg AMPA/kg bw/day by dietary administration and an oral LD_{50} of approximately 8000 mg AMPA/kg bw. It was considered appropriate to proceed with the teratogenicity study using the conventional 'limit' dose of 1000 mg AMPA/kg bw/day as the highest dose level.

Dose preparation and analysis: The test item was formulated as a part-solution, part-suspension, the vehicle being a 0.5 % solution of carboxymethylcellulose (CMC) in distilled water. Fresh formulations were prepared daily, each concentration being prepared separately. The requisite quantity of test item was weighed into a labelled container. The necessary volume of vehicle was added, and mixing was by means of a Silverson laboratory mixer/emulsifier. The undissolved material was maintained in suspension during dosing procedures by means of a magnetic stirrer.

Samples of dosing formulations were analysed at the IRI Analytical Chemistry Laboratory under the provisions of IRI Project No. 353917 (Method Establishment, homogeneity, accuracy and stability) and under Project No. 490421 (concentration accuracy and homogeneity of actual dosing formulations).

For each study replicate, triplicate samples of 1 mL were taken from each concentration on the first day of treatment, then again near the end of the treatment period.

Dosage administration: The mated females were dosed once daily, at approximately the same time each

day, over Days 6-16 inclusive of gestation, where Day 0 was the day of detection of mating.
Doses were administered orally, by gavage, at a volume of 10 mL/kg bw, using a steel dosing cannula. The volume of formulation administered to each female was determined each day by the weight of that animal as measured at the time of administration.

Clinical observations: All animals were checked for viability at the beginning of each day and again as late as possible on each day. All animals were examined for reaction to treatment on each day. The nature, onset, duration and intensity of any signs were recorded, based on a routine examination conducted 1-1.5 h after dosing, with any necessary follow-up examinations.

Bodyweight: Individual body weights were recorded on Days 0, 6, 9, 13, 17 and 20 of gestation.

Food consumption: The weight of the food consumed by each mated female was recorded daily throughout, commencing on Day 4 of gestation (weighed quantity first offered on Day 3 of gestation).

Terminal investigations: On Day 20 of gestation, the animals were killed by carbon dioxide asphyxiation. The contents of the thoracic and abdominal cavities were examined macroscopically for abnormalities. Any abnormal tissue was sampled and preserved in neutral buffered 10 % formalin, to facilitate any necessary histological analysis. The reproductive tract was removed and weighed intact, then opened and the contents were examined. The number of corpora lutea in each ovary and the number and position of all implantation sites in the uterus were recorded. Each implant was classified as being live, a foetal death (death judged to have occurred after ca. Day 16 of gestation), a late embryonic death (death judged to have occurred in the period ca. Day 12- Day 16) or an early embryonic death (death judged to have occurred prior to ca. Day 12).

Foetal observations: Each live foetus was individually identified within the litter and its weight was recorded. The foetuses were examined for externally visible abnormalities prior to fixation. Approximately one half of the foetuses from each uterus were fixed in methylated ethyl alcohol, and the remaining half in Bouin's fluid. Those foetuses fixed in alcohol were subsequently examined by open dissection for visceral abnormalities. The eviscerated carcasses were then cleared in potassium hydroxide and glycerol, and the skeletons were stained with Alizarin Red S. Skeletal structures in these foetuses were examined for abnormalities and variants, including state of ossification.

Those foetuses fixed in Bouin's fluid were examined for soft tissue abnormalities and variants by means of a freehand razor blade sectioning technique derived from that of Wilson.

The sex of each foetus was determined during the dissection procedures.

Statistical analyses: It was considered unnecessary to conduct any formal statistical tests on the data from this study. Interpretation was able to be based on examination of the individual and group mean values.

II. RESULTS AND DISCUSSION

A: ANALYSIS OF DOSE FORMULATIONS

Dose formulation analysis data show that the formulations used in the study were prepared to an acceptable level of accuracy, were homogeneous, and were stable for at least 24 hours.

B: CLINICAL OBSERVATIONS

One female at 1000 mg/kg bw/day was difficult to dose on Day 13 of gestation; salivation was noted at dosing but had ceased by 1 h post-dosing. For a second high dose animal a hard mass was noted associated with the left scapula. Post-mortem examination identified the mass as a 5 x 4 mm cartilaginous/bony outgrowth from left scapula, apparently non-neoplastic. These findings were considered incidental and not associated with treatment. In addition to these, there were sporadic findings common to this strain of animals (e.g. patches of alopecia, skin scabbing), there having been no relationship to treatment in their

incidence.

C. BODY WEIGHT

There was no effect of treatment with AMPA on maternal body weights.

Table 5.8.1-62: AMPA: Teratogenicity study in rats ([redacted], 1992): Summary of maternal body weight and body weight gain.

	Dose level of AMPA (mg/kg bw/day)			
Body weight (g ± S.D.) Day post coitum	**0 (control)**	**100**	**350**	**1000**
6	303 ± 25	303 ± 19	300 ± 27	306 ±23
13	347 ± 27	347 ± 24	344 ± 27	348 ± 26
17	387 ± 31	387 ± 28	384 ± 30	391 ± 31
20	439 ± 37	439 ± 34	439 ± 35	447 ± 33
Body weight gain (g ± S.D.) Day 6-17 post coitum	83.5 ± 11.3	83.5 ± 12.2	83.8 ± 8.5	85.0 ± 15.3

S.D. = Standard deviation

D. FOOD CONSUMPTION

There was no effect of treatment with AMPA on maternal food consumption.

Table 5.8.1-63: AMPA: Teratogenicity study in rats ([redacted], 1992): Summary of maternal food consumption (g/animal/day)

	Dose level of AMPA (mg/kg bw/day)			
Food consumption Day post coitum	**0 (control)**	**100**	**350**	**1000**
6	31	30	30	29
13	34	33	34	35
17	38	36	38	39
19	30	32	34	32
Total consumption Days 6-17 post coitum	413	406	415	424

E. NECROPSY

Gross pathology: Not reported.

Maternal performance:

There was no effect of treatment on any of the examined parameters of pregnancy performance. In particular, there were no notable inter-group differences in the incidence of intra-uterine death, or in mean foetal weight.

Table 5.8.1-64: AMPA: Teratogenicity study in rats ([redacted], 1992): Intergroup comparison of maternal performance

Observation	Dose level of AMPA (mg/kg bw/day)			
	0 (control)	**100**	**350**	**1000**
No. of animals mated	25	25	25	25
No. of animals pregnant	25	25	24	24
No. of premature decedents	0	0	0	0
Pregnancy frequency (%)	100	100	96	96
Mean No. of corpora lutea ± SD	18.2 ± 2.0	17.5 ± 1.9	17.3 ± 1.7	17.9 ± 1.7
Mean No. of implantations ± SD	17.8 ± 1.7	17.0 ± 2.0	16.9 ± 1.9	17.5 ± 1.8

Pre-implantation loss (%)	2	3	3	2
Mean live implantations (%) ± SD	16.6 ± 2.0	15.3 ± 3.5	15.5 ± 2.8	16.8 ± 1.9
Mean dead implantations (%)± SD	1.2 ± 1.4	1.7 ± 2.8	1.4 ± 1.4	0.8 ± 0.8
Mean early embryonic deaths ± SD	1.1 ± 1.4	1.6 ± 2.6	1.1 ± 1.3	0.6 ± 0.8
Mean late embryonic deaths ± SD	0.2 ± 0.4	0.1 ± 0.4	0.3 ± 0.7	0.1 ± 0.3
Mean foetal deaths	0	0	0	0.04 ± 0.2
Live foetal sex ration (m f)	1:0.92	1:1.01	1:1.11	1:0.92
Mean total uterus weight (g) ± SD	98.5 ± 11.5	89.7 ± 19.2	93.0 ±14.5	99.0 ± 11.1
Mean litter mean foetal weight (g) ± SD	3.76 ± 0.19	3.73 ± 0.21	3.78 ± 0.26	3.68 ± 0.18

SD = Standard deviation

F. FOETUSES

There was-no effect of AMPA on the number, growth or survival of the foetuses *in utero*.

Major and minor abnormalities of various kinds occurred sporadically in all treatment groups. None of the incidences exceeded the levels expected from this strain of animals in this laboratory, and no effect of treatment was noted.

Equally, the various parameters of skeletal ossification state were considered not to indicate an effect of treatment. None of the inter-group differences, taking parameters individually or collectively, were considered of sufficient magnitude to be of biological significance.

Table 5.8.1-65: AMPA: Teratogenicity study in rats ([redacted], 1992): Group incidence of major foetal abnormalities

Observation	Dose level of AMPA (mg/kg bw/day)			
	0 (control)	100	350	1000
	Incidence of foetuses (litters)			
Total no. of foetuses examined	414	383	372	402
Total no. of litters examined	25	25	24	24
Cleft palate, oedema; limb, digit and lung abnormalities ± diaphragmatic hernia	0	6 (1)	0	0
Right-sided aortic arch	0	0	0	1 (1)
Abnormalities of atlas and occipital bones	0	1 (1)	0	0
Interventricular septal defect	0	0	1 (1)	0
Partial situs inversus, interventricular septal defect	0	0	0	1 (1)
Forelimbs extended downwards ± carpal flexure, shortened and thickended ribs, shortened body oedema	5 (1)	6 (2)	0	0
Oedema, fused digits, misshapen genital papilla	0	0	0	1 (1)
Hydronephrosis	2 (2)	0	1 (1)	1 (1)

III. CONCLUSIONS

Treatment with AMPA at dose levels of up to 1000 mg/kg bw/day produced no evidence of toxicity in either the dam or the outcome of pregnancy.

3. Assessment and conclusion

Assessment and conclusion by applicant:

This study is considered acceptable although some endpoints that are requested in the OECD 414 TG

from 2018 are not included in this study. Treatment with AMPA at dose levels of up to 1000 mg/kg bw/day produced no evidence of toxicity in either the dam or embryo-fetal development. The dose level of 1000 mg AMPA/kg bw/day was considered the no observed effect level (NOAEL) in this study for both maternal and developmental effects.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.8.1/029
Report author	[redacted]
Report year	1991
Report title	A dose range-finding developmental toxicity study of AMPA in rats
Report No	[redacted]-50146
Document No	n.a.
Guidelines followed in study	Not applicable for this dose range-finding study
Deviations from current test guideline	Not applicable for this dose range-finding study
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

The potential maternal toxicity and developmental toxicity of AMPA were evaluated in rats. AMPA in corn oil was administered to five groups of eight mated Sprague Dawley Crl:CD•BR rats once daily from gestation Days 6 through 15. Dosage levels were 125, 250, 500, 750 and 1000 mg/kg bw/day administered in a constant volume of 10 mL/kg bw. A concurrent control group, composed of eight mated females, received the vehicle, corn oil, on a comparable regimen at 10 mL/kg bw. The route of administration was oral by gastric intubation. Clinical observations and body weights were recorded. All animals were sacrificed on gestation Day 20 for a scheduled Caesarean section. The uteri and ovaries were examined and the numbers of foetuses, early and late resorptions, total implantations and corpora lutea were recorded. Mean gravid uterine weights and net body weight changes were calculated for each group. The kidneys, spleen and liver of each female were weighed. The foetuses were weighed, sexed, examined for external malformations and developmental variations and discarded.

All animals in the study survived to the scheduled necropsy. No compound-related clinical findings were observed at any dose level. There were no adverse effects at any dose level on mean body weights, body weight gains, gravid uterine weights, net body weight changes and organ weights. Treatment with AMPA had no apparent adverse effect on intrauterine growth and survival, and there were no external malformations or developmental variations observed in this study.

Based on the results of this study, dose levels of 150, 400 and 1000 mg/kg bw/day were considered suitable for the subsequent definitive developmental toxicity study of AMPA in rats.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	AMPA
Description:	white solid
Lot/Batch number:	HET-9001-1463T
Purity:	94.38 %
Stability of test compound:	Stable until Jan 1993
2. Vehicle:	Corn oil
3. Test animals:	
Species	Rat
Strain	Sprague-Dawley CD
Age on arrival	Approximately 10 weeks
Body weight at initiation of dosing	265 - 278 g (group mean values)
Source	[redacted]
Housing	Individually, in wire-mesh cages suspended above card board (except for mating when animals were housed pairwise)
Acclimatisation period	4 weeks
Diet	Purina® Certified Rodent Chow #5002, *ad libitum*
Water	Drinking water, *ad libitum*
Environmental conditions	Temperature: 70 - 73 °F (corresponding to 21 - 23 °C) Humidity: 59 ± 86 % Air changes: approximately 10 changes / hour Photoperiod: 12 hours light / 12 hours dark

B. STUDY DESIGN AND METHODS

In-life dates: 19 Jun 1990 – 14 Jul 1990

Mating procedure: At the end of the acclimatization period, animals judged to be in good health and meeting acceptable body weight requirements (a minimum of 220 grams) were placed in a suspended wire-mesh cage with a resident male from the same strain and source for breeding. Resident males were untreated, sexually mature rats utilized exclusively for breeding. A breeding record containing the male and female identification numbers and the dates of cohabitation was prepared. The females were approximately 14 weeks old when paired for breeding.
Positive evidence of mating was confirmed by the presence of a copulatory plug in the vagina or the presence of sperm in a vaginal smear. Each mating pair was examined daily. The day on which evidence of mating was identified was termed day 0 of gestation and the animals were separated.

Animal assignment: The mated females were consecutively assigned in a block design to groups containing eight rats each by the following randomization procedure. The first mated female and the appropriate gestation Day 0 designation were entered on the form and the female was assigned to group 1, the second mated female was assigned to group 2, and the third to group 3, etc. This process was continued daily until eight females were placed into each group. Body weight values ranged from 217 g to 285 g on Day 0 of gestation.

Dose preparation and analysis: An appropriate amount of the test item (AMPA) was weighed for each group into tared weigh boats and transferred to a mortar. The test material was triturated with corn oil, and ground with a pestle until a slurry was obtained. The slurry was transferred to a graduated cylinder via a series of vehicle rinses. A sufficient amount of vehicle was added to attain a volume of 250 mL. The cylinder was inverted several times to ensure adequate mixing and the contents were transferred to a properly labeled storage container. The cylinder was rinsed with an additional 100 mL of vehicle which was also added to the storage container. The preparations were then mixed for approximately 5 minutes on a Polytron PT6000 homogenizer to reduce particle size and to ensure proper mixing. The dosing

preparations were visually inspected for homogeneity prior to dispensation for the first day of dose administration.
Preparations for all dose groups were made once at study start, and were stored at room temperature. The preparations were stirred using a magnetic stir plate and bar each day prior to dispensing and during the dosing procedure.
Analysis of dose formulation was not performed in this dose range-finding study.

Dose administration: The dose formulations were administered orally by gavage, via a 16-gauge stainless steel gavage cannula, once daily for 10 consecutive days initiating on gestation Day 6 and continuing up to and including Day 15 of gestation. A dosage volume of 10 mL/kg bw was used for all dose levels. The control animals received corn oil on a comparable regimen of 10 mL/kg bw. Individual dosages were based on the most recent body weights.

Clinical observations: All animals were observed twice daily for moribundity and mortality. Detailed clinical observations were recorded individually from Days 0 through 20 of gestation (prior to compound administration during the dosing period). Animals were observed for signs of toxicity approximately one hour after treatment throughout the dosing period; all significant findings were recorded.

Bodyweight and gravid uterine weight: Maternal body weights were recorded individually on gestation Days 0, 6, 9, 12, 16, 18 and 20. A group mean body weight was calculated for each of these days. Mean body weight changes were calculated for each corresponding interval and for days 6-16, 16-20 and 0-20.
Gravid uterine weight, net body weight (the Day 20 body weight minus the weight of the uterus and contents) and net body weight change (the Day 0-20 body weight change minus the weight of the uterus and contents) were presented for each gravid female at the scheduled Caesarean section.

Food consumption: Food consumption was not recorded in this study.

Terminal investigations: All maternal animals were sacrificed by carbon dioxide inhalation on gestation Day 20. The thoracic, abdominal and pelvic cavities were opened by a ventral midline incision and the contents examined. In all instances, the post mortem findings were correlated with the ante mortem comments and any abnormalities were recorded. The uterus and ovaries were excised. The number of corpora lutea on each ovary was recorded. The trimmed uterus was weighed, opened and the number and location of all foetuses, early and late resorptions and the total number of implantation sites were recorded. Uteri with no macroscopic evidence of nidation were opened and subsequently placed in 10 % ammonium sulphide solution for detection of early implantation loss as described by Sa1ewski.
The liver, kidneys and spleen from each dam were excised, trimmed and weighed and all findings recorded. The carcasses of the dams, including all organs, were then discarded.

Foetal observations: A detailed external examination of each foetus was conducted to include, but was not limited to, the eyes, palate, and external orifices. Findings were recorded as either developmental variations or malformations. The weight and sex of each foetus were recorded and the foetuses were discarded.

Statistical analyses: All analyses were conducted using two-tailed tests for a minimum significance level of 5 % comparing each treated group to the vehicle control group. Each mean was presented with the standard deviation (S.D.) and the number of animals (N) used to calculate the mean. The following statistical tests were performed: Chi-square test with Yates' correction factor (foetal sex ratios); Fisher's Exact test (malformations and variations); Mann-Whitney U test (early and late resorptions, dead foetuses, post-implantation losses); One-way ANOVA with Dunnett's test (corpora lutea, total implantations, viable foetuses, foetal body weights, maternal body weights and weight changes, maternal net body weight changes and gravid uterine weights, selected organ weights).

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS

Dose formulation analysis was not performed in this dose range-finding study
.

B. CLINICAL OBSERVATIONS

All animals survived to the scheduled sacrifice on gestation Day 20. There were no apparent test item-related clinical effects observed in any of the AMPA treated groups. Faeces, which appeared grey in colour, were observed infrequently during the daily examinations at dose levels of 250 mg/kg bw/day and above. The predominant findings at the 1-hour post-dosing period included red staining around the nose, noted in one or two animals in each of the treated groups, and a low incidence of salivation at dose levels of 250 mg/kg bw/day and above. However, no dose-relationship was apparent and the limited incidence of these findings was not considered to be a conclusive indication of a treatment effect.
Other clinical findings (hair loss, soft stool, yellow anogenital, urogenital and proximal tail staining, rales, red staining or material around the vaginal area, nose and mouth, and decreased defaecation and urination) occurred similarly in the control and treated groups or were generally single occurrences.

C. BODY WEIGHT

No adverse effects on mean body weights or mean body weight gains were apparent in any dose group throughout the study. Mean gravid uterine weights and net body weight changes in the treated groups were also apparently unaffected by test item administration. No statistically significant differences were observed between the control group values and those of the AMPA-treated groups.

Table 5.8.1-66: AMPA: DRF teratogenicity study in rats ([redacted], 1991a): Summary of maternal body weight, body weight gain and gravid uterine weight.

	Dose level of AMPA (mg/kg bw/day)					
	0 (control)	125	250	500	750	1000
No. of females	8	8	8	8	8	7
Body weights (g ± SD) Day post coitum						
0	240 ± 16.1	246 ± 11.9	253 ± 13.3	240 ± 9.5	243 ± 20.8	243 ± 11.6
6	266 ± 16.7	269 ± 13.1	278 ± 14.1	265 ± 13.0	270 ± 20.6	269 ± 14.4
12	277 ± 17.1	287 ± 13.9	293 ± 13.8	279 ± 13.1	281 ± 17.8	282 ± 13.1
16	302 ± 23.5	314 ± 17.8	316 ± 16.6	300 ± 13.7	309 ± 23.8	308 ± 16.5
20	365 ± 27.1	377 ± 21.2	379 ± 18.9	359 ± 32.1	374 ± 26.3	349 ± 21.5
Body weight gain (g ± SD) Day 6-16 post coitum	36 ± 8.9	46 ± 11.1	38 ± 8.7	35 ± 11.1	39 ± 7.2	39 ± 4.7
Gravid uterine weight (g ± SD)	73 ± 12.2	78.9 ± 17.1	77.1 ± 8.7	69.8 ± 29.1	77.2 ± 8.2	78.0 ± 3.7

SD = Standard deviation

D. FOOD CONSUMPTION

Food consumption was not recorded in this study.

E. NECROPSY

Gross pathology: At scheduled sacrifice on gestation Day 20, macroscopic necropsy findings were observed as follows. One female at 500 mg/kg bw/day had a pitted kidney while one control group female had a dilated renal pelvis containing white precipitate. The only other remarkable internal finding was an accessory spleen in one female at 250 mg/kg bw/day. These findings were considered not due to treatment.

Organ weights: Mean liver, kidney and spleen weights in the treated groups were similar to the control group, indicating no apparent adverse effect from AMPA administration.

Caesarean section data: The mean numbers and percentages of post-implantation losses and viable

foetuses in the 125, 250, 500, 750 and 1000 mg/kg bw/day groups were similar to the control group. Mean foetal body weights in the treated groups were also comparable to the control group. No remarkable differences were observed between the control and treated groups in foetal sex ratios or the mean numbers of corpora lutea and implantation sites. It was noted that a single female at 1000 mg/kg bw/day was non-gravid; this was considered not due to treatment.

Table 5.8.1-67: AMPA: DRF teratogenicity study in rats ([redacted], 1991a): Intergroup comparison of maternal performance and Caesarean section data

Observation	Dose level of AMPA (mg/kg bw/day)					
	0 (control)	125	250	500	750	1000
No. of animals mated	8	8	8	8	8	8
No. of premature decedents	0	0	0	0	0	0
No. of animals pregnant	8	8	8	8	8	7
Pregnancy frequency (%)	100	100	100	100	100	87.5
Mean No. of corpora lutea ± SD	16.8 ± 3.11	16.1 ± 2.23	17.3 ± 3.06	16.3 ± 3.33	16.9 ± 2.64	16.3 ± 2.29
Mean No. of implantations ± SD	14.9 ± 2.03	14.6 ± 3.02	15.5 ± 1.93	14.3 ± 5.12	15.1 ± 1.55	15.0 ± 1.29
Mean post-implantation loss ± SD	0.9 ± 0.83	0.4 ± 0.52	1.1 ± 0.99	0.9 ± 0.83	0.9 ± 0.99	0.4 ± 0.79
Mean viable foetuses ± SD	14.0 ± 2.27	14.3 ± 3.28	14.4 ± 1.69	15.3 ± 1.50	14.3 ± 1.83	14.6 ± 0.98
Mean dead foetuses ± SD	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
Mean foetal weight (g) ± SD	3.50 ± 0.15	3.7 ± 0.21	3.5 ± 0.21	3.4 ± 0.24	3.5 ± 0.18	3.6 ± 0.25
Total No. of male female foetuses	51:61	57:57	57:58	60:47	59:55	52:50

SD = Standard deviation

F. FOETUSES

There was-no effect of AMPA on the number, growth or survival of the foetuses *in utero*.

The number of foetuses (litters) available for evaluation numbered 112(8), 114(8), 115(8), 107(7), 114(8) and 102(7) in the control, 125, 250, 500, 750 and 1000 mg/kg bw/day groups, respectively. No external malformations or developmental variations were noted in this study.

III. CONCLUSIONS

The dose level of 1000 mg AMPA/kg bw/day was the no observed effect level in this study for both maternal and developmental effects. Based on the results of this study, dose levels of 150, 400 and 1000 mg/kg bw/day were considered suitable for the subsequent definitive developmental toxicity study of AMPA in rats.

3. Assessment and conclusion

Assessment and conclusion by applicant:

This dose range-finding study is considered acceptable. Treatment with AMPA at dose levels of up to 1000 mg/kg bw/day produced no evidence of toxicity in either the dam or on any embryo-fetal development endpoint.

Assessment and conclusion by RMS:

1. Information on the study

Data point	CA 5.8.1/030
Report author	[redacted]

Report year	1991
Report title	A developmental toxicity study of AMPA in rats
Report No	[redacted]-50159
Document No	M-645464-01-1
Guidelines followed in study	US EPA Pesticide Assessment Guidelines Subdivision F, 83-3; JMAFF 59 NohSan No. 4200, OECD 414 (1981)
Deviations from current test guideline (OECD 414, 2018)	Duration of treatment was shorter than required. The following endpoints were not assessed: weight and histopathological changes of the maternal thyroid glands, foetal anogenital distance (AGD), indication of incomplete testicular descent/cryptorchidism in male foetuses; assessment of dams' thyroid hormones (T4, T3 and TSH)
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

Executive summary

The potential maternal toxicity and developmental toxicity of AMPA were evaluated in this study. AMPA in corn oil was administered orally by gavage to three groups of 25 mated Charles River Crl: CD BR female rats once daily from gestation days 6 through 15. Dose levels were 150, 400 and 1000 mg/kg bw/day administered in a constant volume of 10 mL/kg bw. A concurrent control group, composed of 25 mated females, received the vehicle on a comparable regimen at 10 mL/kg bw. Throughout gestation all females were observed twice daily for appearance and behaviour. Body weights and food consumption were recorded at appropriate intervals. On day 20 of gestation, all females were sacrificed for a scheduled Caesarean section. The liver, kidneys and spleen of each female were weighed. The uteri and ovaries were examined and the numbers of foetuses, early and late resorption, total implantations and corpora lutea were recorded. Mean gravid uterine weights and net body weight changes were calculated for each group. Foetuses were weighed, sexed and examined for external, skeletal and soft tissue malformations and developmental variations.

Maternal survival was unaffected by treatment at all dose levels. No treatment-related gross necropsy findings were observed. Clinical findings which appeared related to test item administration occurred at 400 and 1000 mg/kg bw/day and included mucoid faeces, hair loss and soft stool. Mean body weight gains at 1000 mg/kg bw/day were similar to the control group during the initial six days of dosing (gestation Days 6-9 and 9-12); food consumption in this group was slightly decreased compared to the control group during the initial three days of dosing and was slightly increased during gestation Days 9-12. During gestation Days 12-16, mean body weight gain at 1000 mg/kg bw/day was slightly reduced and food consumption was similar to the control group during this interval. Body weight gain and food consumption values at 150 and 400 mg/kg bw/day were comparable to the respective control values. Mean gravid uterine weights, net body weights and net body weight gains, and mean liver, kidney and spleen weights in the AMPA treated groups were similar to the values in the control group. The mean foetal body weight at 1000 mg/kg bw/day group was slightly decreased. No other indication of a developmental effect was apparent at any dose level. No foetal malformations or developmental variations which could be related to treatment were observed at any dose level.

In conclusion, a marginal degree of maternal toxicity and a marginal degree of developmental toxicity were observed at 1000 mg/kg bw/day; maternal body weight gain and food consumption values were slightly less than those in the control group for a short duration and mean foetal body weight was slightly decreased. Clinical findings (mucoid faeces, soft stool and hair loss) were the only maternal response at

400 mg/kg/day.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	AMPA
Description:	white solid
Lot/Batch number:	HET-9001-1463T
Purity:	94.38 %
Stability of test compound:	Stable until Jan 1993
2. Vehicle:	Corn oil
3. Test animals:	
Species	Rat
Strain	Sprague-Dawley CD
Age on arrival	71 days
Body weight at initiation of dosing	265 - 278 g (group mean values)
Source	[redacted]
Housing	Individually, in wire-mesh cages suspended above card board (except for mating when animals were housed pairwise)
Acclimatisation period	11 days
Diet	Purina® Certified Rodent Chow #5002, *ad libitum*
Water	Tap water, *ad libitum*
Environmental conditions	Temperature: 69 – 72°F (corresponding to 21 – 22 C) Humidity: 61 ± 86 % Air changes: approximately 10-15 changes / hour Photoperiod: 12 hours light / 12 hours dark

B. STUDY DESIGN AND METHODS

In-life dates: 07 Aug 1990 – 31 Aug 1990

Mating procedure: At the end of the acclimatization period, animals judged to be in good health and meeting acceptable body weight requirements (a minimum of 220 grams) were placed in a suspended wire-mesh cage with a resident male from the same strain and source for breeding. Resident males were untreated, sexually mature rats utilized exclusively for breeding. A breeding record containing the male and female identification numbers and the dates of cohabitation was prepared. The females were approximately 12 weeks old when paired for breeding.
Positive evidence of mating was confirmed by the presence of a copulatory plug in the vagina or the presence of sperm in a vaginal smear. Each mating pair was examined daily. The day on which evidence of mating was identified was termed Day 0 of gestation and the animals were separated.

Animal assignment: The mated females were consecutively assigned in a block design to groups containing eight rats each by the following randomization procedure. The first mated female and the appropriate gestation Day 0 designation were entered on the form and the female was assigned to group 1, the second mated female was assigned to group 2, and the third to group 3, etc. This process was continued daily until eight females were placed into each group. Body weight values ranged from 213 g to 261 g on Day 0 of gestation.

Dose preparation and analysis: An appropriate amount of the test item (AMPA) was weighed for each group into tared weigh boats and transferred to a mortar. The test material was triturated with corn oil, and ground with a pestle until a slurry was obtained. The slurry was transferred to a graduated cylinder via a series of vehicle rinses. A sufficient amount of vehicle was added to attain a volume of 800 mL. The cylinder was inverted several times to ensure adequate mixing and the contents were transferred to a

properly labelled storage container. The cylinder was rinsed with an additional 620 mL of vehicle, which was also added to the storage container. The preparations were then mixed for approximately 5 minutes on a Polytron PT6000 homogenizer to reduce particle size and to ensure proper mixing. The dosing preparations were visually inspected for homogeneity prior to dispensation for the first day of dose administration.
Preparations for all dose groups were made once at study start, and were stored at room temperature. A second preparation for the 150 mg/kg bw/day group was made one week later, and stored at room temperature. The preparations were stirred using a magnetic stir plate and bar each day prior to dispensing and during the dosing procedure.
Samples of the test material preparations were analysed at the Monsanto Company. The following aliquots were drawn on the first day of dose formulation: one sample (middle) from the control group (group 1) and two samples (top and bottom) from the mid-dose group (group 3) preparations to verify concentration; and three samples (top, middle, bottom) from the low dose group (group 2) and high dose group (group 4) preparations to verify concentration and homogeneity. One week later, three aliquots (top, middle and bottom) were taken from the low dose group preparation. In the last week of dosing, two aliquots (top and bottom) were taken from the low dose and high dose group preparations. For all dates, samples at room temperature were sent to the sponsor for analysis. The analytical results were reported separately (see CA 4). AMPA concentrations in corn oil dosing solutions were found to be 105 - 120 % of the target levels.

Dose administration: The dose formulations were administered orally by gavage, via a 16-gauge stainless steel gavage cannula, once daily for 10 consecutive days initiating on gestation Day 6 and continuing up to and including Day 15 of gestation. A dosage volume of 10 mL/kg bw was used for all dose levels. The control animals received corn oil on a comparable regimen of 10 mL/kg bw. Individual dosages were based on the most recent body weights.

Clinical observations: All animals were observed twice daily for moribundity and mortality. Detailed clinical observations were recorded individually from Days 0 through 20 of gestation (prior to compound administration during the dosing period). Animals were observed for signs of toxicity approximately one hour after treatment throughout the dosing period; only significant findings were recorded at the post-dosing observation period.

Bodyweight and gravid uterine weight: Maternal body weights were recorded individually on gestation Days 0, 6, 9, 12, 16 and 20. A group mean body weight was calculated for each of these days. Mean body weight changes were calculated for each corresponding interval and also for Days 6-16 and 0-20.
Gravid uterine weight, net body weight (the Day 20 body weight minus the weight of the uterus and contents) and net body weight change (the Day 0-20 body weight change minus the weight of the uterus and contents) were presented for each gravid female at the scheduled Caesarean section.

Food consumption: Individual food consumption was recorded on Days 0, 6, 9, 12, 16 and 20 of gestation. Food intake was calculated as g/animal/day and g/kg bw/day for the corresponding body weight change intervals. On the occasions when food intake could not be measured for one of the days in a given interval, food intake was calculated using the appropriate number of days for that interval.

Terminal investigations: All maternal animals were sacrificed by carbon dioxide inhalation on gestation Day 20. The thoracic, abdominal and pelvic cavities were opened by a ventral midline incision and the contents examined. In all instances, the post mortem findings were correlated with the ante mortem comments and any abnormalities were recorded. The uterus and ovaries were excised. The number of corpora lutea on each ovary was recorded. The trimmed uterus was weighed, opened and the number and location of all foetuses, early and late resorptions and the total number of implantation sites were recorded. Uteri with no macroscopic evidence of nidation were opened and subsequently placed in 10 % ammonium sulphide solution for detection of early implantation loss as described by Sa1ewski.
The liver, kidneys and spleen from each dam were excised, trimmed and weighed and all findings recorded. The carcasses of the dams, including all organs, were then discarded.

Foetal observations: Each foetus was individually sexed, weighed and tagged for identification. A detailed external examination of each foetus was conducted to include, but was not limited to, the eyes, palate, and external orifices and each finding was recorded. Crown-rump measurements were recorded for late resorptions and the tissues were discarded. The sex of each foetus was verified by an internal examination. Each foetus was examined viscerally by a modification of the Stuckhardt and Poppe fresh dissection technique to include the heart and major vessels. Foetal kidneys were examined and graded for renal papillae development by a method described by Woo and Hoar.
Heads from approximately one-half of the foetuses from each female were placed in Bouin's fixative for subsequent soft-tissue examination by the Wilson sectioning technique. The heads from the remaining one-half of the foetuses were examined by a mid-coronal slice. All carcasses were eviscerated and fixed in 95 % isopropyl alcohol. Following fixation in alcohol, each foetus was macerated in potassium hydroxide and stained with Alizarin Red S by a method similar to that described by Dawson. The skeletal examination was conducted utilizing low power magnification provided by a stereomicroscope. External, visceral and skeletal findings were recorded as developmental variations or malformations. The foetal developmental findings were summarized by 1) presenting the incidence of a given finding both as a percentage of the no. of foetuses and the no. of litters available for examination in the group and 2) by considering the litter as the basic unit for comparison and calculating the number of affected foetuses in a litter on a proportional basis.

Statistical analyses: All analyses were conducted using two-tailed tests for a minimum significance level of 5 % comparing each treated group to the vehicle control group. Each mean was presented with the standard deviation (SD) and the number of animals (N) used to calculate the mean. The following statistical tests were performed: Chi-square test with Yates' correction factor (foetal sex ratios); Fisher's Exact test (malformations and variations); Mann-Whitney U test (early and late resorptions, dead foetuses, post-implantation losses); One-way ANOVA with Dunnett's test (corpora lutea, total implantations, viable foetuses, foetal body weights, maternal body weights and weight changes, maternal net body weight changes and gravid uterine weights, selected organ weights); Kruskal-Wallis test (litter proportions of intrauterine data, considering the litter rather than the foetus as the experimental unit).

II. RESULTS AND DISCUSSION

A: ANALYSIS OF DOSE FORMULATIONS

The analytical results were reported separately by the Monsanto Company. AMPA concentrations in corn oil dosing solutions were found to be 105 - 120 % of the target levels.

B: CLINICAL OBSERVATIONS

All animals survived to the scheduled sacrifice on gestation Day 20. The predominant clinical findings observed during the study were mucoid faeces, hair loss and soft stool. Mucoid faeces occurred only in the AMPA treated groups and with very few exceptions, occurred only during the dosing period with an occasional incidence following the dosing period. The incidence of mucoid faeces was increased by numerical progression in the 400 and 1000 mg/kg bw/day groups; the incidence of mucoid faeces in the 150 mg/kg nbw/day group was limited to three consecutive days for a single animal.
Hair loss on various body surfaces (primarily the forelimbs, hindlimbs and lateral abdominal area) and soft stool occurred similarly in the control group and the 150 mg/kg/day group, however, the incidence of each finding was slightly increased and appeared treatment-related in the 400 and 1000 mg/kg bw/day groups.
In some cases, hair loss on the forelimbs was initially noted prior to the treatment period. Hair loss on the hindlimbs and lateral abdominal area was first observed after dosing initiated. Once observed, hair loss generally persisted throughout the treatment and post-treatment periods.
Soft stool generally occurred during the treatment period with an occasional incidence following the treatment period. Other clinical findings observed during the study were infrequent in occurrence or occurred similarly in the control group. No clinical findings were observed at the l-hour post-dosing observation period.

Table 5.8.1-68: AMPA: Teratogenicity study in rats ([redacted], 1991b): Summary of selected clinical findings.

	Dose level of AMPA (mg/kg bw/day)			
	0 (control)	**150**	**400**	**1000**
No. of females	25	25	25	25
Surviving until scheduled Caesarean section	25	25	25	25
Clinical finding (no. of animals affected)				
Hair loss				
Right forelimb	1	2	6	6
Left forelimb	1	3	6	6
Right abdominal area	0	0	3	6
Left abdominal area	1	0	4	6
Right inguinal area	0	0	0	1
Left inguinal area	0	0	0	1
Left lateral abdomal area	0	0	0	1
Right hindlimb	0	0	1	5
Left hindlimb	1	1	0	6
Ventral thoracic area	0	0	0	1
Excreta				
Soft stool	15	19	22	23
Mucoid faeces	0	1	7	18

C. BODY WEIGHT

Mean body weight gains at 1000 mg/kg bw/day were similar to the control group during the initial six days of the dosing period (gestation Days 6-9 and 9-12). During gestation Days 12-16, mean body weight gain at 1000 mg/kg bw/day was slightly reduced (statistically significant at $p<0.01$) compared to the control group. Mean body weight gains at 1000 mg/kg bw/day were similar to the control group when evaluated over the entire treatment period (gestation Days 6-16) and the post-treatment period (gestation Days 16-20). Mean absolute body weight values at 1000 mg/kg bw/day were comparable to the control group throughout the study. No adverse effects on mean body weights and mean body weight gains were observed at 150 and 400 mg/kg bw/day during the study.

Mean gravid uterine weights, net body weight gains and net body weights in the AMPA treated groups were similar to the control group.

Table 5.8.1-69: AMPA: Teratogenicity study in rats ([redacted], 1991b): Summary of gravid female body weight, body weight gain and gravid uterine weight.

	Dose level of AMPA (mg/kg bw/day)			
	0 (control)	**150**	**400**	**1000**
No. of females	24	24	24	24
Body weights (g ± SD) Day post coitum				
0	232 ± 11.5	233 ± 11.1	234 ± 10.0	234 ± 10.7
6	267 ± 12.2	266 ± 13.6	270 ± 11.2	268 ± 13.4
12	281 ± 14.3	285 ± 14.5	289 ± 12.2	285 ± 15.2
16	315 ± 16.1	315 ± 16.3	321 ± 16.0	310 ± 15.4
20	379 ± 19.9	379 ± 211	381 ±23.1	369 ± 26.4
Body weight gain (g ± SD) Days post coitum				
6-9	2 ± 5.2	5 ± 5.0	4 ± 6.5	4 ±7.0
9-12	13 ± 6.6	14 ± 4.0	15 ± 4.9	13 ± 6.5
12-16	33 ± 7.1	30 ± 7.3	32 ± 7.4	26** ± 11.0

6-16	48 ± 8.1	49 ± 6.6	51 ± 11.0	43 ± 10.0
Gravid uterine weight (g ± SD)	78.5 ± 7.53	77.7 ±9.12	76.9 ± 14.36	75.7 ± 7.11

SD = Standard deviation
** significantly different from control p<0.01 (Dunnett's test, two-tailed)

D. FOOD CONSUMPTION

Food consumption (evaluated as g/animal/day and g/kg bw/day) at 1000 mg/kg bw/day was slightly decreased compared to the control group during the initial three days of dosing (gestation Days 6-9); this was statistically significant (p<0.05) when evaluated as g/kg bw/day. During gestation Days 9-12, food intake (g/animal/day and g/kg bw/day) at 1000 mg/kg bw/day was slightly increased (p<0.05) when compared to the control group. Food consumption at1000 mg/kg bw/day was similar to the control group for the remainder of the treatment period (gestation Days 12-16), the entire treatment interval (gestation Days 6-16), and the post-treatment period (gestation Days 16-20).
Food consumption, evaluated as g/animal/day and g/kg bw/day, was apparently unaffected by treatment at dose levels of 150 and 400 mg/kg bw/day. The only statistically significant differences were in the 400 mg/kg bw/day group; food consumption values were slightly increased (g/animal/day and g/kg bw/day) during gestation Days 12-16, 6-16, and 0-20 (p<0.05).

Table 5.8.1-70: AMPA: Teratogenicity study in rats ([redacted], 1991b): Summary of gravid female consumption.

	Dose level of AMPA (mg/kg bw/day)			
	0 (control)	**150**	**400**	**1000**
No. of females	24	24	24	24
Mean food consumption (g/kg bw/day ± SD) Days post coitum				
0-6	82 ± 6.5	83 ± 6.5	85 ± 6.3	82 ± 7.5
6-9	52 ± 8.5	54 ± 7.3	52 ± 12.2	44* ± 10.0
9-12	56 ± 7.1	56 ± 7.4	60 ± 6.7	62* ± 13.1
12-16	61 ± 6.0	60 ± 5.5	66* ±7.6	60 ± 8.5
16-20	80 ± 4.6	80 ± 4.6	81 ± 5.8	81 ± 10.3
6-16	56 ± 5.2	57 ± 5.0	60* ± 6.0	56 ± 6.3
0-20	68 ± 3.9	68 ± 4.4	71* ± 4.4	68 ± 4.4

SD = Standard deviation
* significantly different from control p<0.05 (Dunnett's test, two-tailed)

E. NECROPSY

Gross pathology: At scheduled sacrifice on gestation day 20, no treatment-related gross necropsy findings were observed. Dilated renal pelves were observed in 2, 3 and 1 animal(s) in the control, 150 and 1000 mg/kg bw/day groups, respectively. A single animal in the control group had a white area on the spleen. There were no other macroscopic findings.

Organ weights: Mean liver, kidney and spleen weights in the treated groups were similar to the control group, indicating no apparent adverse effect from AMPA administration.

Caesarean section data:
No adverse effects were observed at any dose level on post-implantation loss, the numbers of viable foetuses, or foetal sex ratios. All values in the 150, 400 and 1000 mg/kg bw/day groups were comparable to the control group when evaluated on a group mean or proportional (%) litter basis. The mean numbers of corpora lutea and implantation sites in the AMPA treated groups were similar to the corresponding control group values.

Table 5.8.1-71: AMPA: Teratogenicity study in rats ([redacted], 1991 b): Intergroup comparison of maternal performance and Caesarean section data

Observation	Dose level of AMPA (mg/kg bw/day)			
	0 (control)	150	400	1000
No. of animals mated	25	25	25	25
No. of premature decedents	0	0	0	0
No. of animals pregnant	24	24	24	24
Pregnancy frequency (%)	96	96	96	96
Mean No. of corpora lutea ± SD	16.1 ± 1.45	17.0 ± 2.51	16.4 ± 1.61	16.5 ± 2.00
Mean No. of implantations ± SD	15.0 ± 1.33	15.3 ± 1.29	15.0 ± 2.40	15.1 ± 1.15
Mean post-implantation loss ± SD	0.7 ± 1.05	0.8 ± 0.83	0.6 ± 0.88	0.6 ± 0.83
Mean viable foetuses ± SD	14.4 ± 1.66	14.5 ± 1.64	14.4 ± 2.55	14.5 ± 1.28
Mean dead foetuses ± SD	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
Mean foetal weight (g) ± SD	3.5 ± 0.25	3.5 ± 0.18	3.4 ± 0.19	3.3* ± 0.31
Total No. of male female foetuses	162:183	182:165	179:167	180:169

SD = Standard deviation
* significantly different from control p<0.05 (Dunnett's test, two-tailed)

F. FOETUSES

Mean foetal body weight at 1000 mg/kg bw/day was slightly and statistically significantly (p<0.05) decreased (3.3 g) when compared to the control group (3.5 g). This reduction was mainly attributable to two litters with mean foetal body weights of 2.4 g and 2.8 g, respectively. Mean foetal body weight at 1000 mg/kg bw/day was similar to the minimum value in the laboratory historical control data (3.3 g). A mean foetal body weight of 3.3 g has been observed in five of 85 (6 %) of the studies in the laboratory historical control data. At 150 and 400 mg/kg bw/day, mean foetal body weights were either identical to or were similar to the control group (3.5 g and 3.4 g, respectively). A mean foetal body weight of 3.4 g has been observed in 20 of 85 studies (24 %) in the laboratory historical control data set.

There was-no effect of AMPA on the number or survival of foetuses.
The numbers of foetuses (litters) available for morphological evaluation were 345 (24), 347 (24), 346 (24) and 349 (24) in the control, 150, 400 and 1000 mg/kg bw/day groups, respectively. The number of foetuses (litters) with malformations were 2 (2), 4 (4), 0 (0) and 2 (2) in these same dose groups, respectively.

External examination: External malformations were observed in the control, 150 and 1000 mg/kg bw/day groups. One foetus in the control group had microphthalmia (unilateral). At 150 mg/kg bw/day, one dam had dizygotic conceptuses. It was observed that the umbilical structures of the resorbed foetus were reduced and conjoined with the umbilical cord of the surviving foetus, and the resorbed foetus was enclosed in a sac. At the subsequent visceral and skeletal examinations, it was noted that this viable conceptus had a soft tissue malformation and variation (an interrupted aortic arch and a distended ureter, respectively) and a skeletal malformation (a vertebral anomaly without an associated rib anomaly). Detailed descriptions of these soft tissue and skeletal anomalies are presented in the table below. At 1000 mg/kg bw/day, two anomalies were observed: one foetus had localized oedema in the neck and thoracic regions, and another foetus had sternoschisis. No external developmental variations were observed at any dose level

Visceral examination: Soft tissue malformations were observed only in the control and the 150 mg/kg bw/day group. One foetus in the control group had hydrocephaly. At 150 mg/kg bw/day, one foetus had situs inversus. A viable dizygotic conceptus at 150 mg/kg/day had a soft tissue malformation and variation (an interrupted aortic arch and distended ureter, respectively) which were already described in the context of the external examination. Soft tissue variations were also observed in single foetuses in the control and the 400 mg/kg bw/day group. In the control group, one foetus had distended ureters. At 400 mg/kg bw/day, one foetus had a haemorrhagic ring around the iris. No other soft tissue malformations or variations were observed.

Skeletal examination: The only skeletal malformations were observed at 150 mg/kg bw/day group. A viable dizygotic conceptus had a skeletal anomaly (a vertebral anomaly without an associated rib anomaly, which was described in the context of the external examination. A single foetus had hemispherical enlargement of rib no. 3. Another foetus had a bent radius (bilateral) and a vertebral anomaly with an associated rib anomaly (left thoracic arch no. 11 and half of the centrum were absent; thoracic centrum no. 10 was malpositioned; and left rib nos. 10 and 11 were fused proximally). Several skeletal variations were observed in the control and treated groups. The most notable variants were sternebra(e) nos. 5 and/or 6 unossified, 14th rudimentary ribs, reduced ossification of the 13th ribs, and 7th cervical ribs; these are commonly observed variants in the laboratory historical control data. No dose-relationship was apparent in the percentage of foetuses (litters) affected in the dose groups and the variations occurred with a similar frequency in the control group. Also, the skeletal variants were well within the ranges observed in the laboratory historical control data. Other variants observed were infrequent occurrences or occurred similarly in the control group.

Summary of foetal examinations: Considering both the incidence and the expression of specific anomalies, the external, soft tissue and skeletal malformations observed in the treated groups appeared to be spontaneous in origin (2, 4, 0 and 2 foetuses in the control, 150, 400 and 1000 mg/kg bw/day groups had malformations). Also the skeletal variants which were observed in the study occurred with a similar frequency in the control group

Table 5.8.1-72: AMPA: Teratogenicity study in rats ([redacted], 1991b): Group incidence of foetal malformations

Observation	Dose level of AMPA (mg/kg bw/day)			
	0 (control)	100	350	1000
	Incidence of foetuses (litters)			
External examination				
No. of foetuses (litters) examined	345 (24)	347 (24)	346 (24)	349 (24)
Dizygotic conceptuses	0 (0)	1 (1)	0 (0)	0 (0)
Sternoschisis	0 (0)	0 (0)	0 (0)	1 (1)
Localized foetal oedema	0 (0)	0 (0)	0 (0)	1 (1)
Microphthalmia and/or anophthalmia	1 (1)	0 (0)	0 (0)	0 (0)
Visceral examination				
No. of foetuses (litters) examined	345 (24)	347 (24)	346 (24)	349 (24)
Interrupted aortic arch	0 (0)	1 (1)	0 (0)	0 (0)
Situs inversus	0 (0)	1 (1)	0 (0)	0 (0)
Hydrocephaly	1 (1)	0 (0)	0 (0)	0 (0)
Skeletal examination				
No. of foetuses (litters) examined	345 (24)	347 (24)	346 (24)	349 (24)
Bent limb bone(s)	0 (0)	1 (1)	0 (0)	0 (0)
Rib(s) - hemispherical enlargement	0 (0)	1 (1)	0 (0)	0 (0)
Vertebral anomaly with or without associated rib anomaly	0 (0)	2 (2)	0 (0)	0 (0)
Combined incidence of malformations	2 (2)	4 (4)	0 (0)	2 (2)

III. CONCLUSIONS

A marginal degree of maternal toxicity and a marginal degree of developmental toxicity were noted at 1000 mg/kg bw/day as clinical signs, transient reductions of maternal body weight gain and food consumption. Similar clinical findings were the only maternal response at 400 mg/kg bw/day. With this exception, the NOAEL (no observable adverse effect level) for maternal toxicity and developmental toxicity was considered to be 400 mg/kg bw/day.

3. Assessment and conclusion

Assessment and conclusion by applicant:

This developmental toxicity study is considered acceptable, although some endpoints that are requested in the OECD 414 TG from 2018 are not included in this study. At 1000 mg/kg bw/day, a marginal degree of maternal and developmental toxicity was noted in form of clinical signs, transient reductions in body weight gain, food consumption and reduced foetal body weights. Clinical signs (alopecia and mucoid faeces) were also noted at 400 mg/kg bw/day. Accordingly, the NOAEL for maternal toxicity was considered to be 150 mg/kg bw/day, while the NOAEL for developmental toxicity was considered to be 400 mg/kg bw/day.

Assessment and conclusion by RMS:

Literature

A literature search for the active substance glyphosate was performed in accordance to the provisions of the EFSA Guidance "Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) 1107/2009" and updated Appendix to this Guidance document. The following publications were found relevant and reliable for this section and the summaries are thus presented below and are part of the general discussion at the beginning of the section.

Table 5.8.1-73: Overview on literature found relevant for evaluation of glyphosate in section 5.8.1

Annex Point	Study	Study type Parameters investigated Test system	Substance Dose levels Exposure	Reliability and relevance comments	Result
CA 5.8.1/031	Kwiatkowska M. *et al.*, 2020	Apoptosis induction in human peripheral blood mononuclear cells	AMPA, Purity: 98 % 0.01 to 5 mM	Reliable with restriction No cytotoxicity tests performed to establish appropriate doses Most effects above physiological ranges, > 1mM Glyphosate impurities tested at unrealistically high concentrations No positive controls to verify assay viability	Low apoptotic potential. Borderline high dose effects.
CA 5.8.1/048 (CA 5.4/009) #	Suárez-Larios *et al.*, 2017	Induction of DNA double strand breaks Cytotoxicity Westernblot analysis of proteins involved in DNA recombination	Glyphosate and AMPA, Purity: not reported 0.4 – 50 µM (information on S9 mix n.a.) 1.5 h exposure	Reliable with restriction AMPA tested not sufficiently characterized	No induction of DNA double strand breaks

		In human peripheral blood lymphocytes			
CA 5.8.1/049 (CA 5.4/011) #	Roustan *et al*., 2014	Photoactivation Micronucleus assay Intracellular ROS In CHO-K1 cells	Glyphosate and AMPA, Purity: not reported 0.005 – 100 µg/mL ± S9 mix 3 h exposure	Reliable with restriction Test substances not sufficiently characterized No historical control data	AMPA: Photoactivation and oxidative stress: equivocal Micronucleus assay: Statistically significantly increased (0.005-0.1 µg/mL) No increase when tested in combination with glyphosate (5-100 µg/mL)
CA 5.8.1/050 (CA 5.4/012) #	Mañas *et al*., 2013	Comet assay (blood and liver) Oxidative stress (TBARs, SOD and CAT activity; liver, kidney, lung and heart) In Balb C mice	AMPA, Purity: 99 %, 100 mg/kg bw/day 14 day admin. in drinking water	Reliable with restriction Only two doses No dose-response Doses much lower than other *in vivo* studies with negative results	AMPA: DNA damage: positive; oxdative stress: decrease SOD all tissues

#: The summary of the publication is provided in section 5.4

1. Information on the study

Data point:	CA 5.8.1/031
Report author	Kwiatkowska, M. *et al.*
Report year	2020
Report title	Evaluation of apoptotic potential of glyphosate metabolites and impurities in human peripheral blood mononuclear cells (*in vitro* study)
Document No	doi.org/10.1016/j fct.2019.110888 E-ISSN: 1873-6351
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	None
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities (Research conducted in an academic laboratory)
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary of the study according to OECD format

The study aimed to assess the effect of glyphosate, its metabolites: aminomethylphosphonic acid (AMPA), methylphosphonic acid and its impurities: PMIDA, N-methylglyphosate, hydroxymethylphosphonic acid and bis (phosphonomethyl)amine on apoptosis induction in human peripheral blood mononuclear cells (PBMCs). PBMCs were exposed to the compounds studied at concentrations ranging from 0.01 to 5 mM for 4 h. It was observed an increase in reactive oxygen species (including hydroxyl radical) and cytosolic

calcium ions levels as well as reduction of transmembrane mitochondrial potential (ΔΨm) in PBMCs exposed to the compounds examined. All substances studied changed PBMCs membrane permeability, activated caspase-8, -9, -3 and caused chromatin condensation, which showed that they were capable of inducing apoptosis both via extrinsic and particularly intrinsic pathway. Generally the study demonstrated that there were no differences between apoptotic changes induced by glyphosate, its metabolites or impurities, and observed changes were provoked by high concentrations of investigated compounds. Since clear changes were only seen at high concentrations, a low apoptotic potential of these compounds was concluded.

I. MATERIALS AND METHODS

Chemicals; The investigated compounds i.e., aminomethylphosphonic acid (AMPA) (purity 98 %), methylphosphonic acid (purity 98 %), N-(phosphonomethyl)iminodiacetic acid (PMIDA) (purity 98 %), N-methylglyphosate, hydroxymethylphosphonic acid (purity 98 %) and bis-(phosphonomethyl)amine (purity 97 %) were synthetized by the Institute of Industrial Organic Chemistry, Warsaw, Poland. Glyphosate [N-(phosphonomethyl)glycine] (purity 95 %) in acid form was bought from Sigma-Aldrich, USA. The investigated compounds were dissolved in phosphate-buffered saline (pH 7.4). Other chemicals were purchased from POCh (Poland) and Roth (Germany) and were of analytical grade. Different concentrations of glyphosate have been selected in this study:

- 0.01 mM (low concentration) that is quite similar to the concentration determined in blood of humans who were not directly exposed to this herbicide,
- 0.05 mM to 0.5 mM (moderate to high concentration) that corresponds to the concentration that may penetrate into human blood as a result of glyphosate formulation poisoning,
- 5 mM to 10 mM (very high concentration) that corresponds to the concentration detected in humans after acute poisoning with glyphosate formulation.

Cells isolation; PBMCs were isolated from leucocyte-buffy coat obtained from blood collected in Blood Bank in Lodz, Poland. Blood was taken from healthy, non-smoking volunteers who showed no signs of infection disease symptoms at the time the blood samples were collected. The study was approved by the Bioethics Committee of the University of Lodz No. KBBN-UŁ/I/3/2013. The final PBMCs density used in the experiments (after addition of glyphosate, its metabolites or impurities) was $2x10^6$ per 1 mL. The viability of the cells was over 95 % as determined by flow cytometry.

Quantitative determination of apoptosis (YO-PRO-1/PI staining); Apoptosis is characterized by changes in cell membrane permeability. Apoptotic cells that have altered membrane permeability (membrane integrity is maintained) are permeable for a marker YOPRO-1 (carbocyanine nucleic acid stain), which exhibits green fluorescence, whereas they are not permeable for the dye propidium iodide (PI) that shows red fluorescence. The cells were treated with glyphosate, its metabolites and impurities in the final concentrations ranging from 0.01 to 10 mM and incubated for 4 h at 37 °C in total darkness. Apoptosis was induced with 10 µM of camptothecin. After the incubation, the samples were centrifuged at 300 g for 5 min at 4 °C, the supernatant was removed, and the cells were supplemented with RPMI with L-glutamine and 10 % FBS. Then, the mixture of YO-PRO-1 and PI (0.1 µM each) was added to the samples, which were incubated for 20 min on ice in total darkness. The samples were analysed by flow cytometry (LSR II, Becton Dickinson) with excitation maximum at 488 nm to visualize the YO-PRO-1 green fluorescence (520/30 bandpass filter) and PI red fluorescence (610/20 bandpass filter). FMC gate on PBMCs has been established for data acquisition and the data were recorded for a total of 10,000 events per sample.

Determination of biochemical and morphological hallmarks of apoptosis

Cytosolic calcium ion level; Calcium ion accumulated in mitochondria and endoplasmatic reticulum is an important secondary messenger in controlling apoptotic cell death. The level of cytosolic calcium ions was analysed by flow cytometry (LSR II, Becton Dickinson) using a fluorescent probe Fluo-3/AM according to manufacturer's protocol. Fluo-3/AM passes through membrane of living cells, in which it is cleavaged by intracellular esterases to Fluo-3. Inside the cell, Fluo-3 exhibits green fluorescence after complexation with calcium ions. PBMCs treated with glyphosate, its metabolites or impurities in the final concentrations ranging from 0.01 to 10 mM were incubated for 4 h at 37 °C in total darkness. Next, the cells were centrifuged at 300 g for 5 min at 4 °C, suspended in Fluo-3/AM (1 µM) solution and incubated at 37 °C for

20 min in total darkness. Then, Hanks' Balanced Salt Solution (HBSS), composed of inorganic salts and supplemented with glucose (with 1 % of BSA) was added to the cells suspension, and PBMCs were incubated for 40 min at 37 °C in total darkness. The cells were centrifuged at 300 g for 5 min at 4 °C and washed twice with HEPES buffer. After centrifugation, PBMCs were suspended in HEPES buffer and incubated for 10 min at 37 °C in total darkness. The samples were analysed using flow cytometer (LSR II, Becton Dickinson) with excitation at 490/500 nm to visualize the Fluo-3 fluorescence. FMC gate on PBMCs has been established for data acquisition and the data was recorded for a total of 10,000 events per sample.

Mitochondrial transmembrane potential (ΔΨm); Mitochondrial dysfunction leading to collapse of transmembrane mitochondrial potential has been shown to participate in the induction of apoptosis. Transmembrane mitochondrial potential was shown as red fluorescence intensity of MitoTracker Red CMXRos (excitation/emission maxima – 579/599 nm). This probe is cell permeable and contains mildly thiol-reactive chloromethyl moiety for mitochondrial labeling. Nigericin and valinomycin (1 μM) were used to increase and decrease ΔΨm, respectively. PBMCs were exposed to glyphosate, its metabolites or impurities for 4 h at 37 °C in total darkness. Then, the samples were centrifuged at 300 g for 5 min at 4 °C, the supernatant was decanted, and the cells were suspended in PBS. The cells were stained with MitoTracker CMXRos in the final concentration of 1 μM for 15 min at 37 °C in total darkness, and then analysed in 96-well plates using a microplate reader (Cary Eclipse, Varian).

Caspase-8, -9 and -3 activity; Caspases are critical enzymes of apoptosis. The caspases are able to break down peptide bonds via cysteine residues in various substrates, and therefore they catalyze the apoptotic cell death irreversibly in mammals including human beings. Analysis of caspase-8 and -3 was executed according to the manufacturer's protocols. The assays were based on the hydrolysis of the peptide substrates such as acetyl-Ile-Glu-Thr-Asp-7-amino-4-methylcoumarin (Ac-IETDAMC) by caspase-8 and acetyl-Asp-Glu-Val-Asp-7-amino-4-methylcoumarin (Ac-DEVD-AMC) by caspase-3, which resulted in the release of the fluorescent 7-amino-4-methylcoumarin (AMC). The excitation and emission wavelengths of AMC were 360 nm and 460 nm, respectively. Caspase-9 colorimetric assay was based on hydrolysis of the substrate acetyl-Leu-Glu-His-Asp-p-nitroaniline (Ac-LEHD-pNA), which led to the release of p-nitroaniline (pNA) that absorbance was determined at 405 nm. Camptothecin (10 μM) was used to induce apoptosis. Detection of caspase-3 and -8 activities was executed using fluorescent microplate reader (Fluoroskan Ascent FL, Labsystem) and determination of caspase-9 activity was performed using absorbance microplate reader (BioTek ELx808, Bio-Tek).

Hoechst 33342/PI staining; Chromatin condensation paralleled by DNA fragmentation is one of the most important criteria, which are used to identify apoptotic cells. Morphological changes of chromatin in PBMCs were assessed by double staining with Hoechst 33342 and PI. The cells were exposed to glyphosate, its metabolites or impurities for 4 h at 37 °C in total darkness. After incubation, PBMCs were centrifuged at 200 g for 3 min at 4 °C. The supernatant was removed, and the cells were suspended in PBS (0.5 ml). Then the mixture of 1 μl of Hoechst 33342 and 1 μl of PI (1 mg/ml each) was added. After 1 min incubation at 37 °C in total darkness, the cells were analysed by fluorescence microscope (Olympus IX70, Japan) at 400x magnification. PMBCs were classified on the basis of their morphological staining characteristics: viable (blue fluorescence), early apoptotic (intensive bright blue fluorescence), late apoptotic (blue-violet fluorescence) and necrotic (red fluorescence).

Determination of reactive oxygen species level

Oxidation of 6-carboxy-2′,7′-dichlorodihydrofluorescein diacetate; ROS play a central role in regulation of the main pathways of apoptosis mediated by mitochondria, death receptors and the endoplasmic reticulum (ER). The rate of 6-carboxy-2′,7′-dichlorodihydrofluorescein diacetate (H2DCF-DA) oxidation was assessed by flow cytometry. 6-CarboxyH2DCF-DA is a compound widely used for the detection of intracellular oxidants production. The probe diffuses across cellular membrane where it is hydrolyzed by intracellular esterases to 6-carboxy-2′,7'dichlorodihydrofluorescein (6-carboxy-H2DCF) that after oxidation, yields highly fluorescent 6-carboxy-2′,7′-dichlorofluorescein (DCF). The cells were treated with glyphosate, its metabolites or impurities for 4 h at 37 °C in total darkness. Next, fluorescent marker was added to PBMCs, which were stained for 15 min at 37 °C in the dark. The final concentration of the fluorescent probe was 10 μM. Positive control consisted of hydrogen peroxide (2 mM). FMC gate on PBMCs has been established for data acquisition, and fluorescence was measured with the excitation and emission maxima of 488 and 530 nm, respectively. The data was recorded for a total 10,000 events per

sample.
Oxidation of hydroxyphenyl fluorosceine; Highly reactive oxygen species (mainly hydroxyl radical) were assessed using flow cytometer (Becton Dickinson, LSR II) and 3-(p-hydroxyphenyl)-fluorescein (HPF). HPF is nonfluorescent until it reacts with hydroxyl radical. As a result of oxidation, the probe exhibits bright green fluorescence (excitation/emission maxima – 490/515 nm). Formation of hydroxyl radical was provoked by the addition of the mixture of ferrous perchlorate(II) (0.1 mM) and hydrogen peroxide (1 mM) to PBMCs suspension. Finally, the cells were treated with HPF in the final concentration of 2 µM and incubated for 15 min at 37 °C in total darkness. The data was recorded for a total of 10,000 events per sample.
Statistical analysis; The statistical analysis was performed with STATISTICA 8 data analysis software (2000 StatSoft, Inc., Tulsa, OK, USA). In this study, one-way analysis of variance (ANOVA) (p and F added to the description of the results) with post hoc multiple comparisons procedure (Tukey test) was used to assess statistical differences in case of normal distribution (significance marked on the charts). The difference was considered to be significant for $P < 0.05$. The individual analysis was performed on blood from 4 to 5 donors, while each experiment was repeated trice.

II. RESULTS

Apoptotic changes; After 4 h incubation, all compounds studied caused an increase in the number of apoptotic cells. Apoptotic changes were observed in PBMCs treated with 0.5 mM and 5 mM of glyphosate, PMIDA and hydroxymethylphosphonic acid. Two glyphosate metabolites: AMPA and methylphosphonic acid as well as two glyphosate impurities: N-methylglyphosate and bis-(phosphonomethyl)amine caused apoptotic changes only at their highest concentration of 5 mM (Fig. 1).

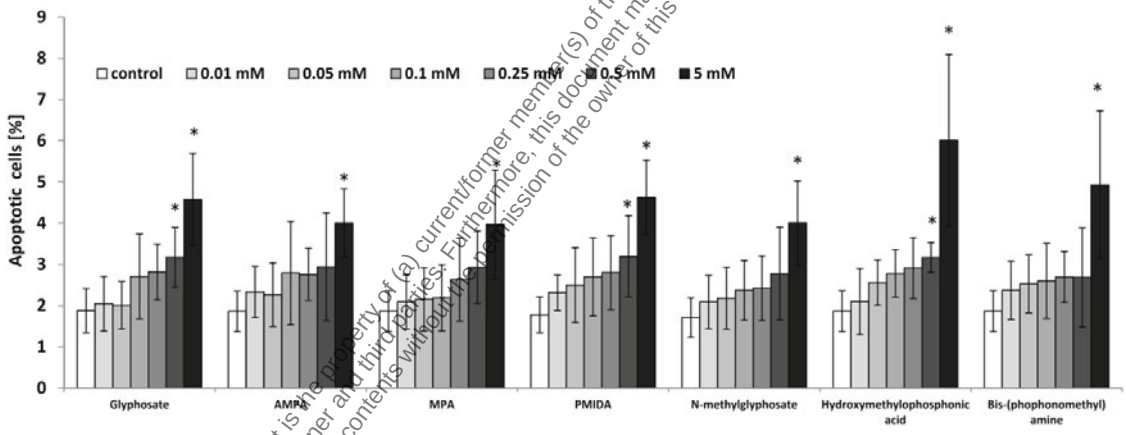


Fig. 1. Changes in the number of apoptotic PBMCs (expressed in per cent) after 4 h incubation with glyphosate, its metabolites and impurities. (*) Statistically significant changes compared to the control ($P < 0.05$).

Morphological changes of chromatin; Cell staining with Hoechst 33342 and PI allowed for the observation of early apoptotic cells (blue color), late apoptotic cells (blue-violet color) and necrotic cells (red color) in the population of PBMCs exposed to the compounds examined for 4 h. Selected photographs showing the presence of individual types of apoptotic and necrotic PMBCs are presented below (Photo 1).

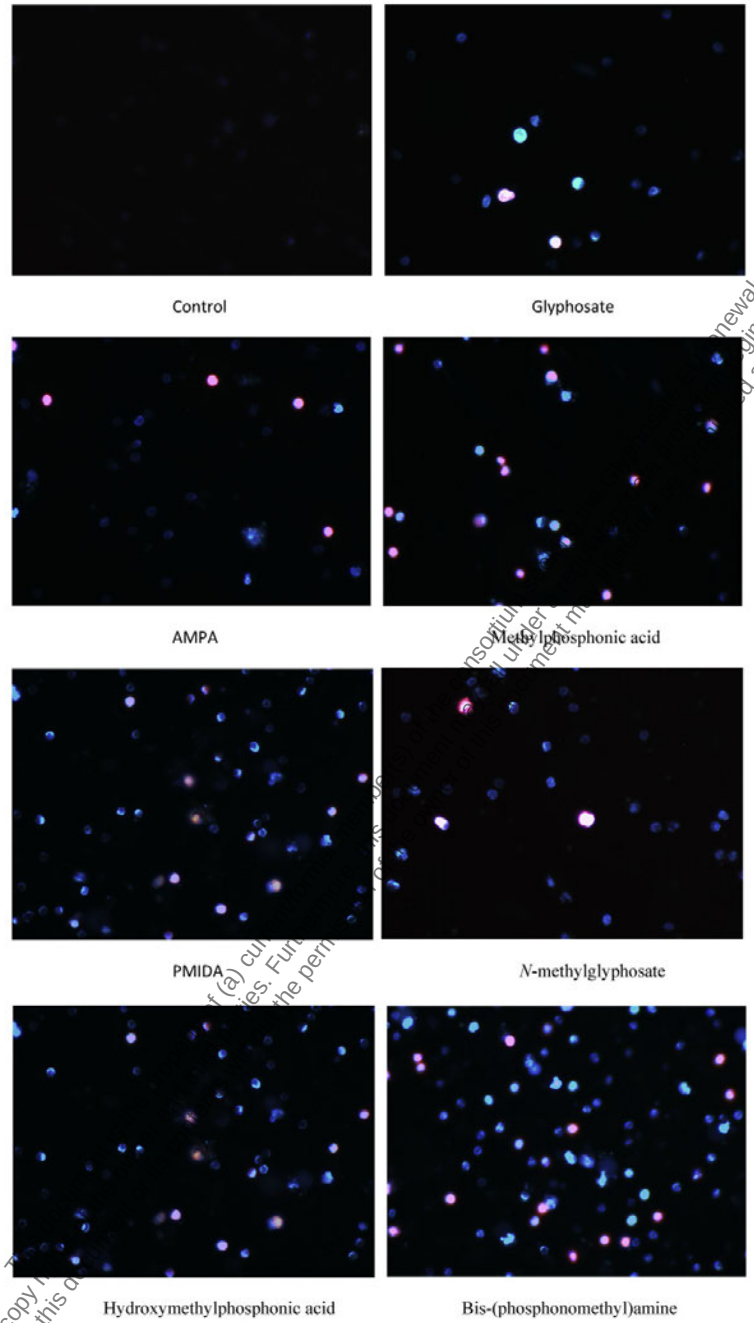


Photo 1. The representative photomicrographs of Hoechst 33324/PI-stained PBMCs pretreated with PBS (control) and with 5 mM of glyphosate, AMPA, methylphosphonic acid, PMIDA, *N*-methylglyphosate, hydroxymethylphosphonic acid and bis-(phosphonomethyl)amine. Viable cells (blue fluorescence), early apoptotic cells (intensive bright blue fluorescence), late apoptotic cells (blue-violet fluorescence) and necrotic cells (red fluorescence).

Cytosolic calcium ion level; A statistically significant increase in cytosolic calcium ion level was observed after incubation of PBMCs with glyphosate and other compounds studied (except for PMIDA). Statistically significant changes were observed for the highest concentration (5 mM) of glyphosate, AMPA and N-methylglyphosate. Glyphosate metabolite - methylphosphonic acid and glyphosate impurity - bis(phosphonomethyl)amine from the concentration of 0.5 mM caused an increase in cytosolic calcium ion level, while hydroxymethylphosphonic acid induced changes in the parameter studied from the concentration of 0.25 mM (Fig. 2).

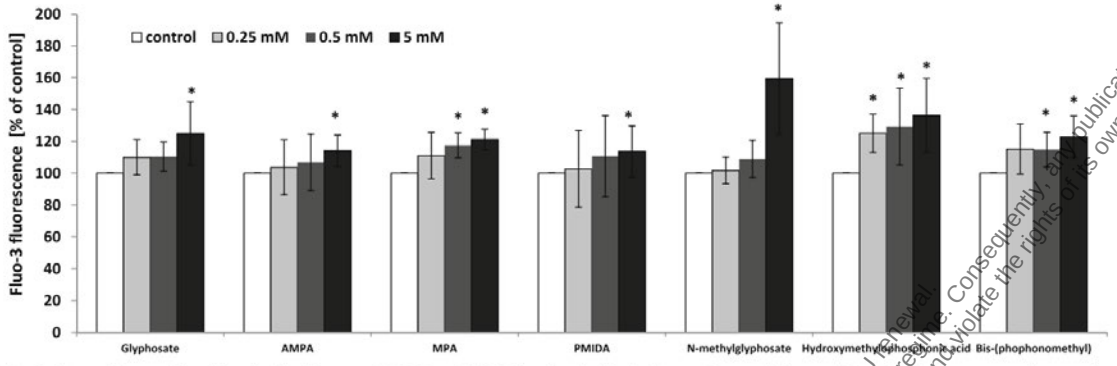


Fig. 2. Changes in cytosolic calcium ion level in control PBMCs and PBMCs incubated with glyphosate, its metabolites and impurities in the concentrations ranging from 0.25 to 5 mM for 4 h (*) Statistically significant changes compared to control ($P < 0.05$).

Transmembrane mitochondrial potential (ΔΨm); Most of the compounds studied caused a decrease in transmembrane mitochondrial potential, while hydroxymethylphosphonic acid (at 0.25 mM) caused statistically significant increase in the parameter examined. Glyphosate and one of its production impurities: bis(phosphonomethyl)amine (from the concentration of 0.05 mM) decreased ΔΨm. Other glyphosate metabolites: AMPA and methylphosphonic acid, and two glyphosate impurities: PMIDA and N-methylglyphosate decreased the parameter studied from the concentration of 0.1 mM (Fig. 3).

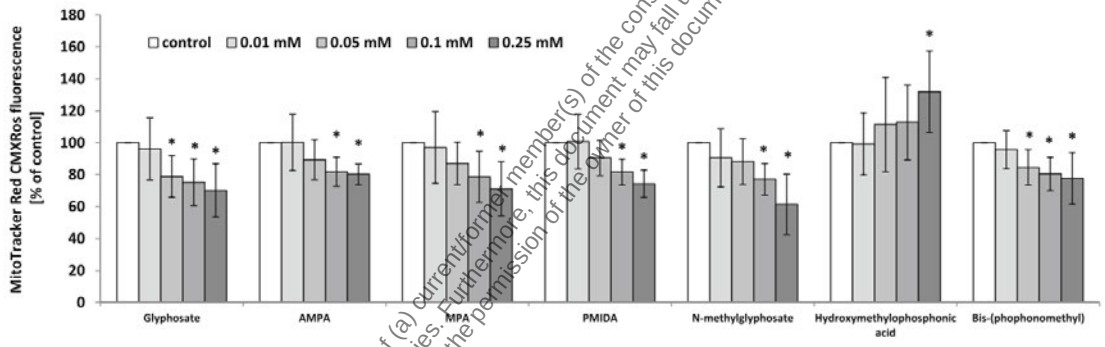


Fig. 3. Changes in transmembrane mitochondrial potential in control PBMCs and PBMCs incubated for 4 h with glyphosate, its metabolites and impurities in the concentrations ranging from 0.01 to 0.25 mM (*) Statistically significant changes compared to control ($P < 0.05$).

Caspase-8 and -9 activity; Glyphosate, AMPA, PMIDA and bis-(phosphonomethyl)amine increased in caspase-8 activity. The changes were observed in PBMCs treated with 0.5 mM of glyphosate and AMPA and with the highest concentration (5 mM) of PMIDA and bis-(phosphonomethyl)amine. Other compounds studied such as methylphosphonic acid, N-methylglyphosate and hydroxymethylphosphonic acid did not cause statistically significant changes in caspase-8 activity (Fig. 4). Glyphosate, its metabolites and impurities caused a substantial increase in caspase-9 activity. Changes in the enzyme activity were observed for the highest concentration (5 mM) of glyphosate, its two metabolites: AMPA and methylphosphonic acid and all glyphosate impurities: PMIDA, N-methylglyphosate, hydroxymethylphosphonic acid and bis(phosphonomethyl)amine (Fig. 5).

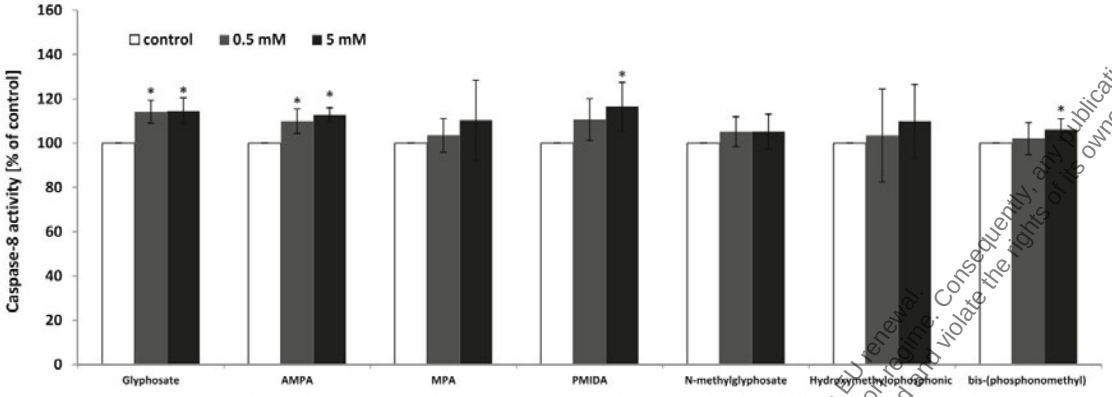


Fig. 4. Changes in caspase-8 activity in PBMCs after 4 h incubation with glyphosate, its metabolites and impurities. (*) Statistically significant changes compared to control ($P < 0.05$).

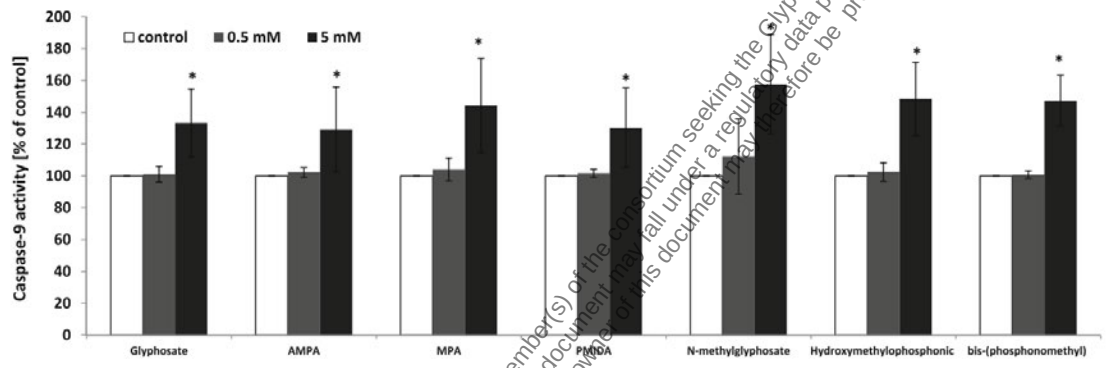


Fig. 5. Changes in caspase-9 activity in PBMCs after 4 h incubation with glyphosate, its metabolites and impurities. (*) Statistically significant changes compared to control ($P < 0.05$).

Caspase-3 activity; An increase in caspase-3 activity was noted in PBMCs treated with 0.25 mM of glyphosate and PMIDA and with 0.5 mM of AMPA, hydroxymethylphosphonic acid and bis-(phosphonomethyl)amine. Other compounds tested: N-methylglyphosate and methylphosphonic acid only at the highest concentration of 5 mM caused an increase in caspase-3 activity (Fig. 6).

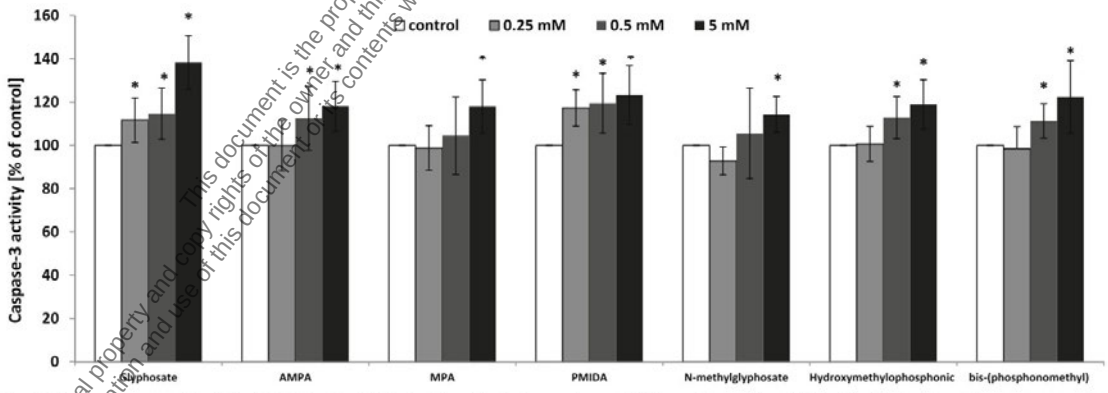


Fig. 6. Changes in caspase-3 activity in PBMCs after 4 h incubation with glyphosate, its metabolites and impurities. (*) Statistically significant changes compared to control ($P < 0.05$).

ROS level; It was observed that glyphosate, its metabolites and impurities induced a statistically significant increase in H2DCFDA oxidation in PBMCs. Changes in ROS level were observed after 4 h of exposure of PBMCs to 0.25 mM of glyphosate, methylphosphonic acid, PMIDA, N-methylglyphosate and hydroxymethylphosphonic acid. Other compounds studied like AMPA and bis-(phosphonomethyl)amine from the concentration of 0.5 mM caused an increase in the H2DCFDA oxidation (Fig. 7).

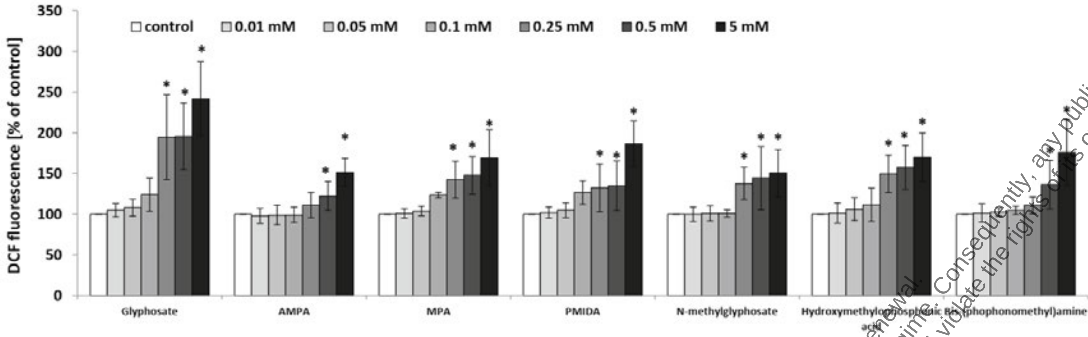


Fig. 7. Changes in total ROS level in PMBCs incubated with glyphosate, its metabolites and impurities for 4 h (*) Statistically significant changes compared to the control (P < 0.05).

Hydroxyl radical level; Glyphosate, its metabolites and impurities increased highly reactive oxygen species level, including hydroxyl radicals in PMBCs. Statistically significant changes were observed for most of the compounds studied from the concentration of 5 mM. An increase in HPF fluorescence was noted in cells treated for 4 h with the highest concentration (5 mM) of glyphosate, its impurities: N-methylglyphosate, hydroxymethylphosphonic acid and bis-(phosphonomethyl)amine and metabolites: AMPA and methylphosphonic acid. The strongest changes in HPF oxidation were caused by PMIDA, which changed this parameter from the concentration of 0.5 mM (Fig. 8).

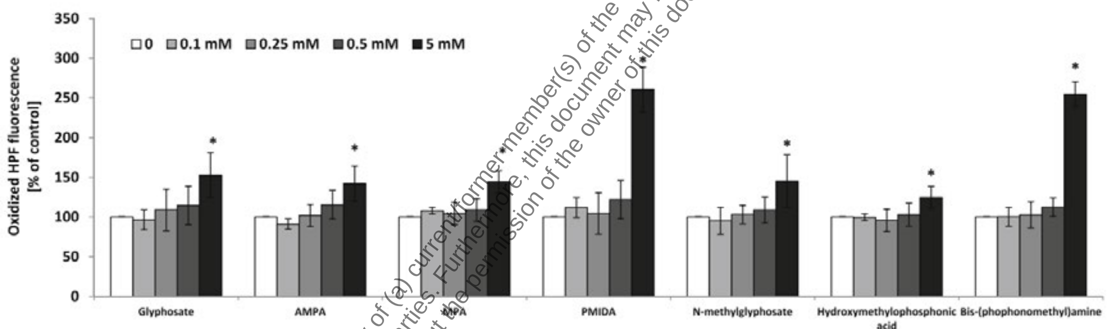


Fig. 8. The level of highly reactive oxygen species (mainly hydroxyl radical) in human PMBCs incubated with glyphosate, its metabolites and impurities for 4 h (*) Statistically significant changes compared to the control (P < 0.05).

Discussion

Apoptotic potential of glyphosate has been evaluated in various cell types; however, the effect of glyphosate, its metabolites (including AMPA and methylphosphonic acid) and its impurities on apoptotic changes in human leucocytes has not been assessed. This study has described apoptotic potential of glyphosate, its metabolites and impurities in human PBMCs. Besides a quantitative determination of apoptotic cells (staining with YO-PRO-1/PI fluorescent probes) the analysis concerned evaluation of the mechanism of action of these substances by measurement of a variety of parameters involved in the programmed cell death. The activities of caspases, of both initiator caspase-8 and -9, as well as executor caspase-3 were determined. Alterations in cytosolic calcium ion and ROS levels were also analysed. Moreover, changes in transmembrane mitochondrial potential and chromatin condensation were assessed. PBMCs were exposed to tested compounds for 4 h, the time necessary to observe apoptotic changes. Flow cytometry analysis has demonstrated a statistically significant increase in the number of apoptotic cells exposed to all compounds studied. Apoptotic changes induced by glyphosate, PMIDA and hydroxymethylphosphonic acid were observed from the concentration of 0.5 mM, while those induced by AMPA, methylphosphonic acid, N-methylglyphosate and bis-(phosphonomethyl)amine from the concentration of 5 mM. A slight increase in the number of apoptotic cells (5.76 %) treated with glyphosate was observed, compared to the control (1.22 %).

It was observed that methylphosphonic acid at lower concentration (0.25 mM) (in comparison to other analysed compounds) caused a statistically significant increase of cytosolic calcium ion level in PBMCs. Other compounds such as methylphosphonic acid and bis(phosphonomethyl)amine induced apoptosis from the concentration of 0.5 mM, while glyphosate, AMPA and N-methylglyphosate from 5 mM. It was also observed that aminomethylphosphonic acid did not cause any statistically significant increase in this parameter.

Our study demonstrated that all compounds analysed caused reduction of transmembrane mitochondrial potential. Glyphosate and its impurity - bis(phosphonomethyl)amine, from the concentration of 0.05 mM, caused reduction of ΔΨm, while other compounds analysed induced the same changes from the concentration of 0.1 mM (except for hydroxymethylphosphonic acid, that caused reduction of the discussed parameter at the concentration of 0.25 mM). An increased ROS production was observed in PBMCs exposed with all analysed compounds. The obtained results are consistent with data published, which observed ROS formation in human PBMCs treated with glyphosate, AMPA and particularly glyphosate preparation - Roundup 360 PLUS. It has been shown that the increase in ROS level, and hydroxyl radical in particular may contribute to DNA damage, and thus apoptotic cell death. For that reason, the formation of highly reactive oxygen species (including hydroxyl radical) was assessed in PBMCs treated with glyphosate, its metabolites and impurities. It has been noted that glyphosate, its impurities: N-methylglyphosate, hydroxymethylphosphonic acid and bis(phosphonomethyl)amine as well as its metabolites: AMPA and methylphosphonic acid at the concentration of 5 mM caused an increase in hydroxyl radical level. The highest increase in this parameter was noted in PBMCs exposed to PMIDA (from 0.5 mM Similarly, our study showed that AMPA at lower concentration did not induce hydroxyl radical formation, and the increase in discussing parameter was noted only at its highest level of 5 mM. The proteolytic activity of caspases involves cleavage of their substrates at aspartate residues. In this study we have observed that glyphosate, its metabolites and impurities caused chromatin condensation in human PBMCs. Based on our own research, and also on studies of other authors it may be suggested that glyphosate, its metabolites and impurities induce apoptosis both via the intrinsic pathway (evidenced by the observed increase in total ROS and hydroxyl radical levels, a decrease in transmembrane mitochondrial potential and an increase in caspase-9 activity), and - to a lesser extent via the extrinsic pathway (evidenced by changes in caspase-8 activity) (except for methylphosphonic acid, Nmethylglyphosate and hydroxymethylphosphonic acid).

III. CONCLUSION

Toxicity of pure glyphosate versus its metabolites and impurities: The results obtained in this study do not indicate an essential role of metabolites and production impurities of glyphosate in toxic (proapoptotic) action of glyphosate and possibly glyphosate-based preparations. Research presented in this paper indicated that both AMPA and MPA exerted a smaller effect on ROS and hydroxyl radical formation than glyphosate. For other analysed parameters, no significant differences have been demonstrated between glyphosate and its metabolites activities. Studies on toxicity of glyphosate metabolite - MPA - are even more limited, while our results clearly demonstrated a relatively low toxicity of this compound. The study also revealed that some of glyphosate impurities were characterized by a slightly stronger proapoptotic potential than the parent compound. They constitute, however, a minor impurities of glyphosate and should not significantly increase toxicity of N-(phosphonomethyl)glycine-based products (contrary to surfactants).
Obtained results clearly indicate low proapoptotic properties of all analysed compounds. Initial clear apoptotic effects are associated with their highest analysed concentrations, which correspond to the concentrations to which a human organism could be exposed only as a result of acute or subacute poisoning with glyphosate.

1. Assessment and conclusion

Assessment and conclusion by applicant:

The study describes in-vitro investigations of glyphosate, its metabolites (AMPA and

methylphosphonic acid) and impurities (PMIDA, N-methylglyphosate, hydroxymethylphosphonic acid, and bis-(phosphonomethyl)amine) on six intermediate endpoints of apoptosis (membrane permeability, cytosolic calcium concentration, mitochondrial transmembrane potential, caspase activity, chromatin condensation, and ROS quantitation by two methods) in human peripheral blood mononuclear cells. The reason for selection of this model is not stated but is possibly as potential target tissue for Non-Hodgkins Lymphoma. The methodologies used are frequently reported in literature but are not a standardized or validated method by GLP standards; there is no OECD guideline. Positive control results are not presented and it is unclear if positive controls were used for all assays; wording is sufficiently poor that it may be inferred that some positive controls were used, e.g. nigericin and valinomycin in the studies of mitochondrial transmembrane potential, campothecin in the caspase assays, although these may alternatively be reagents for the assay. It is unclear if assays were conducted in duplicate or triplicate (the stated term was "trice" which may be either twice or thrice), which may then also influence statistical evaluation. However, the methodology appears basically sound.

Apoptotic or pre-apoptotic activity was seen generally consistently across the assays. While glyphosate, its metabolites, and impurities were seen to increase apoptotic endpoints in these assays (0.5 mM and higher), clear effects occurred only at high concentrations.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because no proper cytotoxicity tests were performed, no positive controls were used and the concentration range at which most of the effects were observed is beyond the acceptable physiological range (> 1 mM). The concentration range at which the glyphosate impurities were tested is the same as that for glyphosate which is not a realistic approach for risk assessment of impurities.

Reliability criteria for *in vitro* toxicology studies

Publication: Kwiatkowska *et al*., 2020	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity (95 %). Source: Sigma-Aldrich, USA.
Only glyphosate acid or one of its salts is the tested substance	N	Also glyphosate impurities were tested.
AMPA is the tested substance	Y	
Study		
Test system clearly and completely described	Y	PBMCs
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	N	
Test concentrations in physiologically acceptable range (< 1 mM)	Partly	0.01, 0.05, 0.25, 0.5, 5, 10 mM
Cytotoxicity tests reported	?	
Positive and negative controls	N	No positive controls.
Complete reporting of effects observed	Y	

Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because no proper cytotoxicity tests were performed, no positive controls were used and the concentration range at which most of the effects were observed is beyond the acceptable physiological range (> 1 mM). The concentration range at which the glyphosate impurities were tested is the same as that for glyphosate which is not a realistic approach for risk assessment of impurities.		

Ongoing studies for AMPA

1. Information on the study

Data point:	CA 5.8.1/045
Report author	[redacted]
Report year	Aug 2020
Report title	Aminomethylphosphonic acid: Reverse Mutation Assay 'Ames Test' using Salmonella typhimurium and Escherichia coli
Report No	
Document No	
Guidelines followed in study	OECD Test Guideline 471
Deviations from current test guideline (OECD 425, 2008)	
Previous evaluation	
GLP/Officially recognised testing facilities	
Acceptability/Reliability:	
Category study in AIR 5 dossier (L docs)	

1. Information on the study

Data point:	CA 5.8.1/046
Report author	[redacted]
Report year	Aug 2020
Report title	Aminomethylphosphonic acid: V79 HPRT Gene Mutation Assay
Report No	
Document No	
Guidelines followed in study	OECD Test Guideline 476
Deviations from current test guideline (OECD 425, 2008)	
Previous evaluation	

GLP/Officially recognised testing facilities	
Acceptability/Reliability:	
Category study in AIR 5 dossier (L docs)	

2. Information on the study

Data point:	CA 5.8.1/047
Report author	[redacted]
Report year	Aug 2020
Report title	Aminomethylphosphonic acid: Micronucleus Test in Human Lymphocytes in vitro
Report No	
Document No	
Guidelines followed in study	OECD Test Guideline 487
Deviations from current test guideline (OECD 425, 2008)	
Previous evaluation	
GLP/Officially recognised testing facilities	
Acceptability/Reliability:	
Category study in AIR 5 dossier (L docs)	

Studies with N-acetyl AMPA

The metabolite N-acetyl AMPA was investigated for acute oral toxicity, subchronic toxicity and mutagenicity. All studies presented are submitted on EU level for the first time.

The oral LD_{50} for N-acetyl AMPA was determined to be > 5000 mg/kg bw (limit test), based on the results of an acute oral toxicity study in female rats. No death occurred, but diarrhoea and general clinical signs of toxicity were observed in all animals ([redacted], 2007, CA 5.8.1/032).

In a 90-day feeding study in rats, no adverse effects were noted on in-life parameters, neurobehavioral evaluation and clinical and anatomic pathology at any dose tested. Therefore, a NOAEL of 1163 and 1400 mg/kg bw/day was derived for males and females, respectively ([redacted], 2008, CA 5.8.1/033).

N-acetyl AMPA was tested negative for mutagenicity in bacteria (Ames test) and mammalian cells (HPRT test) ([redacted], 2007, CA 5.8.1/034; [redacted] 2007, CA 5.8.1/036). Furthermore, no clastogenic/aneugenic activity was observed in an *in vitro* chromosomal aberration test in human lymphocytes and in an *in vivo* micronucleus test in mice ([redacted], 2007, CA 5.8.1/035; [redacted], 2007, CA 5.8.1/037).

Table 5.8.1-74: Studies on toxicity with metabolite N-acetyl AMPA

Annex Point	Study	Study type	Substance(s)	Status	Exposure conditions, dose	Result

					levels	
Acute oral toxicity						
CA 5.8.1/032	[redacted], 2007	Acute oral toxicity, SD rat, ♀	N-Acetyl AMPA (Purity: 79 %)	Valid, Category 1	Limit test	LD_{50} oral > 5000 mg/kg bw (females), diarrhea and some general clinical signs observed
Short-term toxicity						
CA 5.8.1/033	[redacted], 2008	90-day oral toxicity study, SD rats, ♀/♂	N-Acetyl AMPA (Purity: 79 %)	Valid, Category 1	900, 6000, and 18000 ppm (equivalent to 55, 374 and 1163 mg/kg bw/day for males and 68, 455 and 1400 mg/kg bw/day for females)	NOAEL = 18000 ppm (equivalent to 1163 and 1400 mg/kg bw/day for males and females, respectively)
Genotoxicity						
CA 5.8.1/034	[redacted], 2007	Genotoxicity in bacteria (Ames); *S. typhimurium* TA 98, TA 100, TA 1535 and TA 1537, and *E. coli* WP2 uvrA	N-Acetyl AMPA (Purity: 76 %)	Valid, Category 1	1.5 - 5000 µg/plate, ± S9, plate incorporation method	Negative
CA 5.8.1/035	[redacted], 2007	*In vitro* chromosome aberration test, human lympocytes	N-Acetyl AMPA (Purity: 76 %)	Valid, Category 1	191.25 - 1530 µg/mL, ± S9	Negative
CA 5.8.1/036	[redacted], 2007	*In vitro* mammalian cell gene mutation test (HPRT), CHO cells	N-Acetyl AMPA (Purity: 72 %)	Valid, Category 1	100 - 1531 µg/mL, ± S9	Negative
CA 5.8.1/037	[redacted], 2007	*In vivo* micronucleus test, CD-1 mice, ♀/♂	N-Acetyl AMPA (Purity: 72 %)	Valid, Category 1	Single oral doses of 500, 1000 and 2000 mg/kg bw *via* gavage	Negative

AMPA = Aminomethyl Phosphonic Acid

Acute oral toxicity

CA 5.8.1/032

1. Information on the study

Data point:	CA 5.8.1
Report author	[redacted]
Report year	2007

Report title	IN-EY252: Acute Oral Toxicity Study in Rats - Up-and-Down Procedure
Report No	[redacted]-22229
Document No	NA
Guidelines followed in study	U.S. EPA Health Effect Test Guidelines OPPTS 870.1100 (2002); OECD Guideline for the Testing of Chemicals Section 4 (Part 425) (2001)
Deviations from current test guideline (OECD 425, 2008)	None
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive summary

A single dose of IN-EY252 was administered by oral gavage to 3 fasted female rats at a dose of 5000 mg/kg bw. The rats were dosed one at a time at a minimum of 48-hour intervals. The rats were observed for mortality, body weight effects and clinical signs for 14 days after dosing. All rats were necropsied to detect grossly observable evidence of organ or tissue damage.
No deaths occurred. Clinical signs of toxicity were observed in all rats up to 2 days after dosing and included diarrhoea, dark eyes, lethargy, high posture, stained fur/skin, wet fur, ataxia and/or hyper-reactivity. No body weight losses occurred after dosing. No test substance-related gross lesions were found in the study.
Under the conditions of this study, the oral LD_{50} for IN-EY252 was greater than 5000 mg/kg bw for female rats.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	N-Acetyl AMPA (IN-EY252)
Identification:	[(Acetylamino)methyl]phosphonic acid
Description:	White solid
Lot/Batch #:	(IN-EY252-) 003
Purity:	79 %
Stability of test compound:	The test substance appeared to be stable under the conditions of the study. No evidence of instability, such as a change in colour or physical state was observed. Expiry date: 2010-05-16.
2. Vehicle and/ or positive control:	Deionised water / none
3. Test animals:	
Species:	Rat
Strain:	Crl:CD(SD)
Source:	[redacted]

Age:	10 – 11 weeks
Sex:	Female
Weight at dosing:	207.9 – 216.2 g
Acclimation period:	6 days
Diet/Food:	PMI® Nutrition International, LLC Certified Rodent LabDiet® 5002, *ad libitum*
Water:	Water, *ad libitum*
Housing:	Individually in stainless steel, wire-mesh cages suspended above cage boards
Environmental conditions:	Temperature: 18 – 26 °C Humidity: 30 – 70 % Air changes: Not reported 12 hours light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2007-08-28 to 2007-09-12

Animal assignment and treatment:
A single oral dose of IN-EY252, suspended in deionised water, was administered by oral gavage to 3 fasted female rats at a dose of 5000 mg/kg bw. The rats were dosed one at a time at a minimum of 48-hour intervals. The rats were fasted approximately 16 – 18 hours prior to dosing, with food being returned to the rats approximately 3 – 4 hours after dosing. Individual dose volumes were calculated using the fasted body weights obtained prior to dosing. The rats were dosed at a volume of 20 mL per kg of body weight. The dosing suspensions were stirred throughout the dosing procedure.

Table 5.8.1-75: IN-EY252: Acute Oral Toxicity Study in Rats - Up-and-Down Procedure ([redacted], 2007): Study design

Test group	Test substance concentration [mg/kg bw]	Females
IN-EY252	5000	3

Mortality
Viability was checked daily.

Clinical observations
Observations for signs of illness, injury or abnormal behaviour were made daily.
The rats were observed for clinical signs at the beginning of fasting, just before dosing (test day 0), once during the first 30 minutes after dosing and 2 more times on the day of dosing and once each day thereafter for the 14 day observation period.

Body weight
The rats were weighed on test days –1, 0, 7 and 14.

Sacrifice and pathology
On test day 14, the rats were anesthetised by carbon dioxide, euthanised by exsanguination and necropsied to detect grossly observable evidence of organ or tissue damage.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no animals found dead or killed *in extremis* in any group during the treatment period.

B. CLINICAL OBSERVATIONS

Clinical signs of toxicity were observed in all rats up to 2 days after dosing and included diarrhoea, dark eyes, lethargy, high posture, stained fur/skin (abdomen, yellow; inguen, yellow or brown; perineum, yellow or brown), wet fur (perineum), ataxia and/or hyper-reactivity.

C. BODY WEIGHT

No body weight losses occurred after dosing.

D. NECROPSY

Gross pathology

No test substance-related gross lesions were found in the study. The only gross lesion observed, uterus dilatation in rat 3308, was non-specific and not indicative of target organ toxicity.

III. CONCLUSIONS

Under the conditions of this study, the oral LD_{50} for IN-EY252 was greater than 5000 mg/kg bw for female rats.

3. Assessment and conclusion

Assessment and conclusion by applicant:

This study is considered to be valid as it was conducted under GLP and no deviations from the recent guideline could be identified.

Under the conditions of this study, the oral LD_{50} for IN-EY252 was greater than 5000 mg/kg bw for female rats.

Assessment and conclusion by RMS:

Short-term toxicity

CA 5.8.1/033

1. Information on the study

Data point	CA 5.8.1
Report author	[redacted]
Report year	2008
Report title	IN-EY252: Subchronic Toxicity 90-Day Feeding Study in Rats
Report No	[redacted]-23316
Document No	n.a.
Guidelines followed in study	OPPTS 870.3100 (1998); OECD Guideline 408 (1998)
Deviations from current test guideline (OECD 408, 2018)	Clinical chemistry was performed without determining LDL, HDL, T3, T4 and TSH; organ weights were determined without prostate (+seminal vesicles and coagulating glands), thyroid and pituitary gland; histopathology was performed without coagulating glands, gall bladder and male mammary glands.
Previous evaluation	No, not previously submitted
GLP/Officially recognised	Yes

testing facilities	
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive summary

In a 90-day feeding study, IN-EY252 technical was administered to male and female Crl:CD(SD) rats (10 rats/sex/concentration) at concentrations of 0, 900, 6000, and 18000 ppm (equivalent to 0, 55, 374 and 1163 mg/kg bw/day for males and 0, 68, 455 and 1400 mg/kg bw/day for females). Parameters evaluated included body weight, body weight gain, food consumption, food efficiency, clinical signs, abbreviated functional observational battery (FOB), haematology, clinical chemistry, urinalysis, ophthalmology, organ weights and gross and microscopic pathology.

No adverse effects were noted in any of the parameters examined. Test substance-related clinical signs, soft faeces, and dried and/or wet brown material around the anogenital area were observed in 18000 ppm group males and females. Final body weights (test day 91) and overall body weight gains (test day 0 – 91) in males and females were 7.6 % and 5.0 % and 11.5 % and 9.3 %, respectively, lower (statistically non-significant) than controls in the 18000 ppm group. These changes were considered test substance related, but not adverse.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	N-Acetyl AMPA (IN-EY252)
Identification:	[(Acetylamino)methyl]phosphonic acid
Description:	Solid
Lot/Batch #:	(IN-EY252-) 003
Purity:	79 %
Stability of test compound:	The test substance appeared to be stable under the conditions of the study. No evidence of instability, such as a change in colour or physical state was observed. Expiry date: 2010-05-16.
2. Vehicle and/ or positive control:	Diet / none
3. Test animals:	
Species:	Rat
Strain:	Crl:CD(SD)
Source:	[redacted]
Age:	Approximately 8 weeks
Sex:	Male and female
Weight at dosing:	♂ 237 – 333 g; ♀ 164 – 212 g
Acclimation period:	20 days
Diet/Food:	PMI® Nutrition International, LLC Certified Rodent LabDiet® 5002, *ad libitum*
Water:	Reverse osmosis-treated (on-site) drinking water, *ad libitum*

Housing:	Individually in stainless steel, wire-mesh cages suspended above cage boards	
Environmental conditions:	Temperature:	22 ± 3 °C (21.8 – 22.1 °C)
	Humidity:	50 ± 20 % (36.7 – 62.4 %)
	Air changes:	10 air changes / hour
	12 hours light / dark cycle	

B. STUDY DESIGN AND METHODS

In life dates: 2007-08-20 to 2007-11-19/20

Animal assignment and treatment:
The test substance, IN-EY252, was administered on a continuous basis in the basal diet for 90 or 91 days to 3 groups (Groups 2 – 4) of Crl:CD(SD) rats. Dose levels were 900, 6000 and 18000 ppm. A concurrent control group (Group 1) received the basal diet on a comparable regimen. Each group consisted of 10 animals per sex.

Table 5.8.1-76: IN-EY252: Subchronic Toxicity 90-Day Feeding Study in Rats ([redacted], 2008): Study design

Test group	Dietary concentration [ppm]	Mean daily intake mg/kg bw/day	Number of animals	
			Males	Females
Control	0	♂: 0; ♀: 0	10	10
Low	900	♂: 55; ♀: 68	10	10
Intermediate	6000	♂: 374; ♀: 455	10	10
High	18000	♂: 1163; ♀: 1400	10	10

Analysis of the test substance
Prior to the initiation of dose administration, quadruplicate samples (approximately 25 g each) for homogeneity determination were collected from the top, middle and bottom strata of the 900, 6000 and 18000 ppm test diet preparations. In addition, quadruplicate samples (approximately 25 g each) for stability determinations were collected from the middle strata of these same test diet preparations at dietary levels of 900 and 18000 ppm and stored at room temperature or refrigerated for 7 or 15 days. Quadruplicate samples (approximately 25 g each) for concentration analyses were collected from the middle stratum of each test diet preparation, including the diet preparation administered to the control group, prepared on study week 0, study week 6 and study week 12. Two samples from each set of 4 samples collected were shipped frozen to the Sponsor, DuPont Haskell Laboratory for Health and Environmental Sciences, Newark, Delaware, for analysis. The remaining samples from each sample set were kept frozen at [redacted], and were retained as back-up samples. In addition, a 100 g sample of the basal diet was shipped to the sponsor with each shipment of test diet samples. The basal diet was used by the sponsor to prepare matrix-matched analytical chemistry standards for the homogeneity, stability and confirmation of concentration analyses.

Mortality
All animals were observed twice daily, once in the morning and once in the afternoon, for mortality and moribundity.

Clinical observations
Clinical examinations were performed once daily for all animals. The daily observations were performed at approximately the same time each day and were omitted on days of detailed physical examinations. All significant findings were recorded. Detailed physical examinations were conducted on all animals weekly, beginning 1 week prior to test substance administration and prior to the scheduled necropsy. A separate computer protocol was used to record any observations noted outside of the above-specified intervals.

Body weight
Individual body weights were recorded at least weekly, beginning 3 weeks prior to test substance administration (study week -3). Mean body weights and mean body weight changes were calculated for the corresponding intervals. Final body weights were recorded on the day prior to (un-fasted) and on the day of (fasted) the scheduled necropsy.

Food consumption and test substance intake
Individual food consumption was recorded weekly, beginning 3 weeks prior to test substance administration (study week -3 to -2). Food intake was calculated as g/animal/day for the corresponding body weight intervals. Food efficiency (body weight gained as a percentage of food consumed) was also calculated and reported for these intervals.
The mean amounts of test substance consumed (mg/kg bw/day) by each sex of each dietary group was calculated from the mean food consumed (g/kg of bw/day) and the appropriate target concentration of test substance in the food (mg/kg of diet).

Abbreviated Functional Observational Battery (FOB)
Abbreviated functional observational battery (FOB) assessments were recorded for all animals prior to the initiation of dose administration and during study week 12. Testing was performed by the same trained technicians, whenever possible, without knowledge of the animals' group assignments. The FOB was performed in a sound-attenuated room equipped with a white-noise generator set to operate at 70 ± 10 dB, to minimise environmental variations in the test conditions. The FOB used at [redacted] is based on previously developed protocols ([redacted], 1991; [redacted], 1968; [redacted], 1982; [redacted]., 1988; [redacted], 1989; [redacted], 1989). All animals were observed for the following parameters as described below:

1. Home Cage Observations: Feces consistency
2. Sensory Observations: Approach response, startle response, pupil response, touch response and tail pinch response
3. Neuromuscular Observations: Grip strength - hind and forelimb

Locomotor activity
Locomotor activity was assessed for all animals prior to the initiation of dose administration and during study week 12. Locomotor activity, recorded after the completion of the abbreviated FOB, was measured automatically using the SDI Photobeam Activity System (San Diego Instruments, Inc., San Diego, California). This personal computer-controlled system utilised a series of infrared photobeams surrounding a clear plastic, rectangular cage to quantify the motor activity of each animal. Four-sided black plastic enclosures were used to surround the clear plastic boxes and decrease the potential for distraction from extraneous environmental stimuli or activity by technicians or adjacent animals. The black enclosures rested on top of the photobeam frame and did not interfere with the path of the beams. The locomotor activity assessment was performed in a sound-attenuated room equipped with a white-noise generator set to operate at 70 ± 10 dB, to minimise environmental variations in the test conditions. The testing of treatment groups was conducted according to replicate sequence. Each animal was tested separately. Data were collected in 5-minute epochs and the test session duration was 60 minutes. These data were compiled as four 15-minute sub-sessions for tabulation.
Data for ambulatory and total motor activity were tabulated. Total motor activity was defined as a combination of fine motor skills (i.e., grooming, interruption of 1 photobeam) and ambulatory motor activity (interruption of 2 or more consecutive photobeams).

Ophthalmoscopic examination
Ocular examinations were conducted on all animals prior to the initiation of dose administration (study week -3) and near the end of the treatment period (study week 12). All ocular examinations were conducted using an indirect ophthalmoscope and slit lamp biomicroscope preceded by pupillary dilation with tropicamide ophthalmic solution, USP, 0.5 %.

Haematology, clinical chemistry and urinalysis

Blood and urine samples for clinical pathology evaluations (haematology, coagulation, serum chemistry and urinalysis) were collected from all surviving animals at the scheduled necropsy (study week 13). In addition, blood samples for clinical pathology evaluations (haematology, coagulation and serum chemistry) were collected and analysed from the Group 3 male euthanised *in extremis* (prior to euthanisation). The animals (excluding the animal euthanised *in extremis*) were fasted overnight prior to blood collection while in metabolism cages for urine collection. Blood was collected for haematology and serum chemistry evaluation via the retro-orbital sinus of animals anesthetised by inhalation of isoflurane. Blood samples for analysis of coagulation parameters were collected at the time of euthanasia via the *vena cava* of animals' euthanised by inhalation of carbon dioxide. Blood was collected into tubes containing EDTA (haematology), sodium citrate (clotting determinations) or no anticoagulant (serum chemistry).

The following haematological parameters were evaluated: Blood smears, total leukocyte count, erythrocyte count, haemoglobin, haematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count, prothrombin time, activated partial thromboplastin time (APTT), reticulocyte count (percent/absolute) and differential leukocyte count (percent and absolute: Neutrophil, lymphocyte, monocyte, eosinophil, basophil and large unstained cells).

The following clinical chemistry parameters were evaluated: Albumin, total protein, globulin, albumin/globulin ratio (A/G Ratio), total bilirubin (Total Bili), urea nitrogen, creatinine, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, gamma glutamyl-transferase, glucose, total cholesterol, calcium, chloride, phosphorus, potassium, sodium, sorbitol dehydrogenase and triglycerides.

The following urinalysis parameter were evaluated: Specific gravity, pH, urobilinogen, total volume, colour, clarity, protein, glucose, ketones, bilirubin, occult blood, leukocytes, nitrites, microscopy of sediment and osmolality.

Sacrifice and pathology

Gross pathology

A complete necropsy was conducted on all animals. Animals were euthanised by carbon dioxide anaesthesia and exsanguinated. The necropsies included, but were not limited to, examination of the external surface, all orifices, and the cranial, thoracic, abdominal and pelvic cavities, including viscera.

Organ weights

The following organs were weighed from all animals at the scheduled necropsy: Adrenals, brain, epididymides, heart, kidneys, liver, ovaries with oviducts, spleen, testes, thymus and uterus. Paired organs were weighed together. Organ / body weight and organ / brain weight ratios were calculated.

Histopathology

After fixation, protocol-specified tissues were trimmed according to standard operating procedures and the protocol. Trimmed tissues were processed into paraffin blocks, sectioned at 4 to 8 microns, mounted on glass microscope slides and stained with haematoxylin and eosin. The following organs were examined: Adrenals, aorta, bone with marrow (with femur and sternum), bone marrow smear, brain, cervix, epididymides, exorbital lacrimal glands, eyes with optic nerve, oesophagus, stomach, duodenum, jejunum, ileum, Peyer's Patches, caecum, colon, rectum, heart, kidneys, larynx, liver, lungs, lymph nodes (mandibular and mesenteric), nasal cavity, mammary gland (females only), ovaries (with oviducts), pancreas, peripheral nerve (sciatic), pharynx, pituitary, prostate, salivary glands (mandibular), seminal vesicles, skeletal muscle (rectus femoris), skin, spinal cord (cervical, thoracic, lumbar), spleen, testes, thymus, thyroid/parathyroids, tongue, trachea, urinary bladder, uterus, vagina and all gross lesions and masses.

Statistics

Body weight, food intake parameters, abbreviated FOB, clinical pathology, and organ weight data were subjected to a parametric one-way analysis of variance (ANOVA) to determine intergroup differences. If the ANOVA revealed statistically significant ($p<0.05$) intergroup variance, Dunnett's test was used. Functional observational battery parameters that yielded scalar or descriptive data were analysed using

Fisher's Exact Test. Non-parametric statistical analysis was conducted for gamma glutamyl transferase.

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSIS

Homogeneity and stability were verified prior to study start and concentration verification was done 3 times during the study (start, middle and end). The test substance was at target concentrations (95.6 – 109.4 % nominal), homogeneous (94.2 – 102.2 % nominal) throughout the feed, and was stable (88.3 – 111.1 %) for up to 15 days at room temperature. Based on this information, it can be concluded that the animals received the targeted dietary concentrations of test substance during the study.

B. MORTALITY

One 6000 ppm group male was euthanised *in extremis* on study day 36, which was associated with nasal cavity fractures and was unrelated to test substance administration. Clinical observations noted in this male included dermal atonia, gasping, upper and lower incisors malaligned/broken, red material around the left and right eyes, soft faeces, decreased defecation, smaller than normal faeces, dried yellow material around the urogenital area and dried brown material around the anogenital area.
Additionally one control group male was found dead on study day 71. The cause of death was undetermined, as there were no significant or remarkable clinical findings for this animal throughout the study or at the unscheduled necropsy.

None of the deaths was related to treatment.

C. CLINICAL OBSERVATIONS

Clinical findings were primarily limited to the 18000 ppm group males and females and included occurrences of soft faeces and dried and/or wet brown material around the anogenital area. These clinical findings were considered to be test substance-related, but non-adverse. Other clinical signs were common findings for laboratory rats of this age and strain.

D. BODY WEIGHT

There were no adverse effects on body weights or body weight gains. Final body weights (test day 91) were 7.6 % and 5 % lower than controls in the 18000 ppm males and females, respectively. Overall (test day 0 – 91) body weight gains were 11.5 % and 9.3 % lower than controls in the 18000 ppm males and females, respectively (or there were no test substance-related effects on body weights or body weight gains). These changes were not statistically significant and not adverse. No changes were observed in lower groups.

Table 5.8.1-77: IN-EY252: Subchronic Toxicity 90-Day Feeding Study in Rats ([redacted], 2008): Body weight, body weight gain, food consumption and food efficiency

	Dose group [ppm]							
	Males				Females			
	0	900	6000	18000	0	900	6000	18000
Final body weight [g], (% control)	593	↓561 (95 %)	↓563 (95 %)	↓548 (92 %)	305	↓312 (98 %)	↓302 (99 %)	↓290 (95 %)
Body weight gain [g], (% control)	304	↓277 (91 %)	↓282 (93 %)	↓269 (88 %)	118	↑123 (104 %)	↓115 (97 %)	↓107 (91 %)
Food consumption [g], (% control)	29	↓27 (93 %)	↓28 (97 %)	↓28 (97 %)	20	20 (100 %)	20 (100 %)	20 (100 %)
Food efficiency [g bw gain/g FC], (% control)	11	11 (100 %)	↓7 (64 %)	↓10 (91 %)	6	↑7 (117 %)	6 (100 %)	6 (100 %)

E. FOOD COMSUMPTION; FOOD EFFICIENCY AND DAILY INTAKE

There were no test substance-related effects on food consumption and food efficiency. The mean daily intakes for male rats were 0, 55, 374 and 1163 mg/kg bw/day. The mean daily intakes for female rats were 0, 68, 455 and 1400 mg/kg bw/day.

F. OPHTHALMOLOGICAL EXAMINATIONS

There were no test substance-related ophthalmoscopically visible changes in the eyes of either male or female rats at any dietary concentration.

G. NEUROBEHAVIORAL EVALUATIONS

There were no definitive test substance-related effects on sensory or neuromuscular parameters at the study week 12 abbreviated FOB evaluations.

Home cage observations

Test substance-related effects on home cage parameters included a statistically significantly greater number of 18000 ppm group males (10/10 animals) and females (7/10 animals) with partially formed pellets (faeces consistency) in comparison to the control group males (0/10 animals) and females (0/10 animals) during the study week 12 evaluation. These home cage observations are consistent with the abnormal excreta observations (soft faeces and dried and/or wet brown material around the anogenital area) noted in the 18000 ppm group animals during the daily clinical examinations and are considered to be non-adverse.

Sensory Observations

There were no test substance-related effects on sensory parameters when the test substance-treated males and females were compared to the control group at the study week 12 abbreviated FOB evaluations.

Neuromuscular Observations

There were no test substance-related effects on neuromuscular parameters when the test substance-treated males and females were compared to the control group at the study week 12 abbreviated FOB evaluations. Males in the 18000 ppm group had slightly lower forelimb and hind limb grip strength compared to the control group during the study week 12 evaluation. Since the difference was also present at the pre-test evaluation, the slightly lower grip strength during the study week 12 evaluation in the 18000 ppm group males was not considered to be test substance-related. Females in the 18000 ppm group also had slightly lower grip strength compared to the control group during the study week 12 evaluation. Since cumulative body weight gain for the 18000 ppm group males and females was slightly lower compared to the respective control group, the slightly lower grip strength in the 18000 ppm group males and females may also be secondary to the lower cumulative body weight gain in this group

Locomotor Activity

Within-session repeated measures analyses of variance were conducted across the subintervals of each test session for total and ambulatory counts and for overall interval means (representing the entire 60-minute session activity) during each test session. Locomotor activity patterns (mean ambulatory and total motor activity counts) were unaffected by test substance administration. There were no statistically significant differences for the test substance-treated males and females when compared to the control group during study week 12. Values obtained from the four epochs (0 – 15 minutes, 16 – 30 minutes, 31 – 45 minutes and 46 – 60 minutes) evaluated and the overall 60-minute test session values were comparable to the concurrent control values. No remarkable shifts in the pattern of habituation occurred in any of the test substance-treated groups when the animals were evaluated during study week 12.

H. HAEMATOLOGY, CLINICAL CHEMISTRY AND URINALIYIS

There were no test substance-related alterations in haematology, serum chemistry and urinalysis parameters.

Haematology

A statistically significant higher prothrombin time in the 18000 ppm group males was noted at study week

13 but was not toxicologically significant because the increase was very small and well within the [redacted] Historical Control Range Version 2.5 (reference range of 13.0 – 19.6 seconds from 15 studies).

Table 5.8.1-78: IN-EY252: Subchronic Toxicity 90-Day Feeding Study in Rats ([redacted], 2008): Selected haematological parameters

Parameter	Dose group [ppm]							
	Males				Females			
	0	900	6000	18000	0	900	6000	18000
Prothrombin time [s]	13.1 ± 0.56	↑13.4 ± 0.43	↑13.5 ± 0.81	**↑14.3* ± 1.26**	12.6 ± 0.36	↓12.3 ± 0.58	↓12.3 ± 0.60	↓12.4 ± 0.53

*: Statistically significant from control ($p<0.05$)

Clinical Chemistry
There were no test substance-related alterations in serum chemistry parameters.

Urinalysis
Urine pH was 9 % lower in the 18000 ppm group males at study week 13. Eighty percent of the 18000 ppm group males had a urine pH of 6.0, compared with 0 %, 10 % and 20 % of 0, 900 and 6000 ppm group males at study week 13. Urine volume was 51 % lower and osmolality was 41 % higher in the 18000 ppm group males at study week 13 and the small change in urine pH may have been related to concentrated urine and was not adverse.

Table 5.8.1-79: IN-EY252: Subchronic Toxicity 90-Day Feeding Study in Rats [redacted], 2008): Selected urinalysis parameters

Parameter	Dose group [ppm]							
	Males				Females			
	0	900	6000	18000	0	900	6000	18000
pH value	6.7 ± 0.26	↓6.5 ± 0.44	↓6.6 ± 0.39	**↓6.1** ± 0.21**	6.3 ± 0.35	↑6.5 ± 0.41	↓6.1 ± 0.46	↓6.2 ± 0.35
Urine volume [mL]	8.9 ± 4.51	↓6.7 ± 3.92	↓8.7 ± 5.24	↓4.4 ± 1.96	8.7 ± 10.52	↓5.5 ± 4.3	↓4.1 ± 1.73	↓5.6 ± 2.95
Osmolality [mOsm/kg]	1378 ± 418.6	↑1388 ± 521.4	↓1275 ± 531.3	↑1944 ± 618.1	1089 ± 484.7	↑1215 ± 568.9	↑1433 ± 615.4	↓1012 ± 453.3

**: Statistically significant from control ($p<0.01$)

I. NECROPSY

No test substance-related changes in mean organ weights (absolute or relative) and gross lesions were observed at any dietary concentration. There were no test substance-related microscopic findings in the male or female 18000 ppm groups.

Histopathology
Inflammation was multifocally present within the lungs of individuals throughout the control and 18000 ppm groups (6/10 and 8/10 males and 5/10 and 8/10 females in the control and 18000 ppm groups, respectively). A mixed population of inflammatory cells consisting primarily of mononuclear cells (macrophages and lymphocytes) occasionally admixed with low numbers of neutrophils was typically noted in perivascular areas. Occasionally, inflammatory infiltrate extended into adjacent parenchyma and was associated with septal thickening and alveolar infiltration. This idiopathic pulmonary lesion has been described in multiple rat strains, and the most severe lesions occur in 10 – 12 week old animals. Lesions appear to be transient, with apparent resolution in older rats, and etiology remains undetermined although a viral agent is suspected ([redacted] 1997; [redacted]., 1997; [redacted]., 1998). The inflammatory lesion observed in this study is consistent with those reported and the presence of this lesion did not hinder histopathological evaluation of potential test substance-related effects. There were no test substance-related

alterations in the lungs.
Remaining histologic changes were considered to be incidental findings or related to some aspect of experimental manipulation other than administration of the test substance. There was no test substance-related alteration in the prevalence, severity or histologic character of those incidental tissue alterations.

III. CONCLUSIONS

There were no indications of systemic toxicity when IN-EY252 was administered on a continuous basis in the basal diet for 90 or 91 days to 3 groups (Groups 2 – 4) of Crl:CD(SD) rats at dose levels of 900, 6000 and 18000 ppm. IN-EY252 was well tolerated with no adverse effects on clinical observations, body weights, food consumption, FOB parameters and clinical pathology parameters. In addition, there were no ophthalmic, histologic or gross alterations related to test substance administration. Therefore, the no-observed-adverse-effect level (NOAEL) in this study for administration of IN-EY252 on a continuous basis in the basal diet for 90 or 91 days to Crl:CD(SD) rats was 18000 ppm (equivalent to 1163 and 1400 mg/kg bw/day for males and females, respectively).

3. Assessment and conclusion

Assessment and conclusion by applicant:
Despite the deviations in clinical chemistry (LDL, HDL, T3, T4 and TSH not determined), organ weights (prostate + seminal vesicles and coagulating glands), thyroid and pituitary gland not determined) and histopathology (coagulating glands, gall bladder and male mammary glands not examined) this study was conducted under GLP and was considered to be valid.
Based on a lack of adverse effects on in-life parameters, neurobehavioral evaluation and clinical and anatomic pathology in males and females at any dose level, the NOAEL for males and females was 18000 ppm (equivalent to 1163 and 1400 mg/kg bw/day, respectively).

Assessment and conclusion by RMS:

Genotoxicity

CA 5.8.1/034

1. Information on the study

Data point	CA 5.8.1
Report author	[redacted]
Report year	2007
Report title	IN-EY252: Bacterial Reverse Mutation Assay
Report No	AB47BT.503.BTL
Document No	DuPont-22227
Guidelines followed in study	OECD 471 (1998), EEC Commission Directive 2000/32/EC Annex 4D-B.13/14 (2000), US EPA OPPTS 870.5100, JMAFF 12 NouSan 8147 (2000 and 2001)
Deviations from current test guideline	None
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes

Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive summary

[(Acetylamino)methyl]phosphonic acid (IN-EY252, batch: IN-EY252-001, purity: 76 %), metabolite of glyphosate was investigated for its potential to cause gene mutation in bacteria (Ames test). *S. typhimurium* strains TA 98, TA 100, TA 1535 and TA 1537 and *E. coli* strain WP2 uvrA were exposed to the test item, solvent (water) and appropriate positive controls in the presence and absence of metabolic activation (Aroclor 1254-induced rat liver S9 fraction). A preliminary cytotoxicity and mutagenicity experiment was performed to identify cytotoxic concentrations of the test item. In a standard plate test (plate incorporation method), test item concentrations in the range of 1.5 to 5000 µg/plate were applied in the presence and absence of S9 mix. No cytotoxicity was observed in any tester strain up to the highest tested concentration, neither in the presence, nor in the absence of S9 mix.

The preliminary experiment was designated Experiment B1 of the main mutation assay. However, as no bacterial growth was observed on the tester strain plates TA 1535, TA 1537 and WP2 uvrA in the presence and absence of S9 mix, a repeat experiment was conducted with the respective strains (Experiment B2). In addition, a second standard plate test (Experiment B3) was performed using concentrations in the range of 50 – 5000 µg/plate. Both experiments were performed with triplicate plates. After 48 – 72 hours of incubation at 37 ± 2 °C, the bacterial background lawn was examined and the number of revertant colonies were counted for each plate.

Precipitation of the test item in vehicle or cytotoxicity, evident as a reduction in the number of revertant colonies or in the bacterial background lawn were not observed for any strain in any tested concentration, neither with nor without S9 mix.

There was no statistically significant and biologically relevant increase in the number of his^+ or trp^+ revertant colonies observed in any experiment for any strain at any concentration, neither in the presence nor in the absence of metabolic activation. The number of revertant colonies induced by the solvent and the positive controls were within the range of the laboratories historical control data, demonstrating the functionality of the metabolic activation system and the sensitivity of the test.

Under the conditions of the test, [(Acetylamino)methyl]phosphonic acid, metabolite of glyphosate, is not mutagenic in the Ames standard plate test with and without metabolic activation.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	[(Acetylamino)methyl]phosphonic acid (metabolite of glyphosate)
Identification:	IN-EY252
Description:	White powder
Lot/Batch number:	IN-EY252-001
Purity:	76 %
Stability of test compound:	The stability of the test item at storage conditions (at room temperature in the dark with desiccant) was guaranteed until the reported expiry date 25 Apr 2009. The stability of the test item in solvent was confirmed by analytical methods.
Solvent (vehicle) used:	Water

2. Control materials:

Negative control:	A negative control was not employed in this study.

Solvent (vehicle) control: Water

Solvent (vehicle)/final concentration: 0.1 mL per plate.

Positive controls: Please refer to table below.

Strain	Metabolic activation	Mutagen	Conc. [µg/plate]
S. typhimurium strains			
TA 100	-S9	Sodium azide	1.0
	+S9	2-Aminoanthracene[#]	1.0
TA 1535	-S9	Sodium azide	1.0
	+S9	2-Aminoanthracene[#]	1.0
TA 98	-S9	2-Nitrofluorene	1.0
	+S9	2-Aminoanthracene[#]	1.0
TA 1537	-S9	9-Aminoacridine	75.0
	+S9	2-Aminoanthracene[#]	1.0
E. coli strains			
WP2 uvrA	-S9	Methyl methanesulfonate	1000.0
	+S9	2-Aminoanthracene[#]	10.0

[#]: The functionality of the S9 mix batch used was additionally checked with 7,12-dimethylbenz(a)anthracene and showed the expected results.

3. Metabolic activation:

S9 mix was purchased from [redacted] and obtained from the livers of male Sprague-Dawley rats. The animals received a single intraperitoneal injection of Aroclor 1254 at a dose of 500 mg/kg bw. The livers were prepared 5 days after treatment. The S9-mix was thawed prior to each experiment and co-factors were immediately added to prepare S9 mix containing the following components:

S9 mix component	Concentration	Unit
Phosphate buffer (pH 7.4)	100	mM
KCl	33	mM
NADPH-generating system		
Glucose 6-phosphate	5	mM
NADP	4	mM
$MgCl_2$	8	mM
S9	10	% (v/v)

4. Test organisms:

Tester strains				Bacteria batch checked for	
S. typhimurium		***E.coli***			
TA 98	✓	WP2 uvrA	✓	deep rough character (rfa)	✓
TA 100	✓	WP2 uvrA (pKM101)		ampicillin resistance (R factor plasmid)	✓
TA 1535	✓			UV-light sensitivity (absence of uvrB and uvrA genes in *S. typhimurium* and *E. coli* strains, respectively)	✓
TA 1537	✓				
TA 1538				Histidine auxotrophy (automatically via the spontaneous rate)	✓

5. Test concentrations:

(a) Preliminary cytotoxicity and mutagenicity test / Experiments B1 & B2 of the main mutation assay:

Plate incorporation test ± S9 mix:	

Concentrations:	*1.5, 5, 15, 50, 150, 500, 1500 and 5000 µg/plate**	
Tester strains:	TA 98, TA 100, TA 1535, TA 1537 and WP2uvrA	
Replicates:	*Duplicate*	

* *Dosing solutions were adjusted to compensate the purity of the test substance (76 %) by using a correction factor of 1.45.*

(b) Experiment B3 of the main mutation assay:

Plate incorporation test ± S9 mix:	
Concentrations:	*50, 150, 500, 1500 and 5000 µg/plate**
Tester strains:	TA 98, TA 100, TA 1535, TA 1537 and WP2uvrA
Replicates:	*Triplicates*

*: Dosing solutions were adjusted to compensate the purity of the test substance (76 %) by using a correction factor of 1.45.

B: Study design and methods

Dates of experimental work: 01 – 21 Mar 2007
Finalisation date: 23 Jul 2007

1. Standard plate test (plate-incorporation test, SPT):
An aliquot of 0.1 mL test solution, vehicle or positive control, an aliquot of 0.1 mL fresh bacterial culture and 0.5 mL S9 mix (in tests with metabolic activation only) or S9 substitution buffer (in tests without S9 mix) were added to 2 mL of molten selective top agar (supplemented with 0.05 mM L-histidine + 0.05 mM L-biotin or 0.05 mM L-tryptophan). When plating the positive controls, the test item solution was replaced by 50 µL aliquot of the respective positive control. After vortexing, the mixture was overlaid onto the surface of a minimal bottom agar plate. After the overlay had solidified, the plates were inverted and incubated for approx. 48 – 72 hours at 37 ± 2 °C. Following incubation, the bacterial background lawn was examined and the number of his^+ revertant colonies were counted. Plates that were not counted immediately following the incubation period were stored at 2 – 8 °C until evaluation. Each concentration and the controls were tested in triplicates.

2. Cytotoxicity
Toxicity was detected by a

- reduction in the number of spontaneous revertants by > 50 %. The reduction must have been accompanied by an abrupt dose-dependent drop in the revertant count.
- clearing or diminution of the background lawn (= reduced his^- or trp^- background growth)

and recorded for all test groups both with and without S9 mix in all experiments.

3. Statistics
For each replicate plating, the mean and standard deviation of the number of revertants per plate were calculated. No further statistical analysis was performed.

4. Acceptance criteria
The test was considered valid if the following criteria were met:

- All bacterial cultures demonstrated the characteristic mean number of spontaneous revertants in the vehicle controls.
- To ensure that appropriate numbers of bacteria were plated, tester strain cultures must have been greater or equal to 0.3 x 10^9 cells/mL.
- The mean of each positive control exhibited at least a 3.0-fold increase in the number of revertants over the mean value of the respective vehicle control.
- A minimum of 3 non-toxic dose levels were included for the evaluation of data.

5. Evaluation criteria

A test item was considered positive (mutagenic) in the assay if the following criteria were met:

- There was a dose-related increase in the mean number of revertants per plate in at least one tester strain over a minimum of two increasing concentrations of the test substance when compared to the vehicle control.
- The increase in the mean number of revertant colonies per plate were at least 3.0-fold for tester strains TA 1535 and TA 1537 and at least 2.0-fold for tester strains TA 98, TA 100 and WP2uvrA.

An equivocal response was obtained if there was a biologically relevant increase in the number of revertants that partially met the criteria for evaluation as a positive substance. This was for example the case if a dose-responsive increase did not achieve the respective threshold or if a non-dose-responsive increase was equal to or greater than the respective threshold.

A test item was considered negative (non-mutagenic) if it was neither evaluated positive nor equivocal.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Analytical determinations of test item solutions in vehicle were performed for the concentrations 0.5, 5 and 50 mg/mL. Concentrations were measured by high performance liquid chromatography (HPLC) and UV detection. The actual concentrations were between 106 – 110 % of the target, meeting the acceptance criteria of 85 – 115 % of target. There was no test substance in the 0 mg/mL sample. Stability analysis showed that the test substance in vehicle was stable for approx. 4 hours when stored at room temperature.

B. CYTOTOXICITY

In the preliminary toxicity and mutagenicity test / Experiments B1 and B2 of the main mutation assay there was no cytotoxicity observed up to the highest tested concentration, neither in the presence, nor absence of metabolic activation. Due to the lack of bacterial growth on all assay plates, tester strains TA 1535, TA 1537 and WP2 uvrA in the presence and absence of S9 mix were not evaluated in experiment B1, but were re-tested in an independent experiment (Experiment B2).

In the second experiment of the main mutagenicity assay (Experiment B3) there was no cytotoxicity in any tester strain at any tested concentration, neither in the presence nor absence of S9 mix.

C. SOLUBILITY

Precipitation of the test substance in vehicle was not observed.

D. MUTATION ASSAY

There was no biologically relevant increase in the number of his^+ or trp^+ revertant colonies observed in any experiment for any strain at any concentration, neither in the presence nor in the absence of metabolic activation.

The number of revertant colonies induced by the solvent and the positive controls were within the range of the laboratories historical control data, demonstrating the functionality of the metabolic activation system and the sensitivity of the test.

Table 5.8.1-80: [(Acetylamino)methyl]phosphonic acid, metabolite of glyphosate (IN-EY252) - mutagenicity results (Ames test) with and without metabolic activation ([redacted] 2007), preliminary cytotoxicity and mutagenicity assay / Experiment B1 and B2

Preliminary cytotoxicity and mutagenicity test / Experiment B1 and B2: Standard plate test (SPT)										
Strain	**TA 98**		**TA 100**		**TA 1535**[§]		**TA 1537**[§]		**WP2 uvrA**[§]	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Vehicle control										
Water mean	19	26	114	85	17	11	4	7	22	17
± *SD*	*± 4*	*± 1*	*± 24*	*± 2*	*± 4*	*± 1*	*± 1*	*± 2*	*± 10*	*± 1*
HCD[#] mean	18	26	146	156	18	15	7	7	15	18
± *SD*	*± 6*	*± 9*	*± 32*	*± 34*	*± 7*	*± 5*	*± 3*	*± 3*	*± 6*	*± 7*
[range]	*5 - 51*	*8 - 72*	*63 - 253*	*67 - 267*	*4 - 49*	*2 - 45*	*1 - 23*	*1 - 25*	*5 - 58*	*6 - 52*
Test item										
1.5 mean	16	26	137	112	18	9	6	7	18	19
± *SD*	*± 1*	*± 1*	*± 4*	*± 2*	*± 1*	*± 4*	*± 2*	*± 2*	*± 1*	*± 1*
5.0 mean	17	17	126	120	22	13	7	7	19	24
± *SD*	*± 4*	*± 1*	*± 4*	*± 11*	*± 0*	*± 1*	*± 3*	*± 3*	*± 3*	*± 4*
15 mean	14	18	100	114	18	13	4	8	20	20
± *SD*	*± 5*	*± 1*	*± 8*	*± 43*	*± 1*	*± 3*	*± 0*	*± 1*	*± 2*	*± 1*
50 mean	18	23	123	124	19	15	5	6	14	20
± *SD*	*± 5*	*± 4*	*± 3*	*± 9*	*± 3*	*± 6*	*± 1*	*± 1*	*± 8*	*± 1*
150 mean	14	27	147	128	15	15	3	6	19	22
± *SD*	*± 1*	*± 1*	*± 4*	*± 10*	*± 0*	*± 4*	*± 2*	*± 1*	*± 2*	*± 6*
500 mean	15	25	123	101	18	8	4	6	18	17
± *SD*	*± 3*	*± 1*	*± 7*	*± 8*	*± 0*	*± 1*	*± 2*	*± 1*	*± 2*	*± 0*
1500 mean	16	27	137	122	14	13	5	8	25	28
± *SD*	*± 1*	*± 4*	*± 1*	*± 11*	*± 0*	*± 4*	*± 2*	*± 0*	*± 8*	*± 3*
5000 mean	17	28	122	115	14	14	7	11	16	17
± *SD*	*± 5*	*± 1*	*± 8*	*± 11*	*± 3*	*± 2*	*± 4*	*± 1*	*± 1*	*± 2*
Positive control										
[§] mean	221	416	467	492	325	95	326	65	263	133
± *SD*	*± 24*	*± 34*	*± 57*	*± 62*	*± 54*	*± 5*	*± 127*	*± 9*	*± 7*	*± 19*
HCD[#] mean	212	781	586	959	370	139	690	120	151	520
± *SD*	*± 175*	*± 434*	*± 136*	*± 427*	*± 137*	*± 70*	*± 373*	*± 113*	*± 110*	*± 271*
[range]	*44 - 1981*	*42 - 2669*	*239 - 2373*	*168 - 2652*	*31 - 1050*	*21 - 985*	*14 - 2216*	*13 - 2021*	*32 - 1741*	*35 - 1392*

[§] = information on respective positive control is reported in Material and Method section I.A.2

[§] = data were obtained in a separate experiment (Experiment B2) because no bacterial growth was observed for these strains in the first experiment (Experiment B1, data not shown).

[#] = Historical control data generated from 2003 - 2005

Table 5.8.1-81: [(Acetylamino)methyl]phosphonic acid, metabolite of glyphosate (IN-EY252) - mutagenicity results (Ames test) with and without metabolic activation ([redacted], 2007), Experiment B3

Experiment B3: Standard plate test (SPT)										
Strain	**TA 98**		**TA 100**		**TA 1535**		**TA 1537**		**WP2 uvrA**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Vehicle control										
Water mean	15	18	118	146	20	14	6	6	20	24
± *SD*	± *3*	± *1*	± *15*	± *20*	± *1*	± *2*	± *3*	± *2*	± *4*	± *5*
HCD[#] mean	18	26	146	156	18	15	7	7	17	18
± *SD*	± *6*	± *9*	± *32*	± *34*	± *7*	± *5*	± *3*	± *3*	± *6*	± *7*
[range]	*5 - 51*	*8 - 72*	*63 - 253*	*67 - 267*	*4 - 49*	*2 - 45*	*1 - 23*	*1 - 25*	*5 - 58*	*6 - 52*
Test item										
50 mean	15	11	126	143	19	16	7	7	31	18
± *SD*	± *2*	± *2*	± *33*	± *25*	± *2*	± *3*	± *2*	± *1*	± *3*	± *2*
150 mean	14	18	170	145	20	13	6	9	25	21
± *SD*	± *5*	± *4*	± *39*	± *33*	± *3*	± *3*	± *3*	± *2*	± *8*	± *4*
500 mean	14	12	150	110	16	13	5	8	26	27
± *SD*	± *2*	± *4*	± *21*	± *20*	± *3*	± *4*	± *2*	± *3*	± *5*	± *5*
1500 mean	16	18	114	117	17	14	9	10	22	25
± *SD*	± *1*	± *8*	± *9*	± *24*	± *1*	± *3*	± *3*	± *3*	± *7*	± *5*
5000 mean	15	18	117	150	16	13	7	8	23	18
± *SD*	± *3*	± *4*	± *25*	± *19*	± *4*	± *2*	± *5*	± *1*	± *4*	± *1*
Positive control										
[§] mean	162	290	389	438	348	90	331	60	188	190
± *SD*	± *31*	± *39*	± *60*	± *49*	± *90*	± *3*	± *66*	± *17*	± *40*	± *22*
HCD[#] mean	212	781	586	959	370	139	690	120	151	520
± *SD*	± *175*	± *434*	± *136*	± *427*	± *137*	± *70*	± *373*	± *113*	± *110*	± *271*
[range]	*44 - 1981*	*42 - 2669*	*239 - 2373*	*168 - 2625*	*31 - 1050*	*21 - 985*	*14 - 2216*	*13 - 2021*	*32 - 1741*	*35 - 1392*

[§] = information on respective positive control is reported in Material and Method section I.A.2

[#] = Historical control data generated from 2003 - 2005

III. CONCLUSIONS

Based on the experimental findings, [(Acetylamino)methyl]phosphonic acid (IN-EY252), metabolite of glyphosate, was negative for gene mutation in bacteria in the Ames standard plate test in the presence and absence of metabolic activation.

3. Assessment and conclusion

Assessment and conclusion by applicant:

In the present study, [(Acetylamino)methyl]phosphonic acid was negative for gene mutation in bacteria (*S. typhimurium* TA 100, TA 98, TA 1535 and TA 1537 and *E. coli* WP2 uvrA) with and without metabolic activation.

The study was conducted under GLP conditions and in accordance with OECD guideline 471 (1997). There were no deviations from the guideline. The study is therefore considered valid.

Assessment and conclusion by RMS:

CA 5.8.1/035

1. Information on the study

Data point	CA 5.8.1
Report author	[redacted]
Report year	2007
Report title	IN-EY252*: In vitro* Mammalian Chromosome Aberration Test in Human Peripheral Blood Lymphocytes
Report No	AB47BT.341.BTL
Document No	DuPont-22225
Guidelines followed in study	OECD 473 (1997), US EPA OPPTS 870.5375 (1998), EC Commission Directive 2000/32/EC Annex 4A-B10 (2000), JMAFF 12-NouSan-8147 (2000 and 2001)
Deviations from current test guideline	Only 200 cells in metaphase were evaluated, whereas the currently valid OECD 473 (2016) recommends the evaluation of 300 metaphase cells per condition.
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive summary

[(Acetylamino)methyl]phosphonic acid (IN-EY252, batch: IN-EY252-001, purity: 76 %) was tested in a mammalian chromosome aberration test in peripheral human lymphocytes *in vitro* for its potential to induce structural and numerical chromosome aberrations in the presence and absence of metabolic activation (rat liver S9 fraction).

Concentrations for the cytogenicity study were selected based on the results of a preliminary toxicity test, in which no cytotoxicity was observed up to 1530 µg/mL (corresponding to 10 mM) in the presence and absence of S9 mix. Duplicate cultures were exposed to the test item, solvent (water) or positive control (mitomycin C in the absence of S9 mix and cyclophosphamide in the presence of S9 mix) in the presence and absence of metabolic activation. Test item concentrations ranged from 191.25 to 1530 µg/mL.

The cells were exposed for 4 hours in the presence and absence of S9 mix and for 20 hours in the absence of S9 mix. Cells of the 4-hour exposure period were washed with calcium and magnesium-free phosphate buffered saline following treatment and re-incubated in culture medium for the remaining 16 hours of incubation. All cultures were sampled 20 hours after start of exposure and chromosome preparations were made.

A total of 200 metaphase cells per condition were scored for structural and numerical chromosome aberrations. Cytotoxicity was evaluated as mitotic index and assessed for 500 cells per culture.

Precipitation of the test substance in vehicle or culture medium, or cytotoxicity, evident as a ≥ 50 % reduction in the mitotic index were not observed for any treatment schedule or any concentration, neither in the presence, nor in the absence of S9 mix.

There was no statistically significant increase in the number of cells with structural or numerical chromosomal aberrations observed for any treatment schedule, neither in the presence nor in the absence of metabolic activation. The percentage of aberrant cells in the test substance-treated groups was comparable to those of solvent controls at any dose level.

The positive and vehicle controls fulfilled the criteria for a valid test and demonstrated the functionality of the metabolic activation system and the validity of the test.
Under the conditions of the test, [(Acetylamino)methyl]phosphonic acid, metabolite of glyphosate, did not induce chromosomal aberrations in human peripheral lymphocytes *in vitro*, neither in the presence nor in the absence of metabolic activation.

Materials and methods

A. MATERIALS

1. Test material: [(Acetylamino)methyl]phosphonic acid (metabolite of glyphosate)

Identification: IN-EY252
Description: White powder
Lot/Batch number: IN-EY252-001
Purity: 76 %
Stability of test compound: The stability of the test item at storage conditions (at room temperature in the dark with dessicant) was guaranteed until the expiry date 25 Apr 2009. The stability of the test item in solvent was confirmed by analytical methods.
Solvent (vehicle) used: Water

2. Control material:

Negative control: The negative control was actually the solvent control.
Solvent (vehicle) control: Water
Positive control: - S9 mix: Mitomycin C (MMC), 0.6 µg/mL for the 4-hour treatment and 0.3 µg/mL for the 20-hour treatment
+ S9 mix: Cyclophosphamide (CP), 20 µg/mL

3. Metabolic activation:

S9 mix was purchased from [redacted] (Lot no. 2074) and each batch was assayed for sterility and it's ability to metabolise 2-aminoanthracene and 7,12-dimethyl-benzanthracene to mutagens in *Salmonella typhimurium* strain TA 100.
Immediately prior to use, S9 was thawed and mixed with co-factors as follows:

S9 mix component	Concentration	Unit
KCl	6	mM
NADPH-generating system		
Glucose 6-phosphate	1	mM
NADP	1	mM
$MgCl_2$	2	mM
S9	2	% (v/v)

4. Test organism:

Human peripheral blood lymphocytes were obtained from a healthy, non-smoking, 25-year old female (preliminary cytotoxicity test) and from a healthy, non-smoking 30-year old male (main cytogenicity test) and collected in heparinised vessels.

5. Cell culture media:

Medium: RPMI 1640 medium, supplemented with 100 U/mL penicillin,

Incubation:	100 µg/mL streptomycin and 2 mM L-glutamine *Cultures were incubated at 37 ± 1 °C in a humidified atmosphere of 5 ± 1 % CO_2.*
Cell culture establishment prior to exposure:	*0.6 mL of heparinised blood was cultured with 9.4 mL culture medium and 0.1 % phytohaemagglutinin for 44 - 48 hours prior to treatment.*

6. Test concentrations and number of replicates:

(c) Preliminary cytotoxicity assay

Metabolic activation	Duration of exposure (Fixation time)	Concentrations§	Replicates
- S9 mix	4 h (20 h)	0.153, 0.459, 1.53, 4.59, 15.3, 45.9, 153, 459 and 1530# µg/mL	Single culture
- S9 mix	20 h (20 h)	0.153, 0.459, 1.53, 4.59, 15.3, 45.9, 153, 459 and 1530# µg/mL	Single culture
+ S9 mix	4 h (20 h)	0.153, 0.459, 1.53, 4.59, 15.3, 45.9, 153, 459 and 1530# µg/mL	Single culture

#: highest concentration, corresponding to 10 mM
§: Dosing solutions were adjusted to compensate the purity of the test substance by a correction factor of 1.45.

(d) Main cytogenicity test:

Metabolic activation	Duration of exposure (Fixation time)	Concentrations§	Replicates
- S9 mix	4 h (20 h)	191.25, 382.5*, 765* and 1530*# µg/mL	Duplicate
- S9 mix	20 h (20 h)	191.25, 382.5*, 765* and 1530*# µg/mL	Duplicate
+ S9 mix	4 h (20 h)	191.25, 382.5*, 765* and 1530*# µg/mL	Duplicate

* Samples analysed for chromosomal aberrations
#: highest concentration, corresponding to 10 mM
§: Dosing solutions were adjusted to compensate the purity of the test substance by a correction factor of 1.45.

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 08 Mar – 17 Apr 2007
Finalisation date: 18 Jun 2007

2. Preliminary cytotoxicity test:
In a preliminary cytotoxicity test, human peripheral lymphocytes were treated with the test item at concentrations in the range of 0.153 – 1530 µg/mL in the presence and absence of S9 mix. The test was conducted under the same conditions as in the main cytogenicity experiment. A single cell culture per condition was exposed for 4 hours in the presence and absence of S9 mix and for 20 hours in the absence of S9 mix. All cells were harvested 20 hours after start of exposure. Two hours prior to harvest, 0.1 µg/mL colcemid was added to the cultures to arrest cells in metaphase. At harvest, the cells were collected by centrifugation, treated with hypotonic potassium chloride (75 mM), fixed and stained. The number of cells in mitosis was determined per 500 cells scored in order to evaluate the test item effect on mitotic index.

3. Main cytogenicity test:

Treatment:	Following cell culture establishment, duplicate cultures per condition were exposed to the test item, solvent (water) or positive control (mitomycin C in the absence of S9 mix and cyclophosphamide in the presence of S9 mix) in the presence and absence of metabolic activation. The cells were exposed for 4 hours in the presence and absence of S9 mix and for 20 hours in the absence of S9 mix. Cells of the 4-hour exposure period were washed with calcium and magnesium-free phosphate buffered saline (CMF-PBS) following treatment, re-fed with culture medium and returned to the incubator for 16 further hours. For all conditions, chromosome preparations were made 20 hours after start of exposure.
Spindle inhibition:	During the last 2 hours of the culture period, cell division was arrested by addition of 0.1 µg/mL colchicine each culture.
Cell harvest:	The cell cultures were harvested by centrifugation, the pellets were re-suspended in 75 mM potassium chloride and swollen for 20 minutes at 37 ± 1 °C. Afterwards, the cells were gently mixed and approx. 0.5 mL of fixative (methanol : glacial acetic acid, 3 : 1, v/v) was added to each tube. Cells were collected by centrifugation, washed twice in fixative and stored in fixative until preparation.
Slide preparation:	Fixed cells were centrifuged and re-suspended in 0.1 – 0.3 mL of the supernatant fixative above the cell pellet. One or two drops of cell suspension were placed on glass slides and allowed to air-dry. Dried slides were coded, stained with 5 % Giemsa, air-dried and permanently mounted.
Metaphase analysis:	A total number of 200 metaphase cells per condition (100 per duplicate treatment condition) were examined by microscopy. Only metaphase cells with 46 centromers were examined. In case the percentage of aberrant cells reached a significant level (≥ 10 %) before 100 cells were scored, less metaphases were investigated. Chromatide-type and chromosome-type aberrations were recorded, including breaks, exchange figures and complex re-arragements. In addition, pulverised chromosomes and cells and severely damaged cells were recorded. The percentage of polyploidy and endoreduplicated cells was evaluated per 100 cells.
Cytotoxicity:	The mitotic index of each culture was calculated based on the number of cells in metaphase observed per 500 cells scored.

4. Statistics

Statistical analysis of the percent aberrant cells was performed using the Fisher's Exact test. Fisher's Exact test was used to compare pairwise the percent aberrant cells of each treatment group with that of the solvent control. In the event of a positive Fisher's Exact test at any test substance dose level, the Cochran-Armitage test was used to measure dose-responsiveness.

5. Acceptance criteria

The chromosome aberration test was considered acceptable if the following criteria were met:

- The frequency of cells with structural chromosome aberrations in the vehicle control was within the laboratories historical range.
- The percentage of cells with chromosome aberrations in the positive control was statistically increased ($p \leq 0.05$) relative to the solvent control.

6. Evaluation criteria

A test substance was considered positive (clastogenic) in the chromosome aberration test if the following criteria were met:

- The percentage of cells with aberrations was increased in a dose-related manner with one or more concentrations being statistically elevated relative to the solvent control group ($p \leq 0.5$).
- Values that were statistically significant but did not exceed the range of historical solvent control data were judged as not biologically significant.

A test substance not demonstrating a statistically significant increase in aberrations was concluded to be negative.

II. RESULTS AND DISCUSSION

E. ANALYTCAL DETERMINATIONS

Analytical determinations of the test substance in the solvent were performed for the formulations 1.9125, 3.825 and 15.30 mg/mL. The concentrations determined were 111.9 %, 110.8 % and 107.8 % of the nominal concentration, respectively, which were within the accepted range of 85 – 115 % of the target concentration. The formulations proved to be stable for 5 hours at room temperature.

F. CYTOTOXICITY

Preliminary cytotoxicity test

In the preliminary cytotoxicity test, substantial cytotoxicity (at least 50 % reduction in mitotic index when compared to the solvent control) was not observed for any treatment schedule, up to the highest tested concentration of 1530 µg/mL, neither in the presence, nor in the absence of S9 mix.

The osmolality of the highest test substance concentration in treatment medium did not exceed the osmolality of the solvent by more than 20 % and was therefore considered acceptable. The pH of the highest test substance concentration was 7.0.

Based on these results, 1530 µg/mL was chosen as the highest test item concentration for the main cytogenicity study.

Main mutagenicity test

In the main mutagenicity test, cytotoxicity evident as a reduced mitotic index of ≥ 50 % was not observed for any treatment schedule or any concentration, neither in the presence, nor in the absence of S9 mix.

G. SOLUBILITY

Precipitation of the test item in solvent or culture medium was not observed for any treatment schedule, neither in the presence, nor in the absence of metabolic activation.

H. CYTOGENICITY

There was no statistically significant increase in the number of cells with structural or numerical chromosomal aberrations observed for any treatment schedule, neither in the presence nor in the absence of metabolic activation. The percentage of aberrant cells in the test substance-treated groups was comparable to those of solvent controls at any dose level. The number of average aberrations per cell observed for the highest test item concentration after 4 hours of exposure in the presence of S9 mix (0.005 ± 0.071) was increased when compared to those of the solvent controls (0.0 ± 0.0). The value observed for the test item is explained by a single chromosome aberration of gap-type, which is not included in the assessment of total aberration frequency. As the percentage of structural aberrant cells was within the range of historical control data, the observation was considered not relevant. The frequency of chromosomal aberrations observed for the solvent and positive controls was within the range of the laboratory's historical control data. The positive controls (mitomycin C without S9 mix and cyclophosphamide with S9 mix) induced a statistically significant increase in the number of structural chromosome aberrations,

demonstrating the functionality of the metabolic activation system and proving the validity of the test.

Table 5.8.1-82: Results of the cytogenicity test with [(Acetylamino)methyl]phosphonic acid (IN-EY252) ([redacted], 2007)

Compound	Concentration [µg/mL]	No. of metaphases scored	Genotoxicity						Mitotic index [%]
			% structural aberrant cells	% numerical aberrant cells	Total number of structural aberrations		Average aberrations / cell	Judge	
					incl. gaps	excl. gaps			
Without metabolic activation; 4-hour treatment and 20-hours sampling									
Solvent									
Water	0	200	0.0	**0.0**	0.0	**0.0**	**0.000**	negative	12.1
HCD# mean ± SD			0.0 ± 0.1	0.0 ± 0.2					
range			0.0 - 1.0	0.0 - 1.5					
Test item									
	382.50	200	0.0	**0.0**	0.0	**0.0**	**0.000 ± 0.00**	negative	11.5
	765.00	200	0.0	**0.0**	0.0	**0.0**	**0.000 ± 0.00**	negative	11.1
	1530.00	200	0.0	**0.0**	0.0	**0.0**	**0.000 ± 0.00**	negative	10.6
Positive control									
MMC	0.6	100	18	**0**	21**	**21****	**0.210 ± 0.478****	positive	9.0
HCD# mean ± SD			17.8 ± 5.8	0.1 ± 0.3					
range			6.0 - 40.0	0.0 - 2.0					
Without metabolic activation; 20-hour treatment and sampling									
Solvent									
Water	0	200	0.0	**0.0**	0.0	**0.0**	**0.000 ± 0.00**	negative	11.9
HCD# mean ± SD			0.0 ± 0.1	0.0 ± 0.2					
range			0.0 - 1.0	0.0 - 1.5					
Test item									
	382.50	200	0.0	**0.0**	0.0	**0.0**	**0.000 ± 0.00**	negative	11.3
	765.00	200	0.0	**0.0**	0.0	**0.0**	**0.000 ± 0.00**	negative	10.9
	1530.00	200	0.0	**0.0**	0.0	**0.0**	**0.000 ± 0.00**	negative	9.7
Positive control									
MMC	0.30	100	16	**0**	18**	**18****	**0.180 ± 0.435****	positive	8.2
HCD# mean ± SD			17.8 ± 5.8	0.1 ± 0.3					
range			6.0 - 40.0	0.0 - 2.0					
With metabolic activation; 4-hour treatment, 20-hours sampling time									
Solvent control									
Water	0	200	0.0	**0.0**	0.0	**0.0**	**0.000 ± 0.00**	negative	8.2
HCD# mean ± SD			0.0 ± 0.1	0.0 ± 0.2					
range			0.0 - 0.5	0.0 - 1.5					
Test item									

Compound	Concentration [µg/mL]	No. of metaphases scored	Genotoxicity						Mitotic index [%]
			% structural aberrant cells	% numerical aberrant cells	Total number of structural aberrations		Average aberrations / cell	Judge	
					incl. gaps	excl. gaps			
	382.50	200	0.0	**0.0**	0.0	**0.0**	**0.000 ± 0.00**	negative	7.2
	765.00	200	0.0	**0.0**	0.0	**0.0**	**0.000 ± 0.00**	negative	6.9
	1530.00	200	0.5	**0.0**	1.0	**1.0**	**0.005 ± 0.071**	negative	7.9
Positive control									
CP	20.00	100	19	**0**	23**	**23****	**0.230 ± 0.510****	positive	7.0
HCD# mean ± SD			17.3 ± 5.5	0.1 ± 0.4					
range			9.0 - 40.0	0..0 - 2.5					

HCD#: Historical control data from the laboratory obtained in 2003 - 2005

MMC: Mytomicin C, positive control without S9 mix; CP:cyclophosphamide, positive control with S9 mix

Mitotic index: Number of mitotic figures x 100 / 500 cells counted

% of aberrant cells: numerical includes polyploidy and endoreduplicated cells; structural excludes cells with only gaps
** $p \leq 0.01$ using Fisher's Exact Test

III. CONCLUSIONS

Based on the experimental findings there is no evidence for an induction of structural or numerical chromosome aberrations by IN-EY252, metabolite of glyphosate, in peripheral human lymphocytes, neither in the presence nor in the absence of metabolic activation.

3. Assessment and conclusion

Assessment and conclusion by applicant:

In the present study, [(Acetylamino)methyl]phosphonic acid was negative for cytogenicity (chromosome aberrations *in vitro*) in peripheral human lymphocytes with and without metabolic activation.

The test was performed under GLP conditions and in accordance with OECD guideline 473 (1997).

There were only minor deviations when compared to OECD 473 (2016). The number of metaphases investigated was only 200, which was the number to be investigated recommended by the previous OCED 473 (1997). The study is therefore considered valid.

Assessment and conclusion by RMS:

CA 5.8.1/036

1. Information on the study

Data point	CA 5.8.1
Report author	[redacted]
Report year	2007
Report title	IN-EY252: In Vitro Mammalian Cell Gene Mutation Test (CHO/HGPRT)
Report No	DuPont-22224
Document No	-
Guidelines followed in study	OECD 476 (1997), US EPA OPPTS 870.5300, EC Commission Directive 2000/32/EC Annex 4E-B17 (2000), JMAFF (1985)

Deviations from current test guideline	The newly introduced cytotoxicity parameter RS (relative survival) in OECD 476 (2016) and the adjusted cloning efficiency were not re-calculated, since no data on the number of cells after treatment were provided. In the current study, cytotoxicity was evaluated based on cloning efficiency after treatment (CE_1, survival) and after selection (CE_2, viability), in accordance with the previous guideline version. Historical control data for the negative control were reported without 95 % control limits. In addition, acceptability and evaluation criteria specified in the study report were inconsistent with those specified in the current guideline.
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive summary

[(Acetylamino)methyl]phosphonic acid (IN-EY252, batch: IN-EY252-002, purity: 72 %) was tested *in vitro* for its ability to induce forward mutations in mammalian cells by assessing the mutation of the HPRT locus in Chinese hamster ovary (CHO K_1) cells. Duplicate cultures were exposed to the test item, solvent (water) and positive controls (ethylmethane sulfonate for cultures without S9 mix and benzo(a)pyrene for cultures with S9 mix) in the presence and absence of metabolic activation (Aroclor 1254-induced rat liver S9 fraction). Based on the results of a preliminary toxicity test, in which no substantial cytotoxicity was observed up to 1531 µg/mL (corresponding to 10 mM), the concentrations for the main mutagenicity study were selected. [(Acetylamino)methyl]phosphonic acid concentrations in the range of 100 – 1531 µg/mL were applied. After 5 hours of exposure with and without S9 mix, the cells were incubated for 7 days to allow expression of the mutant phenotype. The expression period was followed by a selection period, in which the cells were cultivated in 6-thioguanidine-enriched medium for 10 days. Cytotoxicity was assessed as relative survival and relative viability after the expression and selection period, respectively.

There was no precipitation of the test item in solvent and in culture medium, neither in the presence nor absence of S9 mix. Substantial cytotoxicity was not observed for any condition at any concentration. The relative cloning efficiency at the highest concentration of 1531 µg/mL was 67 % without S9 mix and 83 % with S9 mix.

There was no increase in the number of mutant colonies upon treatment with the test substance, neither in the presence nor in the absence of metabolic activation. For all conditions and at all dose levels, mutant frequencies were below the minimum value for a positive response.

Mutant frequencies of the control cultures remained within the range of the laboratories historical control data. The positive control mutagens ethylmethane sulfonate and benzo(a)pyrene induced mutant frequencies that were at least 3-fold above those of vehicle controls, demonstrating the functionality of the S9 mix and the sensitivity of the test.

Under the conditions of the test, there was no evidence for gene mutation in mammalian cells *in vitro*, neither in the presence nor in the absence of metabolic activation.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: [(Acetylamino)methyl]phosphonic acid

Identification: IN-EY252
Description: White solid
Lot/Batch number: IN-EY252-002

Purity:	72 %
Stability of test compound:	The stability of the test item at storage conditions (with a desiccator) was guaranteed until the expiry date 13 Apr 2010. The stability of the test substance in vehicle was confirmed by analytical methods.

2. Control material:

Negative control:	The negative control was actually the solvent control.
Solvent (vehicle) control:	Water
Positive control:	- S9 mix: Ethylmethane sulfonate (EMS), 0.2 µL/mL in DMSO + S9 mix Benzo(a)pyrene (BaP), 4 µg/mL in DMSO

3. Metabolic activation:

S9 mix was purchased from [redacted]. ([redacted]). The liver homogenate was produced from the livers of male Sprague-Dawley rats that were induced with Aroclor 1254. Immediately prior to use the S9 liver homogenate was thawed and mixed with co-factors as follows:

S9 mix component	Concentration	Unit
Sodium phosphate buffer (pH 7.4)	100	mM
KCl	33	mM
NADPH-generating system		
Glucose 6-phosphate	5	mM
NADP	4	mM
$MgCl_2$	8	mM
S9	10	% (v/v)

4. Test organism:

Chinese hamster ovary (CHO-K_1) cells were obtained from American Type Culture Collection (ATCC) in Manassas, Virginia, USA. The cell stocks were stored in liquid nitrogen and routinely tested for mycoplasma contamination. Cells used in the mutation assay were within three to four subpassages from cleansing in pre-treatment medium supplemented with hypoxanthine, aminopterin and thymidine (HAT) in order to assure karyotypic stability.

5. Cell culture media:

Pre-treatment medium (HAT medium):	Ham's F12 medium supplemented with hypoxanthine, aminopterin and thymidine
Cultivation medium (F12FBS5-Hx):	Ham's F12 medium, supplemented with 5 % dialysed fetal bovine serum, 100 U/mL penicillin and 100 µg/mL streptomycin
Treatment medium (± S9):	*Cultivation medium (F12FBS5-Hx)*
Selection medium:	Cultivation medium (F12FBS5-Hx) supplemented with 10 µM 6-thioguanidine (6TG)
Incubation:	*At 37.5 ± 2 °C in a humidified atmosphere of 5 ± 2 % CO_2*

6. Locus examined: Hypoxanthin guanine phosphoribosyltransferase (HGPRT)

7. Test concentrations and number of replicates:

(c) Preliminary cytotoxicity and mutagenicity test:

Metabolic activation	Duration of exposure	Concentrations§	Replicates
± S9 mix	5 h	5, 10, 25, 50, 100, 250, 500, 1000 and 1531 µg/mL	Triplicate plates from a single culture

§: Dosing solutions were adjusted to compensate the purity of the test substance by a correction factor of 1.389.

(d) Main gene mutation test:

Metabolic activation	Duration of exposure	Concentrations§	Replicates
± S9 mix	5 h	100, 250, 500, 1000 and 1531 µg/mL	Triplicate plates from duplicate cultures

§: Dosing solutions were adjusted to compensate the purity of the test substance by a correction factor of 1.389.

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 30 May – 12 Sep 2007
Finalisation date: 25 Sep 2007

2. Preliminary cytotoxicity and mutagenicity test:
A preliminary test was performed to identify suitable dose levels for the main mutagenicity study. Cell cultures were established identically to the performance in the main mutagenicity test described below. The cells were exposed to nine test substance concentrations in the range of 5 – 1531 µg/mL for 5 hours in the presence and absence of S9 mix at 37 ± 2 °C. The highest concentration was equivalent to a 10 mM concentration. Following exposure, the cells were washed with Hank's Balanced Salt Solution (HBSS), re-incubated in fresh culture medium for approx. 20 hours, harvested and plated for determination of cell survival (cloning efficiency 1). After incubation for 8 days, the formed colonies were fixed, stained and counted.
Based on the results of the preliminary test, the test item concentrations for the main mutagenicity test were selected.

3. Main mutation assay:
Pre-treatment of cells:
For each test group, 5 x 10^5 cells were seeded into 25 cm^2 flasks and incubated for 16 - 24 hours prior to treatment.

Treatment:
On the day of treatment, the medium was exchanged. For treatment in the presence of S9 mix, 20 % of the medium were replaced by S9 mix. Duplicate cultures were exposed to the test item, vehicle (water) and positive controls (0.2 µL/mL ethylmethane sulfonate without S9 mix and 4 µg/mL benzo(a)pyrene with S9 mix) for 5 hours in the presence and absence of metabolic activation at 37 ± 2 °C. Test item concentrations ranged from 100 – 1531 µg/mL. Following exposure, the cells were washed with Hank's Buffered Salt Solution (HBSS) and the culturing was continued for another 18 – 24 hours. After the incubation period, the cells were trypsinised and counted. Triplicates of 100 cells/ 60 mm dish were seeded for the determination of survival (cloning efficiency 1) and duplicate cultures with ≤ 10^6 cells/ 100 mm dish were seeded for the expression of the mutant phenotype.

Expression period:
After treatment, duplicate cultures with ≤ 10^6 cells/ 100 mm dish were seeded and sub-cultured at 2 - 3

day intervals for a 7-day expression period. After the expression period, each culture was divided. Triplicates of 100 cells/ 60 mm dish were seeded for the determination of viability (cloning efficiency 2) and five culture dishes with 2 x 10^5 cells/ 100 mm dish were seeded for the selection of mutants.

Selection period:
For the selection of mutants, five culture dishes with 2 x 10^5 cells/ 100 mm dish were seeded in medium enriched with 10 µM 6-thioguanidine (selection medium). After an incubation period of 10 days, the colonies were fixed, stained and counted.

4. Cytotoxicity:

Cloning efficiency CE_1 (survival)
The survival (cloning efficiency 1) of [(Acetylamino)methyl]phosphonic acid-treated cells relative to solvent controls was determined in parallel to the mutagenicity test. At the end of the exposure period, a sample of each cell culture was collected to assess survival of the cells. Triplicates of 100 cells/60 mm dish were seeded and incubated for 7 days. Afterwards, the colonies were fixed, stained and counted.

Cloning efficiency CE_2 (viability)
The viability (cloning efficiency 2) was determined in parallel to the selection of mutants. After the expression period, triplicates of 100 cells/60 mm dish were seeded in medium without 6-thioguanine to assess cell viability. After an incubation period of 10 days, the developed colonies were fixed, stained and counted.

5. Evaluation:

Cytotoxicity (cloning efficiency CE)
The number of colonies divided by the number of cells plated was calculated for each sample. The absolute cloning efficiency was determined for each test group, as well as the relative cloning efficiency in comparison to the solvent control group.

CE_1 (survival)
The cytotoxicity of the test substance after the exposure period was determined for each test group and is indicated as absolute and relative cloning efficiency (CE_1 and RCE_1, respectively).

CE_2 (viability)
The cytotoxicity of the test substance at the end of the expression period was determined for each test group and is given as absolute and relative cloning efficiency (CE_2 and RCE_2, respectively).

The cloning efficiency (CE, %) was calculated for each test group as follows:

$$\mathrm{CE_{absolute}} = \frac{\text{Total number of colonies}}{\text{Total number of cells plated}} \times 100$$

$$\mathrm{RCE_x} = \frac{\mathrm{CE_{absolute}} \text{ of the test group}}{\mathrm{CE_{absolute}} \text{ of the vehicle control group}} \times 100$$

Mutant frequency (MF)
The cloning efficiency of mutant colonies in selective medium divided by the cloning efficiency in non-selective medium measured for the same culture at the time of selection was calculated for each sample.

Uncorrected mutant frequency:
The uncorrected mutant frequency (MF_{uncorr}) was calculated for each test group as follows:

$$\text{MF uncorrected} = \frac{\text{Total number of mutant colonies}}{\text{Number of seeded cells}} \times 10^6$$

Corrected mutant frequency
The corrected mutation frequency (MF_{corr}) was calculated regarding the values of CE_2:

$$\text{MF corrected} = \frac{\text{MF uncorrected}}{CE_2} \times 100$$

6. Statistics:
Statistical analysis was not performed.

7. Acceptance criteria:
The test was considered valid if the following criteria were met:

- The cloning efficiency of the vehicle control was > 50 % with a spontaneous mutant frequency within the range of the laboratories historical control data.
- The mutant frequency of the positive control was at least 3-fold as compared to the concurrent vehicle control and exceeded 40 mutants per 10^6 cells (minimum value for the positive control in the laboratories historical control data).
- At least 4 analysable concentrations showing mutants were required for evaluation.

8. 8. Evaluation criteria:
A test item was judged positive for gene mutation in mammalian cells if the following criteria were met:

- A mutant frequency of > 40 mutants per 10^6 cells (minimum value for the positive control in the laboratories historical control data) was observed at 2 or more consecutive concentrations of the test substance when compared to the solvent control.
- The observed increase in mutant frequency was accompanied by a concentration-related increase.
- A mutant frequency of > 40 mutants per 10^6 cells (minimum value for the positive control in the laboratories historical control data) was observed at the highest concentration only.

The test item was judged equivocal if a mutant frequency of > 40 mutants per 10^6 cells (minimum value for the positive control in the laboratories historical control data) was observed at any dose level other than the highest concentration.

The test item was judged negative for gene mutation in mammalian cells if a mutant frequency of > 40 mutants per 10^6 cells (minimum value for the positive control in the laboratories historical control data) was not observed at any dose level.

II. RESULTS AND DISCUSSION

1. ANALYTICAL DETERMINATIONS

Analytical determinations were performed using a high performance liquid chromatography (HPLC) with UV/Vis detection. The test item formulations were ± 7 % (preliminary toxicity test) and ± 5 % (main mutation assay) of the nominal concentrations and therefore within the acceptable range (± 10 % of the nominal concentration). There was no test substance in the 0 mg/mL sample. Stability of the test item in vehicle was confirmed for 23 hours at room temperature.

2. CYTOTOXICITY

In the preliminary cytotoxicity test, no cytotoxicity, evident as a cloning efficiency of ≤ 50 %, was observed. The relative cloning efficiency at the highest test item concentration (1531 µg/mL) was 59 % in the absence of S9 mix and 308 % in the presence of S9 mix. Osmolality and pH measurements of test substance in medium at 1531 µg/mL were found to be within an acceptable range (≤ 20 %). Based on these findings, the concentrations for the main mutagenicity study were selected to be in the range of 100 – 1531 µg/mL,

corresponding to 0.65 – 10 mM.
In the main mutagenicity experiment, no substantial cytotoxicity was observed at any dose level, neither in the presence nor absence of S9 mix.

3. SOLUBILITY

There was no precipitation observed up to the highest concentration tested, neither in the presence, nor absence of metabolic activation.

4. MUTANT FREQUENCY

In a first trial, a high spontaneous mutant frequency was observed for the solvent controls in both, cultures with and without S9 mix (data not shown). The test was therefore considered invalid and repeated.
In the second trial, there was no increase in the number of mutant colonies upon treatment with [(Acetylamino)methyl]phosphonic acid, neither in the presence nor in the absence of metabolic activation. For all conditions and at all dose levels, mutant frequencies were below 40 mutants per 10^6 cells (minimum value for the positive control in the laboratories historical control data) and no dose-response relationship was evident.
Mutant frequencies of the solvent control cultures remained within the range of the laboratory's historical control data. The positive control mutagens ethylmethane sulfonate and benzo(a)pyrene induced mutant frequencies that were at least 3-fold above those of vehicle controls, demonstrating the functionality of the S9 mix and the sensitivity of the test.

Table 5.8.1-83: Results of the HGPRT gene mutation assay in mammalian cells with [(Acetylamino)methyl]phosphonic acid (IN-EY252) ([redacted] 2007)

Test group	Total number of mutant colonies[$]	Mutant frequency (per 10^6 cells)	Cloning efficiency			
			CE_1 (survival)		CE_2 (viability)	
		corr.[#]	abs.	rel. [%]	abs.	rel.[§] [%]
Without metabolic activation; 5-hour exposure period						
Solvent						
Water	28	24.7	0.97	100	0.57	100
HCD mean ± SD		6.1 ± 6.6				
range		*0.0 - 24.0*				
Test item [µg/mL]						
100	26	19.3	1.12	116	0.67	118
250	34	22.4	0.89	92	0.76	133
500	25	18.5	0.98	101	0.68	119
1000	13	8.7	0.85	87	0.75	132
1531	14	10.5	0.80	82	0.67	118
Positive control [µL/mL]						
EMS 0.2	267	188.0	0.66	68	0.71	125
HCD mean ± SD		216.0 ± 148.5				
range		*75.9 - 880.1*				
With metabolic activation; 5-hour exposure period						
Solvent						
Water	34	26.2	0.40	100	0.65	100
HCD mean ± SD		5.5 ± 5.4				
range		*0.0 - 19.0*				

Test group	Total number of mutant colonies[$]	Mutant frequency (per 10^6 cells)	Cloning efficiency			
			CE$_1$ (survival)		CE$_2$ (viability)	
		corr.[#]	abs.	rel. [%]	abs.	rel.[§] [%]
Test item [µg/mL]						
100	24	20.9	0.59	146	0.57	88
250	22	16.1	0.61	152	0.68	105
500	21	16.6	0.83	206	0.63	97
1000	27	27.2	0.86	212	0.50	77
1531	29	17.5	0.77	192	0.83	128
Positive control [µg/mL]						
(B(a)P) 4.0	102	106.3	0.32	79	0.48	74
HCD mean ± SD		206.3 ± 118.5				
range		*89.7 - 379.8*				

[#] = correction on the basis of the absolute cloning efficiency 2 at the end of the expression period

[$]: number of mutant colonies 10 days after seeding 2 x 10^5 cells/100 mm dish, total of 5 cultures

[§]: calculated with total cloning efficiency data (solvent control = 100 %)

HCD: Historical control data, generated in the laboratory from 1989 - 2005

III. CONCLUSIONS

Based on the experimental findings and under the conditions of the test, [(Acetylamino)methyl]phosphonic acid did not induce gene mutations in the HGPRT locus of CHO-K$_1$ cells, neither in the presence nor in the absence of metabolic activation and is therefore considered negative for mutagenicity in mammalian cells *in vitro*.

3. Assessment and conclusion

Assessment and conclusion by applicant:

In the present study, [(Acetylamino)methyl]phosphonic acid was negative for mutagenicity at the HGPRT locus in CHO-K$_1$ cells with and without metabolic activation.
The study was conducted in compliance with GLP and according to OECD guideline 476 (1997). There were only minor deviations when compared to OECD 476 (2016), which were considered to not compromise the validity of the study. The study is therefore considered valid.

Assessment and conclusion by RMS:

CA 5.8.1/037

1. Information on the study

Data point	CA 5.8.1
Report author	[redacted]
Report year	2007
Report title	IN-EY252: Mouse Bone Marrow Micronucleus Test
Report No	[redacted]-22226
Document No	NA
Guidelines followed in study	OECD 474 (1997), US EPA OPPTS 870.5395 (1998), EEC Directive 2000/32/EC B12 (2000), JMAFF 12 Nousan (2000)
Deviations from current	According to the current guideline OECD 474 (2016), at least 4000

test guideline	polychromatic erythrocytes per animal should be evaluated for the presence of micronuclei. However, in the present study only 2000 polychromatic erythrocytes were evaluated, since that was required in the previous OECD guideline (1997). Bone marrow exposure, indicated by a reduced polychromatic to normochromatic erythrocyte ratio, was not confirmed and there was no systemic toxicity observed, but dose levels included limit concentrations specified in the current guideline. In addition, no 95 % control limits were reported for the historical control data.
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive summary

[(Acetylamino)methyl]phosphonic acid (IN-EY252, batch: IN-EY252-002, purity: 72 %) was tested for its potential to induce micronuclei in male and female Crl:CD1 (ICR) mice. Based on the results of a preliminary toxicity study, dose levels for micronucleus tests were selected. Groups of 5 mice per sex per dose level were administered a single dose of 500, 1000 or 2000 mg/kg bw by oral gavage. For the high dose group, 2 additional mice/sex/sampling time point were treated and served as backup (scoring of micronuclei was not performed). Similar constituted groups received the vehicle (deionised water) or the positive control (30 mg/kg bw cyclophosphamide).

Body weights of the animals were collected prior to treatment and 24 and 48 hours post dosing. The animals were observed for clinical signs of toxicity and mortality 1, 3.5, 24 and 48 hours after treatment. About 24 and 48 hours after dosing, the animals of the test item-treated and vehicle control groups were sacrificed and bone marrow smears were prepared. For animals of the positive control group, bone marrow was sampled 24 hours after dosing. For each animal, 2000 polychromatic erythrocytes (PCE) were scored for the presence of micronuclei. In addition, the proportion of PCE among 1000 erythrocytes, expressed as ratio PCE / normochromatic erythrocytes (NCE) was determined.

Treatment with [(Acetylamino)methyl]phosphonic acid induced no mortality in the animals and no clinical signs of toxicity were noted at any dose level. In addition, body weight and body weight gain were not affected in any dose group at any sampling time point. Based on the ratio of PCE / NCE, there was no evidence for bone marrow toxicity.

There was no statistically significant and no biologically relevant increase in the frequency of micronucleated PCE (mPCE) when compared to vehicle control animals at any dose level and for any sampling time.

Incidences of mPCE in solvent and positive control animals were within the range of the laboratory's historical control data and demonstrated the validity and sensitivity of the test system.

Based on the experimental findings of the present study and under the conditions of the test, the test item did not induce micronuclei in the bone marrow of male and female mice *in vivo*.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	[(Acetylamino)methyl]phosphonic acid
Identification:	IN-EY252
Description:	White solid
Lot/Batch #:	IN-EY252-002

Purity:	72 %
Stability of test compound:	The stability of the test item at storage conditions (with a desiccator) was guaranteed until the expiry date 13 Apr 2010. The stability of the test substance in vehicle was confirmed by analytical methods.
Solvent (vehicle) used:	Deionised water

2. Control materials

Solvent (vehicle) control:	Deionised water
Positive control:	Cyclophosphamide, 30 mg/kg bw in deionised water

3. Test animals:

Species:	Mouse
Strain:	Crl:CD1 (ICR)
Sex:	Male and female
Source:	[redacted]
Age at study initiation:	Approx. 7 weeks
Mean weight at dosing:	28.2 – 37.5 g (males) and 21.4 – 27.6 g (females)
Acclimation period:	At least 5 days
Diet/Food:	LLC Certified Rodent LabDiet® 5002 (PMI® Nutrition International), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually

4. Environmental conditions:

Temperature:	18 - 26 °C
Humidity:	30 - 70 %
Air changes:	Not specified
Photoperiod:	12-hour light and dark cycle

5. Test concentrations and treatment groups:

a) Preliminary toxicity study

Dose levels:	2000 mg/kg bw
Concentrations:	Not specified
Dose volume:	Not specified
Number of animals:	3 males
Route of administration:	Oral gavage

b) Main micronucleus test

Dose levels:	500, 1000 and 2000 mg/kg bw[36]
Concentrations:	50, 100 and 200 mg/mL
Dose volume:	10 mL/kg bw
Number of animals:	5/sex/group and additional 2/sex/sampling time point as backup for the high dose group (additional animals were not used for scoring of micronuclei)
Route of administration:	Oral gavage

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 22 May – 23 Jul 2007
Finalisation date: 07 Sep 2007

2. Animal assignment and treatment:

Preliminary toxicity study:
In a preliminary range-finding test 3 male animals were administered a single dose of 2000 mg/kg bw by oral gavage. The animals were observed for clinical signs of toxicity and mortality immediately after dosing and daily for two days. Based on the results of the preliminary toxicity study, the dose levels for the micronucleus test were selected.

Main micronucleus test:
Groups of 5 mice per sex and dose level were administered a single dose of 500, 1000 or 2000 mg/kg bw. For the high dose group, 2 additional mice/sex/sampling time point were treated and served as backup (scoring of micronuclei was not performed). The test substance was dissolved in deionised water and administered by oral gavage at a constant dosage volume of 10 mL/kg bw. Similar constituted groups of 5 mice/sex received the vehicle (deionised water) or the positive control (30 mg/kg bw cyclophosphamide).
Individual body weights were collected prior to treatment and 24 and 48 hours post dosing. The animals were observed for clinical signs of toxicity and mortality 1, 3 - 5, 24 and 48 hours after treatment. About 24 and 48 hours after dosing, the animals of the test item-treated and control groups were sacrificed by CO_2 asphyxiation and bone marrow samples were generated. Animals of the positive control group were sacrificed 24 hours after administration.

3. Slide preparation:

After sacrifice, both femurs of the animals were removed, and marrow was aspirated with the aid of a syringe containing fetal bovine serum (FBS). After centrifugation, the pellet was re-suspended in FBS and a small drop was placed on pre-cleaned microscope slides. Smears were made using a Mini Prep® blood smearing instrument. At least 3 slides were prepared for each animal. The slides were air-dried, fixed in methanol and stained in acridine orange.

4. Slide evaluation:

[36]: Dosing solutions were adjusted to compensate the purity of the test substance by a correction factor.

Slides were coded and evaluated by fluorescent microscopy. For each animal, 2000 polychromatic erythrocytes (PCE) were scored for the presence of micronuclei. Cells containing more than one micronucleus were scored as single micronucleated PCE (mPCE). In addition, the proportion of PCE among 1000 erythrocytes, expressed as ratio PCE / normochromatic erythrocytes (NCE) was determined.
If no increase in the incidence of mPCE was observed at the 24-hour sampling time point at any dose level, slide evaluation for the 48-hour sampling was performed for animals of the high dose and control group only.

5. Statistics:

Data for the amount of micronucleated polychromatic erythrocytes (mPCE) among 2000 erythrocytes and the ratio of PCE among normochromatic erythrocytes (NCE) were transformed prior to analysis using an arcsine square root or Freeman-Tukey function. Transformed data for PCE and MNPCE frequencies were analyzed separately for normality of distribution and equal variance using the Shapiro-Wilk and Levene's tests, respectively.
For those data that were normally distributed and had equal variance, parametric statistics (e.g. analysis of variace (ANOVA) and Dunnett's test) were performed using the transformed data. For those data that were normally distributed but had unequal variance, a robust ANOVA and unequal-variance Dunnett test was done. For those data that were not normally distributed, nonparametric statistics (e.g., Kruskal-Wallis and Dunn's tests) utilizing non-transformed data was performed. The individual animal was considered the experimental unit. All data analyses was one-tailed and conducted at a significance level of 5 %.

6. Acceptance criteria:

The study was considered valid if the following criteria were met:

- In the vehicle control group, the mean frequency of micronucleated polychromatic erythrocytes (mPCE) was within the range of the laboratories historical control range.
- The positive control induced a statistically significant increase in the frequency of mPCE as compared to the vehicle control group.

7. Evaluation criteria:

The test item was considered positive if the following criteria were met:

- The group mean number of micronucleated polychromatic erythrocytes (mPCE) was statistically significantly increased at one or more concentrations when compared to the concurrent vehicle control value.
- An accompanying statistically significant dose-response increase in mPCE was observed.

The test item was considered negative if the following criteria were met:

- There was no statistically significant, dose-related increase in the group mean mPCE above the concurrent vehicle control at any concentration.
- The mPCE values were within reasonable limits of the recent (past 3 years) laboratory historical control range.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The dosing formulations were analysed by high performance liquid chromatography (HPLC) and UV/Vis detection. The data indicated that the achieved concentration of test substance in vehicle was ± 5 % of the nominal concentration, which was considered acceptable. Stability of the test substance in vehicle was confirmed for 5 hours at room temperature.

B. PRELIMINARY TOXICITY STUDY

Data on clinical signs of toxicity in the preliminary toxicity study were not included.

C. MAIN MICRONUCLEUS TEST

Systemic toxicity:

Mortality:

No mortality occurred.

Clinical signs of toxicity:

Clinical signs of toxicity were not observed at any time point or at any dose level.

Body weight and body weight gain:

There were no significant changes in body weight or body weight gain in either male or female animals of any test group.

Evaluation of bone marrow slides:

Upon treatment with the test substance at any dose level and sampling time point, the ratio of polychromatic (PCE) to normochromatic erythrocytes (NCE) was not affected, indicating that no bone marrow toxicity was evident.

In addition, there was no statistically significant and no biologically relevant increase in the frequency of mPCE when compared to vehicle control animals at any dose level and for any sampling time.

Incidences of mPCE in solvent and positive control animals stayed within the range of the laboratory's historical control data and proved the validity and sensitivity of the test system.

Table 5.8.1-84: Summary of genotoxicity data obtained with [(Acetylamino)methyl]phosphonic acid (IN-EY252) in the micronucleus test in mice ([redacted] 2007)

Treatment	Dose (mg/kg bw)	Sampling time	Males		Females	
			mPCE ± SD / 2000 PCE	PCE / NCE	mPCE ± SD / 2000 PCE	PCE / NCE
Solvent control						
Deionised water	/	24 h	1.2 ± 1.3	1.149 ± 0.187	3.0 ± 1.6	1.362 ± 0.400
	/	48 h	1.2 ± 0.8	1.146 ± 0.298	4.2 ± 1.6	1.306 ± 0.330
HCD mean ± SD	/	not specified	2 ± 2	1.27 ± 0.48	3 ± 2	1.35 ± 0.47
range	/		*0 - 8*	*0.198 - 3.184*	*0 - 10*	*0.144 - 2.731*
Test item						
Test item	500	24 h	2.8 ± 2.5	1.367 ± 0.201	1.6 ± 1.5	1.533 ± 0.350
	1000	24 h	1.6 ± 1.8	1.376 ± 0.322	1.6 ± 0.9	1.406 ± 0.228
	2000	24 h	1.8 ± 1.3	1.257 ± 0.238	2.0 ± 3.9	1.423 ± 0.135
	2000	48 h	2.6 ± 2.1	1.610 ± 0.359	1.8 ± 1.6	1.576 ± 0.226
Positive control						
CPA	30	24 h	16.2 ± 4.6*	1.217 ± 0.478	17.6 ± 3.8*	1.109 ± 0.189
HCD mean ± SD	not specified	24 h	26 ± 14	1.31 ± 0.38	21 ± 11	1.37 ± 0.47
range	not specified		*8 - 81*	*0.603 - 2.623*	*4 - 67*	*0.143 - 2.534*

PCE: polychromatic erythrocytes; mPCE: micronucleated polychromatic erythrocytes; NCE: normochromatic erythrocytes

HCD: Historical control data, generated in the laboratory in 16 male and 16 female studies, conducted from 2002 - 2006

CPA: cyclophosphamide; * statistically significant when compared to solvent control

III. CONCLUSIONS

Based on the experimental findings [(Acetylamino)methyl]phosphonic acid did not induce micronuclei in bone marrow of male and female mice and is therefore considered negative for clastogenicity *in vivo*.

3. Assessment and conclusion

Assessment and conclusion by applicant:

In the present study, [(Acetylamino)methyl]phosphonic acid was negative for clastogenic and aneugenic effects in the bone marrow of male and female Crl:CD1 (ICR) mice *in vivo*.
The study was conducted under GLP conditions and in accordance with OECD guideline 474 (1997). Only 2000 polychromatic erythrocytes were evaluated, since that was required in the previous OECD guideline (1997). Bone marrow toxicity, indicated by a reduced polychromatic to normochromatic erythrocyte ratio, or systemic toxicity were not observed. However, the test was performed at limit dose levels in line with the current guideline. The deviations were considered to be of minor degree and do not compromise the validity of the study. The study is therefore considered valid.

Assessment and conclusion by RMS:

Studies with N-acetyl glyphosate

The metabolite N-acetyl glyphosate was investigated for metabolism/pharmacokinetics, acute oral toxicity, subchronic toxicity and mutagenicity. All studies presented are submitted on EU level for the first time.

In an ADME study in male rats, it was shown, that N-acetyl glyphosate is rapidly absorbed from the gastrointestinal tract. The mean maximum concentrations (Cmax) in blood and plasma were 2.93 and 5.31 µg equivalents/g at 1 and 2 hours post-dose, respectively. Radioactivity was eliminated from blood and plasma with half-life values of 20.1 and 15.6 h, respectively.
Approx. 66 % of the total radioactivity was excreted in urine and 26 % in faeces. Over 90 % of the radioactivity was eliminated within 48 h post-dose. No metabolites were detected in plasma and urine.
A metabolite found at less than 0.25 % of the administered radioactivity in faeces was glyphosate, which was formed by N-deacetylation ([redacted] 2004, CA 5.8.1/038). This is further confirmed, by metabolite analysis conducted in a 90-day study in rats ([redacted], 2007, CA 5.8.1/040). Here it was shown that N-acetyl glyphosate was marginally metabolised to glyphosate and N-acetyl AMPA, as only very small quantities (close to LOD) were detected in urine and plasma samples at day 82/83.
The analytical results from the 90-day study (CA 5.8.1/040) of urine and plasma samples from rats confirm that N-acetyl glyphosate was marginally metabolised to glyphosate and N-acetyl AMPA, as only very small quantities (close to LOD) were detected in urine and plasma samples at day 82/83.

The oral LD_{50} for N-acetyl glyphosate was determined to be > 5000 mg/kg bw (limit test), based on the results of an acute oral toxicity study in female rats. Mortality occurred in 20 % of the males and 40 % of females. Further, liquid/soft faeces and general clinical signs were observed in all surviving animals ([redacted] 2004, CA 5.8.1/039).
In a 90-day feeding study in rats, no adverse effects were noted on in-life parameters, neurobehavioral evaluation and clinical and anatomic pathology at any dose tested. Therefore, a NOAEL of 1157 and 1461 mg/kg bw/day was derived for males and females, respectively ([redacted], 2007, CA 5.8.1/040).

N-acetyl glyphosate was tested negative for mutagenicity in bacteria (Ames test) and mammalian cells (HPRT test) ([redacted], 2004, CA 5.8.1/041; [redacted] 2006, CA 5.8.1/043). Furthermore, no clastogenic activity was observed in an *in vitro* chromosomal aberration test in CHO cells and in an *in vivo* micronucleus test in mice ([redacted], 2004, CA 5.8.1/042; [redacted], 2006, CA 5.8.1/044).

Table 5.8.1-85: Toxicity studies with N-acetyl glyphosate

Annex	Study	Study type	Substance(s)	Status	Exposure	Result

Point					conditions, dose levels	
Absorption, Distribution, Metabolism and Excretion						
CA 5.8.1/038	[redacted], 2004	ADME study, SD rat, ♂	[^{14}C]-N-acetyl-glyphosate (Purity: 99.18 %)	Supportive, Category 1	Single oral dose of 15 mg free acid equivalent/kg bw *via* oral gavage	Cmax: 2.93 and 5.31 µg eq/g at 1 and 2 h, respectively; $T_{1/2}$ blood/plasma: 20.1 and 15.6 hours; >90 % of dose excreted within 48h: 26 % *via* faeces, 66 % *via* urine; Minimal metabolism: < 1 % glyphosate in faeces, no metabolites in plasma or urine
Acute oral toxicity						
CA 5.8.1/039	[redacted] 2004	Acute oral toxicity, SD rat, ♀/♂	N-Acetyl Glyphosate, sodium salt (Purity: 84.3 %)	Valid, Category 1	Limit test	LD_{50} oral > 5000 mg/kg bw (males and females); mortality of 20 % in the males and 40 % in the females, liquid/soft faeces and general clinical signs observed
Short-term toxicity						
CA 5.8.1/040	[redacted], 2007	90-day oral toxicity study, SD rats, ♀/♂	N-Acetyl Glyphosate (Purity: 63 %)	Valid, Category 1	180, 900, 4500 or 18000 ppm (equivalent to 0, 11.3, 55.7, 283 and 1157 mg/kg bw/day for males and 0, 13.9, 67.8, 360 and 1461 mg/kg bw/day for females)	NOAEL = 18000 ppm (equivalent to 1157 and 1461 mg/kg bw/day in males and females, respectively)

Genotoxicity						
CA 5.8.1/041	[redacted] 2004	Genotoxicity in bacteria (Ames); *S. typhimurium* TA 98, TA 100, TA 1535 and TA 1537, and *E. coli* WP2 uvrA	N-Acetyl Glyphosate, sodium salt (Purity: 84.3 % for sodium salt, 67.4 % for free acid)	Valid, Category 1	100 - 5000 µg/plate, ± S9, plate incorporation method	Negative
CA 5.8.1/042	[redacted] 2004	*In vitro* chromosome aberration test, CHO cells	N-Acetyl Glyphosate, sodium salt (Purity: 84.3 % for sodium salt, 67.4 % for free acid)	Valid, Category 1	960 - 2800 µg/mL, ± S9	Negative
CA 5.8.1/043	[redacted], 2006	*In vitro* mammalian cell gene mutation test (HPRT), CHO cells	N-Acetyl Glyphosate (Purity: 63 %)	Valid, Category 1	250 - 2091 µg/mL, ± S9	Negative
CA 5.8.1/044	[redacted] 2006	In vivo micronucleus test, CD-1 mice, ♀/♂	N-Acetyl Glyphosate (Purity: 63 %)	Valid, Category 1	Single oral doses of 500, 1000 and 2000 mg/kg bw *via* gavage	Negative

Absorption, Distribution, Metabolism and Excretion

CA 5.8.1/038

1. Information on the study

Data point:	CA 5.8.1
Report author	[redacted]
Report year	2004
Report title	Mass Balance, Metabolism, and Pharmacokinetics of [^{14}C]N-acetyl-glyphosate Following Administration of a Single Oral Dose to Rats
Report No	[redacted] 7535-100 Amended Report, [redacted] 56245A ([redacted]
Document No	NA
Guidelines followed in study	40 CFR 160, Guideline OPPTS 870.7485
Deviations from current test guideline	Yes, no full range ADME study, only males, only one dose
Previous evaluation	No, not previously submitted
GLP/Officially recognised	Yes

testing facilities	
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive summary

The pharmacokinetics, absorption, elimination, and metabolism were studied in male rats following a single oral administration of [^{14}C]-N-acetyl-glyphosate at a nominal dose of 15 mg free acid equivalent/kg bw. Blood was collected from four rats per time point up to 72 hours post-dose. Excreta were collected from five animals at specified intervals through 7 days post-dose. Plasma, urine, and faeces were analysed for unchanged parent compound and metabolites.

The mean total recovery of radioactivity was 95.5 %, with 66.1 % in urine, 26.4 % in faeces, 2.79 % in cage wash and wipe, and 0.23 % in residual carcass. More than 90 % of the total radioactivity was excreted within 48 hours. The mean maximum concentrations (Cmax) in blood and plasma were 2.93 and 5.31 µg equivalents/g 1 and 2 hours after application, respectively. Radioactivity was eliminated from blood and plasma with half-life values of 20.1 and 15.6 hours, respectively. Comparison of blood and plasma values for area under the curve (AUC) indicated that [^{14}C]-N-acetyl-glyphosate distributed preferentially into plasma.

Unchanged [^{14}C]-N-acetyl-glyphosate recovered in urine and faeces represented over 99 % of the administered radioactivity. A metabolite, glyphosate, was detected in faeces and represented less than 1 % of the total radioactivity. Plasma radioactivity consisted entirely of unchanged [^{14}C]-N-acetyl-glyphosate.

I. MATERIALS AND METHODS

A. MATERIALS

1. Non-labelled test material: N-acetyl-glyphosate

Identification: N-acetyl-glyphosate, sodium salt

Description: Not reported

Lot #: 123K5012 (PTRL West Log No. 1247W-001)

Purity: 84.3 % sodium salt (67.4 % based on free acid)

Stability of test compound: Responsibility of sponsor

2. Radiolabelled test material: [^{14}C]-N-acetyl-glyphosate

Identification: N-acetylglyphosate-phosphonomethyl-^{14}C

Position of radiolabel:

Lot #: 123K9416

Radiochemical purity: 99.181 % (confirmed by HPLC prior to dose preparation)

Specific activity: 8.0 mCi/mmol

Stability of test compound:	Stability was confirmed by pre- and post-dose HPLC analysis of radiochemical purity values
3. Analytical standard:	
Identification:	Glyphosate-phosphonomethyl - ^{14}C sodium salt
Lot No.	012K9430/31
Specific activity:	2.2 mCi/mmol
Radiochemical purity:	99.237 %
4. Vehicle:	Sterile water for injection
5. Test animals:	
Species:	Rat
Strain:	Sprague-Dawley (Crl:CD(SD)IGS BR)
Source:	[redacted]
Age:	10 weeks at dosing
Sex:	Males
Weight at dosing:	266 to 292 g at dosing
Acclimation period:	At least 4 days; all animals appeared clinically healthy throughout acclimation
In-life dates:	09 to 16 Mar 2004
Diet/Food:	Certified Rodent Diet #8728CM (Harlan Teklad, Inc.), *ad libitum*
Water:	Water, *ad libitum*
Housing:	During acclimatisation: Individual, suspended, stainless steel, wire mesh cages. During test period: Animals designated for collection of excreta were housed in individual Nalgene cages designed for the separation and collection of urine and faeces; Animals designated for pharmacokinetic analyses were housed in individual, suspended, stainless steel wire-mesh cages.
Environmental conditions:	Temperature: 22 ± 4 °C Humidity: 50 ± 20 % Air changes: not stated 12-hour light/dark cycle

B. STUDY DESIGN

Animal assignment and treatment

Administration

Group	Number of male rats	Route of dosing	Frequency of dosing	Dose level (mg/kg bw)	Dose Volume (mL/kg bw)	Radioactive dose (µCi/kg)	Sampling
1	5	Oral gavage	Single	15	5	100	Faeces, urine, cage wash, carcass
2	40*	Oral	Single	15	5	100	Blood

		gavage					

*4 animals per blood sampling time point

Animals were allocated to the different dose groups randomized based on body weight. Animals were fasted overnight through approximately 4 hours after application of 15 mg [^{14}C]-N -acetyl-glyphosate/kg bw.

Observation of animals

Cage-side observations

Animals were observed for mortality and signs of pain and distress twice daily. Cage side observations for general health and appearance were done once daily.

Body Weights

Animals were weighed at randomization and on the day of dose administration.

Animal assignment and treatment: Excretion/mass balance study (Group 1)

Each of five male rats received a single gavage administration of 15 mg [^{14}C]-N-acetyl-glyphosate/kg bw. Urine and faeces were collected at 0 - 6, 6 - 12, and 12 - 24 hour intervals and at 24-hour intervals through 168 hours. The weight of each sample was recorded. After the last excreta collections, cages were washed and wiped with a solution of methanol: 1 % trisodium phosphate (TSP) in water (50:50, v:v) and gauze pads. The cage wash samples and gauze were collected into separate plastic containers and the weight of each cage wash sample was recorded. Animals were sacrificed with an overdose of halothane anaesthesia and the residual carcass from each animal was retained.

Animal assignment and treatment: Pharmacokinetic study (Group 2)

Each male rat received a single gavage administration of 15 mg [^{14}C]-N-acetyl-glyphosate/kg bw. Four animals per time point were sacrificed via exsanguination (cardiac puncture) under halothane anaesthesia. Samples were collected pre-dose, and at 0.5, 1, 2, 4, 8, 12, 24, 48, and 72 hours post-dose. At sacrifice blood was collected via syringe and needle into tubes containing K_3EDTA anticoagulant from each animal. Blood was aliquoted for radio-analysis and centrifuged to obtain plasma.

Sample Preparation and radioanalysis

All sample combustions were done in a Model 307 Sample Oxidizer (Packard Instrument Company) and the resulting $^{14}CO_2$ was trapped in a mixture of Perma Fluor and Carbo-Sorb. Ultima Gold XR scintillation cocktail was used for samples analysed directly. All samples were analysed for radioactivity in liquid scintillation counters (Packard Instrument Company) for at least 5 minutes or 100,000 counts. Each sample was homogenized before radioanalysis (unless the entire sample was used for analysis). All samples were analysed in duplicate if sample size allowed. If results from sample duplicates (calculated as ^{14}C dpm/g sample) differed by more than 10 % from the mean value, the sample was re-homogenized and re-analysed (if the sample size permitted). This specification was met for all sample aliquots that had radioactivity greater than 100 dpm. Scintillation counting data (cpm) were automatically corrected for counting efficiency using the external standardization technique and an instrument-stored quench curve generated from a series of sealed quenched standards.

Blood and Plasma

Duplicate weighed aliquots of blood and plasma were analysed by LSC.

Urine and Cage Wash

Duplicate weighed aliquots of urine were analysed directly by LSC. If no urine sample was visible, a sufficient amount of water was added to thoroughly rinse the container, the sample was mixed by shaking, and duplicate aliquots were taken and analysed by LSC.

Faeces

A sufficient amount of methanol:water (50:50, v:v) was added to facilitate homogenization. Duplicate weighed aliquots were combusted and analysed by LSC.

Cage Wipe
A sufficient amount of methanol:water (50:50, v:v) was added to cover each sample. Samples were allowed to extract, mixed by gentle shaking, and duplicate weighed aliquots were analysed by LSC.

Carcass (residual)
Each sample was cut into pieces, which were frozen in liquid nitrogen and homogenized in a Wiley Mill that had been precooled with dry ice. Additional dry ice was ground to clear any remaining sample from the mill. After the dry ice had sublimed, the homogenate was digested in 1N sodium hydroxide. Duplicate weighed aliquots were taken and hydrogen peroxide was added in a dropwise fashion. After any foaming had dissipated, Ultima Gold XR scintillation cocktail was added, the aliquots were allowed to sit overnight and analysed by LSC.

Characterisation of Metabolites
Group 1 urine samples collected at 0 - 6, 6 - 12, 12 - 24, 24 - 48, and 48 - 72 hours post-dose were pooled across animals by time point. (Urine collected from Animal No. C16498 was excluded from the pool due to suspected cross-contamination of radioactivity between urine and faeces for this animal). Each pool was analysed by LSC. The pools were microfuged, and the supernatants were analysed by LSC and HPLC to determine the metabolic profile. The HPLC eluents for each pooled sample were also analysed by LSC.

Group 1 faeces samples collected from 6 - 12, 12 - 24, and 24 - 48 hours post-dose were pooled across animals by time point. (Faeces collected from Animal No. C16498 were not included in the pools; however, samples collected from this animal at 12 - 24 and 24 - 48 hours post-dose were analysed separately.) In general, aliquots from each pool or sample were oxidized and analysed by LSC. Approximately 1 to 5 g of each faeces pool or sample was extracted twice with approximately 10 mL of methanol. The samples were sonicated, shaken, and centrifuged. The supernatants were combined and analysed by LSC. The extraction solvent was evaporated under nitrogen. The remaining solids were then extracted twice with approximately 10 mL of mobile phase A (50 mM potassium phosphate, monobasic, pH 2:methanol 96:4, v:v). Samples were sonicated, vortex mixed, and centrifuged. The resulting mobile phase A supernatants were combined and analysed by LSC and HPLC. For two samples, the 6 to 12-hour pool and the 24-hour single sample, the methanol residues and the mobile phase A extracts were combined and the solvent was evaporated under nitrogen. The samples were reconstituted in mobile phase A:methanol (9:1, v:v), and microfuged. The resulting supernatants were analysed by HPLC to determine the metabolite profile. The HPLC column eluents for each pooled sample were also analysed by LSC. Following extraction and centrifugation, remaining solids were allowed to air dry, weighed, combusted, and analysed by LSC to determine the total extraction recovery.

Group 2 plasma samples collected at 0.5, 1, 2, 4, 8, 12, and 24 hours post-dose were pooled across animals by time point and the radioactivity in each pool was analysed by LSC. Approximately 3 to 6 mL of each plasma pool was extracted twice with approximately 3 times the volume of methanol. Samples were vortex mixed, sonicated, and centrifuged. The extracts were combined, analysed by LSC, and evaporated to near dryness. Plasma supernatants from the 8-, 12-, and 24-hour pools were further cleaned (using C18 solid phase extraction cartridge and eluting with water and methanol) then again reduced to dryness. Each sample was reconstituted in an appropriate volume of mobile phase A (50 mM potassium phosphate, monobasic, pH 2:methanol 96:4, v:v) and methanol (6:4 v:v). The samples were microfuged and each supernatant was analysed by HPLC to determine the metabolite profile. The post-extraction residues were dried, weighed, combusted, and analysed by LSC to determine the total extraction recovery.

Dose Preparation, Verification and Stability
The oral dose of [^{14}C]-N -acetyl-glyphosate was formulated as a solution in sterile water for injection at a nominal concentration of 3 mg/mL. The day before dose administration, 1.80 mL (containing 47.5 mg free acid) of [^{14}C]-N-acetyl-glyphosate and 331.8 mg N-acetyl-glyphosate salt were transferred into a serum

vial and combined with 88.2 mL of the dose vehicle. Duplicate weighed aliquots were taken from the dose formulation prior to and following dose administration, and were analysed by liquid scintillation counting (LSC) to determine the concentration of radioactivity and homogeneity. Stability was demonstrated by analysing pre-dose and post-dose aliquots by HPLC.

LC/MS and LC/MS/MS Analyses
Selected urine samples, and extracts of faeces and plasma were analysed using liquid chromatography mass spectrometry (LC/MS) ionised via negative electro spray ionisation. Based on the fulls can LC/MS data, a number of LC/MS/MS analyses were performed for structural elucidation. The LC/MS /MS analyses used the same instrumentation and conditions as for the negative ion full-scan LC/MS with minor modifications.

Data Analyses
Statistical analyses were limited to simple expressions of variation, such as mean and standard deviation. Dose tables were compiled with mean and standard deviation values calculated using Excel, Version 8.0e (Microsoft Corporation). Data tables were generated by Debra, Version 5.2a (LabLogic Systems Ltd., Sheffield, UK). Debra is an automated and validated data capture and management system for data collection in absorption, distribution, and excretion studies using radiolabeled test article. Debra captures data from balances and scintillation counters. The maximum concentration (Cmax) in blood and plasma and the time to reach maximum concentration (Tmax) were obtained by visual inspection of the raw data. Pharmacokinetic parameters calculated included half-life (t1/2), area under the concentration -time curve from time 0 to the last measurable time point (AUC_{0-t}), and area under the concentration-time curve from 0 to infinity ($AUC_{0-\infty}$). Pharmacokinetic parameters were calculated by using WinNonlin Professional Edition (Pharsight Corporation, Mountain View, CA).

Data evaluation
If results of sample analysis are below twice background, Debra reports the result as less than the limit of quantitation (BLQ). Values of BLQ are included as 0 in calculations of mean and variance. The concentration associated with the limit of quantitation is calculated as follows:
Limit of Quantitation (µg equivalents/g) = 2× Background (dpm)+Aliquot weight (g) f /Dose specific activity (dpm/µg)

The percent of administered dose associated with the limit of quantitation is calculated as follows:
Limit of quantitation (% of dose) = Limit of Quantitation (µg equivalents/g) × Sample weight (g) × 100/ µg administered

II. RESULTS AND DISCUSSION

A. TEST ARTICLE AND DOSE FORMULATION ANALYSIS

Radiochemcial purity and Stability
The HPLC analyses showed the radiochemical purity of [^{14}C]-N-acetyl-glyphosate to be 99.8 % prior to dosing. The mean radiochemical purity values obtained from HPLC analysis of pre-dose and post-dose aliquots from the dose solution were 99.9 % and 99.2 %, respectively, confirming stability of the test article under the conditions of the study. Representative stability chromatograms were presented.

Concentration and Homogeneity
The LSC analysis results indicated the dose solution was homogenous during the dosing period.

Specific Activity
The specific activity of the [^{14}C]-N-acetyl-glyphosate as provided by the sponsor was 37.9 µCi/mg. The mean specific activity of [^{14}C]-N-acetyl-glyphosate in the dose solution was determined to be 7.05 µCi/mg.

B. DOSE ADMINISTRATION

The volume of radiolabelled dose formulation to be administered to each animal by oral gavage was calculated based on the body weight taken on the day of dose administration. The actual amount administered was determined by weighing the dose syringe before and after dose administration. All animals were fasted overnight through approximately 4 hours post-dose.

Table 5.8.1-86: Mean body weights and radioactivity doses administered to male rats dosed orally with [^{14}C]-N-acetyl-glyphosate at a target dose of 15 mg/kg

Group	Number of male rats		Body weight (g)	Dose weight (g)	Actual dose administered				
					(dpm)	(mg/animal)	(mg/kg)	(µCi/animal)	(µCi/kg)
1	5	Mean	280	1.39	66457821	4.25	15.1	30.0	107
		SD	8	0.05	2375031	0.15	0.2	1.1	2
2	40	Mean	277	1.38	66246716	4.24	15.3	29.8	108
		SD	8	0.04	2114489	0.14	0.2	1.0	1

SD: Standard deviation. Dose concentration was 4.79 x 10^7 dmp/g. Dose specific activity was 7.05 µCi/mg.

C. CAGE-SIDE OBSERVATIONS

All animals appeared healthy and exhibited no overt signs of toxicity throughout the study.

D. EXCRETION AND MASS BALANCE

The mean total recovery of radioactivity from the five animals in Group 1 was 98.0 % with 57.2 % in urine, 37.6 % in faeces, 2.91 % in cage wash and wipe, and 0.26 % in residual carcass. More than 90 % of the total radioactivity was eliminated within 48 hours, with renal excretion being the primary elimination route. For animal No. C16498, 82.4 % of the administered radioactivity was detected in faeces, indicating probable urine contamination of the faeces. Excluding this animal, mean total recovery was 95.5 %, with 66.1 % in urine, 26.4 % in faeces, 0.23 % in carcass, and 2.79 % in cage wash/wipe.

Table 5.8.1-87: Percent of radioactive dose in urine, faeces, cage wash, cage wipe and carcass at specified intervals after a single oral administration of [^{14}C]-N-acetyl-glyphosate to male rats (n=5) at 15 mg/kg bw

Collection interval (hours)	% of applied radioactive dose			
	Mean	SD	Mean[a]	SD[a]
Urine				
0 - 6	40.5	17.5	47.7	8.0
6 - 12	11.8	7.8	13.6	7.7
12 - 24	2.13	0.70	2.06	0.79
24 - 48	1.35	0.66	1.37	0.77
48 - 72	0.48	0.35	0.43	0.39
72 - 96	0.29	0.19	0.26	0.21
96 - 120	0.42	0.37	0.46	0.42
120 - 144	0.17	0.09	0.18	0.10
144 - 168	0.12	0.10	0.13	0.12
Subtotal	57.2	23.5	66.1	14.3
Faeces				
0 - 6	0.00	0.00	0.00	0.00
6 - 12	5.56	7.65	6.95	8.07
12 - 24	24.2	18.7	16.2	6.8
24 - 48	7.20	10.3	2.68	2.1
48 - 72	0.22	0.18	0.17	0.16
72 - 96	0.17	0.18	0.11	0.14

Table 5.8.1-87: Percent of radioactive dose in urine, faeces, cage wash, cage wipe and carcass at specified intervals after a single oral administration of [^{14}C]-N-acetyl-glyphosate to male rats (n=5) at 15 mg/kg bw

Collection interval (hours)	% of applied radioactive dose			
	Mean	SD	Mean[a]	SD[a]
96 - 120	0.16	0.12	0.16	0.14
120 - 144	0.05	0.04	0.05	0.04
144 - 168	0.03	0.01	0.03	0.02
Subtotal	37.6	27.7	26.4	13.5
Cage wash, cage wipes and carcass				
168 (cage wash)	0.61	0.31	0.69	0.28
168 (cage wipe)	2.30	2.30	2.10	2.61
168 (carcass)	0.26	0.07	0.23	0.04
Total	**98.0**	**5.7**	**95.5**	**1.2**

SD: Standard deviation. a: Calculated excluding values for Animal No. C16498, which had suspected urine contamination of faeces

E.PHARMACOKINETICS

Following a single oral administration, the mean maximum concentrations (C_{max}) in blood and plasma were 2.93 and 5.31 µg equivalents/g after 1 and 2 hours, respectively. The values for half-life ($t_{1/2}$), and area under the curve (AUC_{0-t}, and $AUC_{0-\infty}$) were 20.1 hours, 12.0 µg equivalents-hour/g, and 12.1 µg equivalents- hour/g in blood and 15.6 hours, 20.7 µg equivalents-hour/g and 20.8 µg equivalents-hour/g in plasma.

Table 5.8.1-88: Concentrations of radioactivity in blood and plasma at specified times after a single oral administration of [^{14}C]N-acetyl-glyphosate to male rats at 15 mg/kg bw

Collection time point (hours)	Blood		Plasma	
	µg Eq [^{14}C]N-acetyl-glyphosate/g		µg Eq [^{14}C]N-acetyl-glyphosate/g	
	Mean	SD	Mean	SD
0.5	2.17	0.65	3.89	1.04
1	2.93	1.25	5.29	2.24
2	2.84	0.87	5.31	1.60
4	0.983	0.164	1.79	0.29
8	0.0973	0.0325	0.142	0.061
12	0.0442	0.0041	0.0493	0.0076
24	0.0243	0.0012	0.0166	0.0029
48	0.00994	0.00669	0.00895	0.00090
72	0.00548	0.00635	BLQ	BLQ

SD: Standard deviation; Eq: Equivalents. BLQ = below limit of quantitation. Four rats were sacrificed per sampling time

Table 5.8.1-89: Pharmacokinetic parameters for radioactivity in blood and plasma collected from male rats after a single oral administration of [^{14}C]-N-acetyl-glyphosate at 15 mg/kg bw

Matrix	T_{max} (hours)	C_{max} (µg eq/g)	$t_{1/2}$ (hours)	$AUC_{(0-t)}$ (hours µg Eq/g)	$AUC_{(0-\infty)}$ (hours µg Eq/g)
Blood	1	2.93	20.1	12.0	12.1
Plasma	2	5.31	15.6	20.7	20.8

Eq: Equivalents

F. METABOLITE CHARACTERISATION

Nearly 100 % of the radioactivity was recovered in pooled urine after micro-centrifugation for HPLC analysis. Total extraction recoveries for pooled faeces ranged from 93.2 to 106 %, with 92.9 to 101 % extracted and 0.15 to 5.75 % remaining in the post extraction solid. The total extraction recoveries for pooled plasma collected from 0.5 to 8 hours post-dose ranged from 97.1 to 105 %, with 88.0 to 103 % extracted and 1.35 to 9.05 % remaining in the post extraction solid. Due to low levels of radioactivity in the samples collected at 12 and 24 after dosing, recoveries were low in these samples. Extracted radioactivity from selected samples was analysed by using HPLC with radioactivity flow detection and LC/MS/MS.
One radiolabelled component was detected by HPLC analysis of urine and extracts of faeces and plasma. This component had retention times similar to the [^{14}C]-N-acetyl-glyphosate reference standard when the samples and the standard were co-injected. LC/MS/MS analysis of this component in urine, plasma, and faeces provided molecular ion and production spectra that were virtually identical to that of the [^{14}C]-N-acetyl-glyphosate reference standard.

Urine
The HPLC profile in urine showed only unchanged [^{14}C]-N-acetyl-glyphosate. A total of 65.2 % of the administered [^{14}C]-N-acetyl-glyphosate was excreted in urine.

Faeces
The HPLC profile in faeces showed mostly unchanged [^{14}C]-N-acetyl-glyphosate, with trace amounts of a metabolite. This metabolite was identified as glyphosate by co-chromatography of a faeces extract with the [^{14}C]-glyphosate reference standard; the metabolite was not confirmed by LC/MS analysis, due to the low levels of radioactivity in faeces samples.

Plasma
The HPLC profile for plasma showed only unchanged [^{14}C]-N-acetyl-glyphosate in samples collected from 0.5 to 12 hours post-dose.

Table 5.8.1-90: Detected metabolites in pooled samples following a single oral administration of [^{14}C]-N-acetyl-glyphosate at 15 mg/kg bw to male rats (n=5)

Pooled sample type	Proposed identification	Retention time (minutes)	Percent of Sample Radioactivity				
			0 - 6h	6 - 12h	12 - 24h	24 - 48h	48 - 72h
Urine	N-acetyl-glyphosate	9.2 – 9.6	99.8	100	100	100	100
Faeces	Glyphosate	5.4-6.0	No sample	ND	0.47 (0.45)	ND (0.75)	No sample
	N-acetyl-glyphosate	8.1-8.7		100	99.5 (99.6)	100 (99.3)	
			Percent of radioactive Dose				
			0 - 6h	6 - 12h	12 - 24h	24 - 48h	48 - 72h
Urine	N-acetyl-glyphosate	9.2 – 9.6	47.7	13.6	2.06	1.37	0.43
Faeces	Glyphosate	5.4-6.0	No sample	ND	0.07 (0.24)	ND (0.19)	No sample
	N-acetyl-glyphosate	8.1-8.7		13.9	15.5 (52.3)	2.59 (24.6)	
			Percent of Sample Radioactivity				
			0.5h	1h	2h	4h	8h	12h
Plasma	N-acetyl-glyphosate	9.3 – 9.9	100	100	100	100	100	100
			Concentration (µg Eq [^{14}C]-N-acetyl-glyphosate/g)					
			0.5h	1h	2h	4h	8h	12h
	N-acetyl-glyphosate	9.3 – 9.9	4.13	5.40	5.25	1.86	0.126	0.287

ND = not detected. Values in brackets () were detected in the individual sample from Animal No. C16498

III. CONCLUSIONS

Following a single oral administration of [^{14}C]-N-acetyl-glyphosate to male rats, approximately 66 % of the total radioactivity was excreted in urine and approximately 26 % in faeces. Over 90 % of the radioactivity was excreted within 48 hours post-dose. Minimal metabolism occurred, with unchanged [^{14}C]-N-acetyl-glyphosate representing more than 99 % of the total administered radioactivity detected in urine and faeces. Trace amounts of [^{14}C]-glyphosate, representing less than 1 % of the total radioactivity, were detected in faeces. [^{14}C]-N-acetyl-glyphosate was the only circulating radioactive component in plasma, and was eliminated from plasma with a half-life of 15.6 hours.

3. Assessment and conclusion

Assessment and conclusion by applicant:

A single oral dose of [^{14}C]-N-acetyl-glyphosate at 15 mg free acid equivalent/kg body weight was administered to five male Sprague-Dawley rats. Urine and faeces were collected at different time points. Upon sacrifice the cage was and carcasses were analysed for radioactivity. The mean total recovery of radioactivity after a collection period of 7 days was 95.5 % with 66.1 % in urine, 26.4 % in faeces, 2.79 % in cage wash and wipe, and 0.23 % in residual carcass.

Pharmacokinetics
Blood was collected over a sufficient time period as only trace amounts were detectable at 72 hours post-dose. [^{14}C]-N-acetyl-glyphosate was the only circulating radioactive component in plasma, and was eliminated from plasma with a half-life of 15.6 hours. The mean maximum concentrations (Cmax) in blood and plasma were 2.93 and 5.31 µg equivalents/g at 1 and 2 hours post-dose, respectively. Radioactivity was eliminated from blood and plasma with half-life values of 20.1 and 15.6 hours, respectively. Comparison of blood and plasma values for area under the curve (AUC) indicated that [^{14}C]-N-acetyl-glyphosate distributed preferentially into plasma.
The limit of quantitation was not explicitly stated in the report, but was <0.01 µg Equivalents [^{14}C]-N-acetyI-glyphosate/g for blood and plasma.

Absorption
N-acetyl-glyphosate is quickly absorbed after oral application as indicated by the urinary excretion. 47.7 % of the applied radioactivity was excreted within 6 h after application. 63.4 % of the applied radioactivity was excreted within 24 h (excluding cage wash). This supported by Tmax values being 1 and 2 hours in blood and plasma, respectively.

Elimination
Approximately 66 % of the total radioactivity was excreted in urine and approximately 26 % in faeces. Over 90 % of the radioactivity was eliminated within 48 hours post-dose.

Metabolism
No metabolites were detected in plasma and urine. Plasma and urine radioactivity consisted entirely of unchanged [^{14}C]-N-acetyl-glyphosate. Unchanged [^{14}C]-N-acetyl-glyphosate recovered in urine and faeces represented over 99 % of the administered radioactivity. A metabolite found at less than 0.25 % of the administered radioactivity in faeces was glyphosate, which was formed by N-deacetylation.

Conclusion:
The study mainly focused on metabolism, elimination and pharmacokinetics of N-acetyl-glyphosate after single oral administration to male rats. Methods and results are acceptable and can be used as reliable supplementary data.

Assessment and conclusion by RMS:

Acute oral toxicity

CA 5.8.1/039

1. Information on the study

Data point	CA 5.8.1
Report author	[redacted]
Report year	2004
Report title	Acute Oral Toxicity Study in Rats with N-Acetyl-Glyphosate, Sodium Salt (Acute Toxic Class Method)
Report No	7535-103
Document No	NA
Guidelines followed in study	US EPA 870.1100 – Acute Oral Toxicity (1998); OECD 423 (2001)
Deviations from current test guideline (OECD 423, 2001)	None
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities[1,2]	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive summary

The toxicity of the test article, N-acetyl-glyphosate (as the free acid), was evaluated following a single oral dose to the rat. The test article was suspended in cell culture grade water and administered by oral gavage at a dose level of 5000 mg/kg bw to five rats/sex. The rats were observed for mortality, body weight effects and clinical signs for 15 days after dosing. All animals were subjected to an abbreviated gross necropsy examination of the external features of the carcass, external body orifices, the abdominal, thoracic and cranial cavities and organs/tissues.

Mortality was observed in one female at the 4-hour post-dose observation interval and in one male and one female during the a.m. check on Day 2. All the remaining males and females survived until the scheduled sacrifice. Clinical observations observed in the males included hypoactivity, irregular respiration, liquid or soft feces, light-brown perineal staining, brown nasal crust and/or squinted eyes. Clinical observations observed in the females included slight hypoactivity, irregular respiration, liquid or soft feces and/or light-brown perineal staining. All clinical signs of toxicity were resolved in all surviving animals by Day 3.

All surviving animals gained weight from initiation of dosing to study termination. At necropsy, findings were noted in the one male and two females that were found dead prior to the terminal sacrifice, however no abnormal findings were noted in the remaining animals at necropsy. Findings in one male and one female involved the lungs (mottled or discolored bright-red), liver (discolored black), stomach (soft and/or with yellow fluid), abdominal cavity (fluid/clear fluid), and duodenum, jejunum and ileum (fluid). Finding in the second male involved the stomach (gel-like clear liquid and red walls), abdominal cavity (reddish-liquid), duodenum, jejunum and ileum (fluid).

Under the conditions of this study, the oral LD_{50} of the test article is greater than 5000 mg/kg bw. The mortality rate was 20 % in the males and 40 % in the females dosed at 5000 mg/kg.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	N-acetyl-glyphosate, sodium salt
Description:	White powder
Lot/Batch #:	123K5012 (1247W-001)
Purity:	84.3 % sodium salt
Stability of test compound:	The test substance appeared to be stable under the conditions of the study (sponsor's responsibility). Expiry date: 2006-02-10.

2. Vehicle and/ or positive control:	Cell culture grade water (Bio Whittaker/Cambrex; Lot No. 01102570; Expiration Date: 2005-08-04) / none

3. Test animals:

Species:	Rat
Strain:	Crl:CD®(SD)IGS BR
Source:	[redacted]
Age:	8 – 12 weeks
Sex:	Male and female
Weight at dosing:	♂ 223 – 247 g; ♀ 231 – 242 g
Acclimation period:	5 days
Diet/Food:	PMI® Nutrition International, LLC Certified Rodent LabDiet® 5002, *ad libitum*
Water:	Water, *ad libitum*
Housing:	Individually in sanitary, stainless-steel, screen-bottomed, hanging, wire cages
Environmental conditions:	Temperature: 18 – 26 °C Humidity: 50 ± 20 % Air changes: ≥10 air changes / hour 12 hours light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2004-04-01 to 2004-04-16

Animal assignment and treatment:
A limit test using five animals/sex at a dose level of 5000 mg/kg bw was conducted. The animals were fasted overnight (17 to 20 hours before test material administration) and for approximately 4 hours after dosing. Dose calculations were adjusted by a factor of 67.4 % to obtain a dose of the free acid. On the day of dosing the test material was suspended in the vehicle, forming an opaque, viscous, white, homogeneous suspension. Individual doses were calculated based on each animal's fasted body weight taken just before test material administration and a dose volume of 10 mL/kg bw.
The test article was administered orally via a gavage needle attached to a disposable syringe. An assigned syringe and oral gavage needle was used for each dosing group. Prior to dose administration to each animal, the gavage needle was wiped with paper towels.

Table 5.8.1-91: Acute Oral Toxicity Study in Rats with N-Acetyl-Glyphosate, Sodium Salt (Acute Toxic Class

Method) ([redacted], 2004): Study design and mortality

Test group	Dose level [mg/kg bw]	Mortality / number of animals	
		Males	Females
N-acetyl-glyphosate, sodium salt	5000	1/5	2/5

Analysis of the test substance
The analysis of the test substance was in the responsibility of the sponsor.

Mortality
The animals were observed for general health and mortality at least once daily during acclimation and twice daily (at least 4 hours apart) for the duration of the study.

Clinical observations
Observations were performed for clinical signs of toxicity pre-dose on the day of dosing (Day 1), continuously for the first hour post-dose and at approximately 4 and 8 hours post-dose. Daily observations were performed thereafter until the day prior to sacrifice.

Body weight
The animals were weighed pre-dose on the day of dosing (Day 1; fasted), on Days 8 and 15, and at termination (Day 16; fasted).

Sacrifice and pathology
After 15 days of observation, all surviving animals were food fasted overnight, weighed (Day 16) and anesthetised with an appropriate barbiturate.

Necropsy
All animals were subjected to an abbreviated gross necropsy examination of the external features of the carcass, external body orifices, the abdominal / thoracic / cranial cavities and organs / tissues. Any abnormalities were recorded. After necropsy, the animals were discarded; no tissues were saved.

Statistics
Other than the calculation of the LD_{50} and the descriptive statistics of the body weights (when applicable) no statistical analyses were required.

II. RESULTS AND DISCUSSION

A. MORTALITY
Mortality was observed in one female at the 4 hour post-dose observation interval and in one male and one female during the a.m. check on Day 2. All the remaining males and females survived until the scheduled sacrifice. Please refer to the table above.

B. CLINICAL OBSERVATIONS
Clinical observations observed in the males included slight and distinct hypoactivity, irregular respiration, liquid or soft faeces, brown nasal crust, light-brown perineal staining and/or squinted eyes. Clinical observations observed in the females included slight hypoactivity, irregular respiration, liquid or soft faeces and/or light-brown perineal staining. All clinical signs of toxicity were resolved in all surviving animals by Day 3.

C. BODY WEIGHT
All surviving animals gained weight from initiation of dosing to study termination.

D. NECROPSY

Findings were noted in the one male and two females that were found dead prior to the terminal sacrifice, however no abnormal findings were noted in the remaining animals at necropsy. Findings in one male and one female involved the lungs (mottled or discoloured bright-red), liver (discoloured black), stomach (soft and/or with yellow fluid), abdominal cavity (fluid/clear fluid), and duodenum, jejunum and ileum (fluid). Finding in the second male involved the stomach (gel-like clear liquid and red walls), abdominal cavity (reddish-liquid), and duodenum, jejunum and ileum (fluid).

Table 5.8.1-92: Acute Oral Toxicity Study in Rats with N-Acetyl-Glyphosate, Sodium Salt (Acute Toxic Class Method) ([redacted], 2004): Necropsy findings

Animal number	Sex	Dose level [mg/kg bw]	Day of death	Necropsy Observation
5045	Male	5000	Terminal	No abnormal findings
5046	Male		Terminal	No abnormal findings
5047	Male		Terminal	No abnormal findings
5048	Male		Day 2	Lungs – mottled Liver – discoloured black Stomach – soft Abdominal cavity – clear fluid Duodenum, jejunum, ileum – fluid
5049	Male		Terminal	No abnormal findings
5050	Female		Terminal	No abnormal findings
5051	Female		Day 2	Lungs – discoloured bright-red Liver – discoloured black Stomach – soft with yellow fluid Abdominal cavity – fluid Duodenum, jejunum, ileum – fluid
5052	Female		Day 1	
5053	Female		Terminal	No abnormal findings
5054	Female		Terminal	No abnormal findings

III. CONCLUSIONS

Under the conditions of this study, the oral LD_{50} of the test article is greater than 5000 mg/kg bw. The mortality rate was 20 % in the males and 40 % in the females dosed at 5000 mg/kg bw. The test material, N-acetyl-glyphosate, sodium salt was toxic when administered once by oral gavage at a dose level of 5000 mg/kg bw (as the free acid) in male and female rats under the conditions of this assay.

3. Assessment and conclusion

Assessment and conclusion by applicant:
This study is considered to be valid as it was conducted under GLP and no deviations from the recent guideline could be identified.
Under the conditions of this study, the oral LD_{50} of N-acetyl-glyphosate, sodium salt is greater than 5000 mg/kg bw.

Assessment and conclusion by RMS:

Short-term toxicity

CA 5.8.1/040

1. Information on the study

Data point	CA 5.8.1

Report author	[redacted] [redacted] supplement author)
Report year	2007
Report title	IN-MCX20: Subchronic Toxicity 90-Day Feeding Study in Rats
Report No	[redacted]-19008
Document No	NA
Guidelines followed in study	OPPTS 870.3100 (1998); OECD Guideline 408 (1998); Method B.26, Directive 2001/59/EC (2001); MAFF 2-1-9 Notification 12 Nousan 8147 and Notification 13 Seisan 1739 (2000 and 2001)
Deviations from current test guideline (OECD 408, 2018)	Clinical chemistry was performed without determining LDL, HDL, T3, T4 and TSH; organ weights were determined without prostate (+ seminal vesicles and coagulating glands), thyroid and pituitary gland; histopathology was performed without cervix, coagulating glands, gall bladder and male mammary glands.
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive summary

The objective of this study was to assess the potential sub-chronic toxicity of IN-MCX20 in rats. Five groups of young adult male and female Crl:CD(SD) rats (10/sex/group) were administered diets that contained 0, 180, 900, 4500 or 18000 ppm IN-MCX20 (equivalent to 0, 11.3, 55.7, 283 and 1157 mg/kg bw/day for males and 0, 13.9, 67.8, 360 and 1461 mg/kg bw/day for females) for approximately 90 days (95 days in males and 96 days in females). Samples of the diets were analysed to assess homogeneity, stability, and concentration of the test substance in the diet. Body weights, food consumption and detailed clinical observations were evaluated weekly. Ophthalmological and neurobehavioral (abbreviated functional observational battery, grip strength, motor activity) evaluations were performed on all rats prior to the start of dietary exposure and near the end of the exposure period. Clinical pathology endpoints (haematology, coagulation, clinical chemistry and urinalysis with metabolic analysis of IN-MCX20 (N-acetyl glyphosate) and its possible metabolites, IN-B2856 (glyphosate) and IN-EY252 (N-acetyl AMPA)) were evaluated at the end of the exposure period. After approximately 90 days of dietary exposure, the surviving rats were sacrificed and given a gross and microscopic pathological examination.

Analysis of the diets demonstrated that IN-MCX20 was present at the targeted concentrations and was stable under relevant storage conditions. The homogeneity of the test substance in the initial diet preparations, in particular at the 180 ppm concentration, indicated a coefficient of variance (CV) of >10 %. However, most individual diet samples were within the targeted concentration range, and analysis of subsequent diet preparations supported adequate homogeneity. Therefore, it was concluded that the rats were exposed to the targeted concentrations of test substance. The overall mean daily intake of IN-MCX20 in the 0, 180, 900, 4500 or 18000 ppm groups was 0, 11.3, 55.7, 283 and 1157 mg/kg bw/day, respectively, for male rats and 0, 13.9, 67.8, 360 and 1461 mg/kg bw/day, respectively, for female rats.

No adverse test substance-related effects on any body weight or nutritional parameters were observed. Statistically significantly lower overall mean body weight gain (86 % of control) was observed in 18000 ppm males but was not considered adverse as it was not associated with a statistically significant difference in mean final body weight or in overall mean food consumption or food efficiency.

No test substance-related deaths occurred and no clinical, ophthalmological or neurobehavioral observations were attributed to exposure to the test substance. There were no adverse effects on clinical pathology parameters, organ weights, gross pathology, or microscopic pathology in male or female rats.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	Disodium N-acetyl-N-(phosphonomethyl)glycine
Identification:	IN-MCX20
Description:	White solid
Lot/Batch #:	27419
Purity:	63 %
Stability of test compound:	The test substance appeared to be stable under the conditions of the study and the study was completed prior to the expiration date on the Certificate of Analysis. No evidence of instability, such as a change in colour or physical state, was observed.
2. Vehicle and/ or positive control:	Diet / none
3. Test animals:	
Species:	Rat
Strain:	Crl:CD(SD)
Source:	[redacted]
Age:	7 weeks
Sex:	Male and female
Weight at dosing:	♂ 235.1 – 236.5 g; ♀ 173.8 – 175.9 g
Acclimation period:	6 days
Diet/Food:	PMI® Nutrition International, LLC Certified Rodent LabDiet® 5002, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in stainless steel, wire-mesh cages suspended above cage boards
Environmental conditions:	Temperature: 18 – 26 °C Humidity: 30 – 70 % Air changes: Not reported 12 hours light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2007-05-18 to 2007-02-16

Animal assignment and treatment:
The test substance, IN-MCX20, was administered on a continuous basis in the basal diet for 95 or 96 days to 4 groups of (Crl:CD(SD)) rats. Dose levels were 180, 900, 4500 and 18000 ppm (equivalent to 0, 11.3, 55.7, 283 and 1157 mg/kg bw/day for males and 0, 13.9, 67.8, 360 and 1461 mg/kg bw/day for females). A concurrent control group received the basal diet on a comparable regimen. Each group consisted of 10 animals per sex.

Table 5.8.1-93: IN-MCX20: Subchronic Toxicity 90-Day Feeding Study in Rats ([redacted] 2007): Study design

Test group	Dietary concentration [ppm]	Mean daily intake mg/kg bw/day	Number of animals	
			Males	Females
Control	0	♂: 0; ♀: 0	10	10
Low	180	♂: 11.3; ♀: 13.9	10	10

Intermediate low	900	♂: 55.7; ♀: 67.8	10	10
Intermediate high	4500	♂: 283; ♀: 360	10	10
High	18000	♂: 1157; ♀: 1461	10	10

Analysis of the test substance
IN-MCX20 was initially hand mixed with 500 grams of chow. Subsequently, this pre-mix was added to the diet and thoroughly mixed for a period of time that, by experience, was considered adequate to ensure homogeneous distribution in the diet. The manufacturer and production code of the feed was recorded in the study records. Diets were prepared weekly and refrigerated until used.
Duplicate samples (concentration and homogeneity verification) of all dietary concentrations were collected at the initial diet preparation and analysed to verify the concentration of IN-MCX20 in the diets. Duplicate samples were taken from the lowest and highest dietary concentration of the initial diet preparation and analysed to verify the stability of IN-MCX20 in the diets. The stability of IN-MCX20 in feed was demonstrated by comparing samples stored at room temperature or refrigerated with the mean measured values of the concentration (middle homogeneity) verification samples. Because of difficulties encountered with development of an analytical method, insufficient diet samples collected at study start were available to verify homogeneity in the diet. The analytical results of the middle homogeneity samples from this time point were used to support concentration at study start. Additional diet samples were collected from the top, middle, and bottom of the diet mixer of all dietary concentrations during the middle of the study and evaluated to confirm homogeneity of mixing, as well as to verify concentration at this time point. Toward the end of the study, additional samples were taken to verify concentration.

Mortality
Cage-site examinations to detect moribund or dead rats were conducted at least twice daily throughout the study.

Clinical observations
Cage-site examinations to detect abnormal behaviour and/or appearance among rats were conducted at least twice daily throughout the study. Abnormal behaviour/appearance was recorded by exception.
An additional cage-site evaluation was conducted daily, except on the days when detailed clinical observations were conducted, at approximately the same time (± 2 hours). Acute clinical signs of systemic toxicity were recorded.
At every weighing, each rat was individually handled and examined for abnormal behavior and appearance. Detailed clinical observations in a standardised arena were also evaluated on all rats. The detailed clinical observations included (but were not limited to) evaluation of fur, skin, eyes, mucous membranes, occurrence of secretions and excretions, autonomic nervous system activity (lacrimation, piloerection and unusual respiratory pattern), changes in gait, posture, response to handling, presence of clonic, tonic, stereotypical or bizarre behavior. Any abnormal clinical signs noted were recorded.

Body weight
All rats were weighed once per week.

Food consumption and test substance intake
The amount of food consumed by each rat over each weighing interval was determined throughout the study. From these determinations and body weight data, mean daily food consumption, mean food efficiency, and mean daily intake of IN-MCX20 were calculated.

Abbreviated Functional Observational Battery (FOB)
Sensory and motor function assessments were conducted by evaluating grip strength, responses to approach/touch, sharp auditory stimulus, and tail pinch, and pupillary constriction prior to administration and in week 13. Fore- and hind-limb grip strength was measured by a strain gauge device (Chatillon® Digital Force gauge). Responses to approach/touch, sharp auditory stimulus, and tail pinch were made while the animal was in a standard arena. Pupillary constriction was assessed after a beam of light was directed into each eye prior to removing the rats from the motor activity chambers because the darkened room in

which the apparatus was located facilitated observing the response.

Motor activity evaluation
Following the abbreviated functional observational battery, assessment of motor activity (MA) was conducted prior to treatment and in week 13. Rats were individually tested in 1 of 30 identical, automated activity monitors (Coulbourn® Infrared Motor Activity System). Group and sex were counterbalanced across the monitors and time of day to the fullest extent possible. The infrared monitoring device enables measurement of 2 dependent variables: duration of movement and number of movements. A continuous movement was counted as 1 movement regardless of duration. Each test session was 60 minutes in duration, and the results were expressed for the total session, as well as for 6 successive 10-minute blocks.
Presence of diarrhoea and polyuria on the cage boards below the motor activity monitor were also recorded following each motor activity session.

Ophthalmoscopic examination
Ophthalmology examinations were conducted by a veterinary ophthalmologist. Both eyes were examined by focal illumination and indirect ophthalmoscopy. The examinations were conducted under subdued lighting after mydriasis had been produced with a 1 % tropicamide solution. On test day -15, the initial examination was performed on all rats received for the study, prior to selection and grouping. All surviving rats were examined on test day 85 prior to the scheduled sacrifice.

Haematology, clinical chemistry and urinalysis
A clinical pathology evaluation was conducted on all animals on test days 95 – 96. The day before collection of samples for the clinical pathology evaluation, the animals were placed in metabolism cages. These animals were fasted after 3 p.m. for at least 15 hours and urine was collected from each animal. Blood samples for haematology and clinical chemistry measurements were collected from the orbital sinus of each animal while the animal was under carbon dioxide anaesthesia. Blood samples for coagulation parameters were collected at sacrifice from the abdominal vena cava of each animal while the animal was under carbon dioxide anaesthesia.
Additional blood collected from the vena cava was placed in a serum tube, processed to serum, and frozen at approximately -80 °C. Serum will be discarded without analysis because further tests were not required to support experimental findings. Bone marrow smears were prepared at sacrifice from all surviving animals. Bone marrow smears were stained with Wright-Giemsa stain, but analysis was not necessary to support experimental findings. All blood samples were evaluated for quality by visual examination. Results were maintained in the study records and reported only if the sample was analysed.

The following haematological parameters were evaluated: Red blood cell count, red cell distribution width, haemoglobin, absolute reticulocyte count, haematocrit, platelet count, mean corpuscular (cell) volume, white blood cell count, mean corpuscular (cell) haemoglobin, differential white blood cell count, mean corpuscular (cell) haemoglobin concentration, microscopic blood smear examination, prothrombin time and activated partial thromboplastin time.
The following clinical chemistry parameters were evaluated: Aspartate aminotransferase, alanine aminotransferase, sorbitol dehydrogenase, alkaline phosphatase, total bilirubin, urea nitrogen, creatinine, cholesterol, triglycerides, glucose, total protein, albumin, globulin, calcium, inorganic phosphorus, sodium, potassium and chloride.
The following urinalysis parameters were evaluated: Quality, colour, clarity, volume, osmolality, pH, glucose, ketone, bilirubin, blood, urobilinogen, protein and microscopic urine sediment examination.

Metabolite analysis
Additionally pooled urine samples for each treatment group were taken on Day 82 (males) and Day 83 (females) and individually plasma samples were taken on the same days to analyse N-MCX20 (N-acetyl glyphosate) and its possible metabolites, IN-B2856 (glyphosate) and IN-EY252 (N-acetyl AMPA).
The rat urine samples were prepared by adding 0.935 mL of 10 % formic acid to 0.05 mL of urine sample. The mixture was vortexed and 0.015 mL of $HClO_4$ was added. Finally, it was centrifuged after vortexing and the solution was transferred to a LC vial for LC/MS/MS analysis. The amount of 10 % formic acid

was reduced to 0.185 mL when analyte concentrations in the samples were very low (i.e., samples from the 180 and 900 ppm groups) and a more sensitive LC/MS/MS method was used to analyse these samples.
The rat plasma samples were prepared by adding 0.05 mL of trifluoroacetic acid to 0.05 mL of plasma sample. The mixture was vortexed well and 0.4 mL of 10 % formic acid was added. Finally, it was centrifuged after vortexing and the solution was transferred to a LC vial for LC/MS/MS analysis.
The individual stock solutions of IN-MCX20, IN-B2856 and IN-EY252 were prepared in NANOpure® water. The stock solutions were then used to prepare calibration standards in the control rat urine or plasma matrices. Each calibration standard contained all three analytes at the similar concentrations.

Sacrifice and pathology
After approximately 90 days on study (test day 95 for males and 96 for females), all male and female rats from each exposure group (0, 180, 900, 4500 and 18000 ppm) were sacrificed and necropsied for evaluation of sub-chronic toxicity. Rats sacrificed by design were fasted after 3 p.m. on the afternoon preceding the day of sacrifice. The order of sacrifice for scheduled deaths was stratified across dose groups within each sex. Rats were euthanised by carbon dioxide anaesthesia and exsanguination. Gross examinations were performed on all rats. Gross observations, final body weights and organ weights were recorded.

Organ weights
The following organs were weighed from all animals at the scheduled necropsy: Liver, kidneys, heart, spleen, thymus, adrenal glands, brain, testes, epididymides, ovaries (including oviducts), and uterus (including cervix). Group mean values and organ weight ratios (% body weight and % brain weight) were calculated.

Histopathology
The following organs were examined: Adrenals, aorta, bone with marrow (with femur and sternum), bone marrow smear, brain, epididymides, eyes, oesophagus, stomach, duodenum, jejunum, ileum, Peyer's Patches, caecum, colon, rectum, heart, kidneys, larynx, liver, lungs, lymph nodes (mandibular and mesenteric), nasal cavity, mammary gland (females only), ovaries, pancreas, peripheral nerve, pharynx, pituitary, prostate, salivary glands, seminal vesicles, skeletal muscle, skin, spinal cord, spleen, testes, thymus, thyroid/parathyroids, trachea, urinary bladder, uterus, vagina and all gross lesions and masses.

Statistics
Significance was judged at $p<0.05$. Separate analyses were performed on the data collected for each sex.

Table 5.8.1-94: IN-MCX20: Subchronic Toxicity 90-Day Feeding Study in Rats ([redacted] 2007): Statistical analysis

Parameter	Preliminary Test	Method of Statistical Analysis	
		If preliminary test is not significant	If preliminary test is significant
Body weight Body weight gain Food consumption Food efficiency Clinical pathology Organ weight	Levene's test for homogeneity and Shapiro-Wilk test for normality	One-way analysis of variance followed by Dunnett's test	Kruskal-Wallis test followed by Dunn's test
Survival Incidence of FOB Descriptive parameters	None	Sequential application of Cochran-Armitage test for trend	
Motor activity Grip strength	Levene's test for homogeneity and Shapiro-Wilk test for normality	Repeated measures analysis of variance followed by Linear contrasts	Sequential application of the Jonckheere-Terpstra trend test

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSIS

The results of diet analysis from samples submitted mid-study demonstrate that the test substance was homogeneously distributed (coefficients of variance [CV] = 4 – 10 %) and was at the targeted concentrations (± 13.7 % of nominal) in all diets. Analysis of diet samples from the end of the diet also demonstrated that all diets were at targeted concentrations (± 17.8 % of nominal). No test substance was detected in any control diet samples.

The results of diet analysis from samples submitted at study start demonstrate that the test substance was stable in the diet under relevant storage conditions (up to 21 days at room temperature or refrigerated). A few of the stability samples were not within the targeted concentration range, including 0-day room temperature 180 ppm sample (65.0 % of nominal) and 14-day room temperature 18000 ppm sample (140.6 % of nominal). All other stability samples were within 20.6 % of nominal. Except for the 180 ppm diet, the mean concentrations in fresh diets collected at study start were within 15.8 % of nominal.

The low value (65 % of nominal) measured in the 0-day room-temperature 180 ppm diet sample did not represent instability of the test substance. This is supported by the fact that this low value was measured in the freshly collected sample stored frozen until analysed. In addition, other 180 ppm samples collected from the same diet mixing and analysed for stability assessment were within an acceptable range, and there was no reduction in concentration over time for samples stored at either room temperature or refrigerated for either concentration.

Since the stability analysis did not demonstrate any breakdown of test substance in the diet matrix, it can be assumed that the concentration measured in all samples was comparable to the concentration at the time of mixing. Therefore, the concentration and homogeneity of test substance in the samples of diets collected at study start, at the time of mixing, can be estimated based on the mean and CV of all of the stability samples. Based on these stability sample data, the mean concentration of test substance in the two diets was estimated to be at the targeted levels (± 9 %) for both diets.

Although the initial homogeneity, based on these stability sample data, was outside the Haskell targeted range (CV <10 %), this was driven mainly by one outlier value in each diet. Excluding the one high value for the 18000 ppm diet, the CV for this diet was within the Haskell targeted range. These data, combined with the homogeneity data from the mid-study samples, support that the diets were at the targeted range and fairly homogeneously mixed, especially at the higher concentrations. The homogeneity data in the 180 ppm diet may represent true lower homogeneity at this concentration. However, most of the samples at this concentration at study start fell within the acceptable targeted concentration range (± 20 % of nominal). Since this concentration was below the NOAEL, the analytical results are not critical in evaluating the results of exposure to the test substance.

Thus, the animals were considered to have received the targeted concentration of test substance in the diets over the course of the study.

B. MORTALITY

No test substance-related deaths occurred. One male in the 4500 ppm group was found dead on test day 42. This death was attributed to trauma and not to exposure to the test substance. All other rats survived to scheduled sacrifice.

C. CLINICAL OBSERVATIONS

No clinical observations were attributed to test substance exposure. All observed signs were those typically seen in rats of this age and in this type of study.

D. BODY WEIGHT

No adverse effects on body weight or body weight gain were observed in male rats. Final (test day 91) mean body weight in the 18000 ppm group was 92 % of control (not statistically significant) and mean body weight in this group was not significantly different from control on any test day. Statistically significantly lower overall (test day 0 – 91) body weight gain was observed in male rats in the 18000 ppm group (86 % of control). However, while mean weekly body weight gain was lower than control over most

weekly intervals, the difference was only statistically significant over one weekly interval (test days 35 – 42), and body weight gain in this group exceeded that of control over two subsequent weekly intervals (not statistically significant). Therefore, this lower body weight gain was considered test substance-related but not adverse, as it didn't result in significantly lower mean body weight, and was not associated with a significant reduction in food consumption or food efficiency. No test substance-related effects on body weight or body weight gain were observed in any other male group. A statistically significant difference (reduction) in mean body weight gain was observed in the male 180 ppm group over test days 35 – 42. However, this difference was not considered test substance-related as there was no dose response.
No effects on body weight or body weight gain were observed in any female group. Final body weight and overall body weight gain in the female 18000 ppm group were 99 % and 97 % of control, respectively. Neither difference was statistically significant. No statistically significant difference in mean body weight or body weight gain was observed in any female dose group on any test day.
Lower body weight gain and/or body weight loss was observed in all male and female dose groups over the test day 77 – 84 interval. This lower weight gain is attributed to the stress of blood and urine collection on test days 82 (males) or 83 (females).

Table 5.8.1-95: IN-MCX20: Subchronic Toxicity 90-Day Feeding Study in Rats ([redacted], 2007): Selected body weight gain values

	Dose group [ppm]									
	Males					Females				
	0	180	900	4500	18000	0	180	900	4500	18000
Mean body weight gain Day 35 – Day 42 [g]	32.9 ± 7.1	**↓17.7[§] ± 14.6**	↓25.4 ± 5.0	↓28.1 ± 5.4	**↓24.4[§] ± 4.0**	11.1 ± 10.0	↑11.8 ± 5.3	↑13.1 ± 9.7	↑12.4 ± 7.8	↓10.5 ± 6.4
Mean body weight gain Day 77 – Day 84 [g]	-0.9 ± 11.7	↑5.0 ± 6.2	↓-3.7 ± 7.0	↓-2.1 ± 4.1	↓-1.5 ± 4.9	-4.2 ± 4.6	↑0.4 ± 7.6	↑-0.6 ± 4.3	↑-4.0 ± 6.3	↑1.5 ± 4.9
Mean body weight gain Day 0 – Day 91 [g]	347.3 ± 51.9	↓327.5 ± 27.7	↓338.0 ± 46.1	↓324.1 ± 31.9	**↓298.6 * ± 36.8**	110.9 ± 18.7	↑127.4 ± 17.0	↑120.5 ± 28.4	↑127.3 ± 21.9	↓107.3 ± 14.4

*: Statistically significant from control ($p \leq 0.05$; Dunnett/Tamhane-Dunnett test); §: Statistically significant from control ($p \leq 0.05$; Dunn's test)

E. FOOD COMSUMPTION, FOOD EFFICIENCY AND DAILY INTAKE

No adverse effects on food consumption were observed in male or female rats. Overall food consumption in the 18000 ppm male and female groups was 93 % and 105 % of control, respectively. Neither difference was statistically significant. Statistically significantly lower mean food consumption, compared to control, was observed over one weekly interval each in all male dose groups except the 900 ppm group. Statistically significantly higher mean food consumption was observed in the 4500 ppm and 18000 ppm female groups over one or three weekly intervals. None of these differences was considered test substance related as overall mean food consumption was not significantly different from control.
No adverse effects on food efficiency were observed in male or female rats. Overall food efficiency in the 18000 ppm male and female groups was 92 % of control in both males and females. Neither difference was statistically significant. Mean weekly food efficiency was significantly lower than control in 18000 ppm males over test days 7 – 14 and in 180 ppm males over test days 35 – 42. These differences were not considered adverse as overall food efficiency was not significantly lower than control. No statistically significant differences in weekly food efficiency were observed in any female group during the study.
Mean daily intake of IN-MCX20 in males in the 0, 180, 900, 4500 and 18000 ppm groups was 0, 11.3, 55.7, 283 and 1157 mg/kg bw/day, respectively. Mean daily intake of IN-MCX20 in females in the 0, 180, 900, 4500 and 18000 ppm groups was 0, 13.9, 67.8, 360 and 1461 mg/kg bw/day, respectively.

Table 5.8.1-96: IN-MCX20: Subchronic Toxicity 90-Day Feeding Study in Rats ([redacted], 2007): Selected food consumption values

	Dose group [ppm]

	Males					Females				
	0	**180**	**900**	**4500**	**18000**	**0**	**180**	**900**	**4500**	**18000**
Mean food consumption Day 28 – Day 35 [g]	30.6 ± 2.6	↓29.0 ± 1.6	↓29.5 ± 3.2	**↓27.9* ± 1.6**	**↓27.7* ± 2.7**	19.4 ± 2.1	↑21.2 ± 2.3	↑19.9 ± 2.4	**↑22.3$ ± 2.9**	↑20.4 ± 2.0
Mean food consumption Day 35 – Day 42 [g]	30.8 ± 3.5	**↓27.4* ± 2.9**	↓28.3 ± 3.0	↓28.2 ± 2.0	↓27.9 ± 2.6	18.9 ± 2.1	↑19.9 ± 1.6	↑20.5 ± 3.5	**↑22.7* ± 2.5**	↑21.2 ± 2.4
Mean food consumption Day 42 – Day 49 [g]	30.9 ± 2.9	↓28.1 ± 1.8	↓29.3 ± 3.4	↓28.9 ± 1.8	↓28.9 ± 2.7	19.3 ± 2.0	↑20.4 ± 1.7	↑19.8 ± 3.2	**↑21.8$ ± 2.0**	↑20.0 ± 1.7

*: Statistically significant from control (p≤0.05; Dunnett/Tamhane-Dunnett test); $: Statistically significant from control (p≤0.05; Dunn's test)

Table 5.8.1-97: IN-MCX20: Subchronic Toxicity 90-Day Feeding Study in Rats ([redacted], 2007): Selected food efficiency values

	Dose group [ppm]									
	Males					Females				
	0	**180**	**900**	**4500**	**18000**	**0**	**180**	**900**	**4500**	**18000**
Mean food efficiency Day 7 – Day 14 [g]	0.254 ± 0.031	↓0.245 ± 0.018	↑0.258 ± 0.034	↓0.244 ± 0.012	**↓0.226 * ± 0.019**	0.130 ± 0.038	↓0.129 ± 0.042	0.130 ± 0.030	↑0.144 ± 0.036	↓0.111 ± 0.030
Mean food efficiency Day 35 – Day 42 [g]	0.152 ± 0.026	**↓0.086 $ ± 0.084**	↓0.128 ± 0.021	↓0.142 ± 0.024	↓0.124 ± 0.012	0.080 ± 0.084	↑0.085 ± 0.036	↑0.085 ± 0.049	↓0.078 ± 0.047	↓0.071 ± 0.042

*: Statistically significant from control (p≤0.05; Dunnett/Tamhane-Dunnett test); $: Statistically significant from control (p≤0.05; Dunn's test)

F. OPHTHALMOLOGICAL EXAMINATIONS

No ophthalmological observations were attributed to test substance exposure. All observed signs were those typically seen in rats of this age and in this type of study.

G. NEUROBEHAVIORAL EVALUATIONS

Abbreviated Functional Observational Battery

There were no test substance-related or statistically significant effects for fore- or hind-limb grip strength and for any behavioural parameter in either males or females administered dietary concentrations of 180, 900, 4500 or 18000 ppm of the test substance.

Motor Activity

There were no test substance-related or statistically significant effects on duration of movement or number of movements in either males or females administered dietary concentrations of 180, 900, 4500 or 18000 ppm of the test substance.

H. HAEMATOLOGY, CLINICAL CHEMISTRY AND URINALIYIS

Haematology

There were no adverse changes in haematological parameters in male or female rats. The following statistically significant changes in mean haematological parameters were not adverse or not related to exposure to the test substance.

Mean monocyte count was mildly decreased at test day 95 in males fed 18000 ppm (mean was 61 % of the control group). Individual monocyte counts were within the overall range observed historically in similarly aged control rats, but several males fed 18000 ppm had values that were below the 95 % historical reference interval. This change was of uncertain relationship to treatment. Regardless, decreases in monocyte counts have no clinical significance; therefore, this change was considered to be non-adverse.

Minimally increased mean neutrophil count at test day 95 in males fed 4500 ppm was considered to be unrelated to treatment and non-adverse, because it was not dose-related.

Table 5.8.1-98: IN-MCX20: Subchronic Toxicity 90-Day Feeding Study in Rats ([redacted], 2007): Selected haematological parameters

Parameter	Dose group [ppm]									
	Males					Females				
	0	180	900	4500	18000	0	180	900	4500	18000
Monocytes [×10³/µL]	0.28 ± 0.11	↓0.25 ± 0.06	↓0.27 ± 0.11	↓0.27 ± 0.11	**↓0.17* ± 0.07**	0.22 ± 0.10	↓0.20 ± 0.09	↓0.21 ± 0.06	0.22 ± 0.11	↓0.23 ± 0.13
Neutrophils [×10³/µL]	1.36 ± 0.41	↑1.72 ± 0.37	↑1.68 ± 0.40	**↑2.04* ± 0.88**	↑1.41 ± 0.51	1.42 ± 1.04	↓1.15 ± 0.58	↓1.10 ± 0.31	↓1.31 ± 0.54	↓1.15 ± 0.47

*: Statistically significant from control (p≤0.05; Dunnett/Tamhane-Dunnett test)

There were no treatment-related or statistically significant changes in coagulation parameters in male or female rats.

Clinical Chemistry

There were no adverse or treatment-related changes in clinical chemistry parameters in male or female rats. Decreased glucose at test day 95 in males fed 900 ppm was considered to be unrelated to treatment and non-adverse because it was not dose-related.

Table 5.8.1-99: IN-MCX20: Subchronic Toxicity 90-Day Feeding Study in Rats ([redacted], 2007): Selected clinical chemistry parameters

Parameter	Dose group [ppm]									
	Males					Females				
	0	180	900	4500	18000	0	180	900	4500	18000
Glucose [mg/dL]	116 ± 21	↓106 ± 16	**↓99[$] ± 7**	↓115 ± 19	↓106 ± 20	96 ± 9	↑100 ± 10	↑97 ± 13	↑105 ± 16	↓92 ± 5

[$]: Statistically significant from control (p≤0.05; Dunn's test)

Urinalysis

There were no statistically significant or treatment-related changes in urinalysis parameters in male or female rats.

Urinalysis – Metabolic analysis

Rat Urine Sample Results

The urinary concentrations of IN-MCX20 increased with the increasing dietary levels of this test substance. Concentrations of IN-B2856 and IN-EY252 were detected above the limit of detection at higher dietary levels (900 to 18000 ppm) but at or below the limit of detection in urine samples from the 180 ppm dietary group. Further, the concentrations of these metabolites were much higher in urine samples from male rats than from corresponding female rats at 4500 and 18000 ppm. Neither IN-MCX20 nor either metabolite was detected in urine from control rats.

Rat Plasma Sample Results

The concentrations of IN-MCX20 also increased with the increasing dietary levels of this test substance. Concentrations of IN-MCX20 were <1.0 µg/mL for males and females in the 180 ppm dietary group. The concentrations increased from ~2 µg/mL up to ~14 µg/mL for the other dietary groups. In contrast to urine samples, little to none of IN-B2856 or IN-EY252 was detected for all dietary levels. Neither IN-MCX20 nor either metabolite was detected in plasma from control rats. The concentrations of IN-B2856 reported for 4500 ppm females were greater than those reported for the 18000 ppm females, although all concentrations were very low (close to the limit of detection). The reason for this could be that samples from different groups were analysed on different days, and the fact that the instrument responses were greater on the day the 4500 ppm rat plasma samples were analysed resulting in better quantitation, which is very common for LC/MS instruments. The standard deviation (± 0.26) for the 4500 ppm female samples indicates these results were not statistically significantly different from the 18000 ppm female samples.

Collectively, these analytical results of urine and plasma samples from rats exposed to INMCX20 confirm

that this test substance is metabolised to small quantities of IN-B2856 and INEY252.

I. NECROPSY

There was no test substance-related mortality. A single male rat from the intermediate high-dose group (4500 ppm) was found dead on day 42. Important macroscopic findings were nose fracture and red discoloration of the stomach. The death was considered accidental and unrelated to test substance administration. All other rats survived until scheduled termination.

Organ weights

There were no treatment-related organ weight changes following the exposure period.

Gross pathology

At the terminal sacrifice, there were no treatment-related macroscopic findings.

Histopathology

Following the dosing phase, there were no treatment-related microscopic findings. Subacute/chronic inflammation, biliary hyperplasia and cholangio-fibrosis in the liver of a single intermediate high-dose male rat were considered spontaneous and incidental with no apparent relationship to the test substance exposure.

III. CONCLUSIONS

The no-observed-adverse-effect level (NOAEL) for male and female rats was 18000 ppm, equivalent to 1157 and 1461 mg/kg bw/day in males and females, respectively. The NOAEL is based on a lack of adverse, test substance-related effects in either sex at 18000 ppm, the highest concentration tested.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Statistically significantly lower overall mean body weight gain (86 % of control) was observed in 18000 ppm males but was not considered adverse as it was not associated with a statistically significant difference in mean final body weight or in overall mean food consumption or food efficiency.

Despite the deviations in clinical chemistry (LDL, HDL, T3, T4 and TSH not determined), organ weights (prostate + seminal vesicles and coagulating glands), thyroid and pituitary gland not determined) and histopathology (cervix, coagulating glands, gall bladder and male mammary glands not examined) this study was conducted under GLP and was considered to be valid.

Therefore, the no-observed-adverse-effect level (NOAEL) for male and female rats was 18000 ppm, equivalent to 1157 and 1461 mg/kg bw/day in males and females, respectively. The NOAEL is based on a lack of adverse, test substance-related effects in either sex at 18000 ppm, the highest concentration tested.

Further, the study provides additional information about metabolism of N-acetyl glyphosate. It was shown that N-acetyl glyphosate was marginally metabolised to glyphosate and N-acetyl AMPA, as only very small quantities (close to LOD) were detected in urine and plasma samples at day 82/83.

Assessment and conclusion by RMS:

Genotoxicity

CA 5.8.1/041

1. Information on the study

Data point	CA 5.8.1/041
Report author	[redacted]

Report year	2004
Report title	*Salmonella-Escherichia coli* / Mammalian Microsome Reverse Mutation Assay with a Confirmatory Assay with N-Acetyl-Glyphosate
Report No	7353-101
Document No	VER04-COV-03
Guidelines followed in study	OECD 471 (1997)
Deviations from current test guideline	None
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive summary

N-acetyl-glyphosate sodium salt (batch: 123K5012, purity: 84.3 % as sodium salt; 67.4 % as free acid) metabolite of glyphosate, was assessed for gene mutation in bacteria (Ames test). *Salmonella typhimurium* strains TA 98, TA 100, TA 1535 and TA 1537 and *Escherichia coli* strain WP2 uvrA were exposed to the test item, solvent (deionised water) and appropriate positive controls in the presence and absence of metabolic activation (Aroclor 1254-induced rat liver S9 fraction). Dose levels were selected based on the results of a preliminary toxicity test in strain TA98, in which no cytotoxicity was observed up to 5000 µg/plate.

In the main mutagenicity study, two independent standard plate tests (plate incorporation method) were performed. Test item concentrations were in the range of 100 – 5000 µg/plate. After an incubation period of 52 ± 4 hours at 37 ± 2 °C, the bacterial background lawn was examined and the number of his^+ or trp^+ revertant colonies were counted.

There was no precipitation and no cytotoxicity observed for any tester strain at any concentration, neither in the presence nor in the absence of S9 mix.

In the first experiment, no increase in the mean number of revertant colonies was observed for any tester strain at any concentration, neither with nor without metabolic activation. In the confirmatory experiment, there was a 3.3-fold increase in the number of revertant colonies for strain TA 1537 in the absence of S9 mix. The increase was not dose-related and did not meet the criteria for a positive evaluation, therefore the observation was considered to be incidental. In addition, the mean value of spontaneous revertants obtained for the vehicle control with strain TA 98 in the absence of S9 mix was not within the acceptable range specified in the protocol. Therefore, a third experiment was performed for strain TA 98 in the absence of S9 mix. In the repeat mutagenicity test, all data were acceptable and there was no positive increase in the mean number of revertants per plate. In addition, the number of spontaneous revertants induced by the solvent control was as expected for all other strains in both experiments.

The number of revertant colonies induced by the positive controls were within the range of the laboratories historical control data, demonstrating the functionality of the metabolic activation system and the sensitivity of the test.

Based on the experimental findings, N-acetyl-glyphosate sodium salt, metabolite of glyphosate, did not induce gene mutations in bacteria and is therefore considered negative in the Ames standard plate test in the presence and absence of metabolic activation.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: N-acetyl-glyphosate, sodium salt

Identification: Not specified

Description: White powder

Lot/Batch number: 123K5012

Purity: 84.3 % (N-acetyl-glyphosate sodium salt) and 67.4 % (N-acetyl-glyphosate as free acid)

Stability of test compound: The stability of the test item at storage conditions (at room temperature with desiccant) and the stability of the test item in solvent was not specified.

Solvent (vehicle) used: Deionised water

2. Control materials:

Negative control: A negative control was not employed in this study.

Solvent (vehicle) control: Deionised water

Solvent (vehicle)/final concentration: 0.05 mL per plate

Positive controls: Please refer to table below.

Strain	Metabolic activation	Mutagen	Conc. [µg/plate]
S. typhimurium strains			
TA 98	-S9	2-Nitrofluorene	1.0
	+S9	Benzo[a]pyrene	2.5
TA 100	-S9	Sodium azide	2.0
	+S9	2-Aminoanthracene	2.5
TA 1535	-S9	Sodium azide	2.0
	+S9	2-Aminoanthracene	2.5
TA 1537	-S9	ICR-191	2.0
	+S9	2-Aminoanthracene	2.5
E. coli strains			
WP2 uvrA	-S9	4-Nitroquinoline-N-oxide	1.0
	+S9	2-Aminoanthracene	25.0

3. Metabolic activation:

S9 mix was purchased from [redacted]. (Lot no. 1615 and 1626). The homogenate was prepared from the livers of male Sprague-Dawley rats that had been induced with a single intraperitoneal injection of Aroclor 1254 at a dose of 500 mg/kg bw. The S9 mix was thawed prior to each experiment and co-factors were immediately added to prepare S9 mix containing the following components:

S9 mix component	Concentration	Unit
Phosphate buffer (pH 7.4)	100	mM
KCl	33	mM
NADPH-generating system		
Glucose 6-phosphate	5	mM
NADP	4	mM
$MgCl_2$	8	mM
S9	10	% (v/v)

4. Test organisms:

Tester strains				Bacteria batch checked for	
S. typhimurium		*E.coli*			
TA 98	✓	WP2 uvrA	✓	deep rough character (rfa)	✓
TA 100	✓	WP2 uvrA (pKM101)		ampicillin resistance (R factor plasmid)	✓
TA 1535	✓			UV-light sensitivity (absence of uvrB and uvrA genes in *S. typhimurium* and *E. coli* strains, respectively)	✓
TA 1537	✓				
TA 1538				Histidine auxotrophy (automatically via the spontaneous rate)	✓

5. Test concentrations:

(e) Preliminary cytotoxicity assay:

Plate incorporation test ± S9 mix:	
Concentrations:	*6.67 - 5000 µg/plate**
Tester strains:	TA100 and WP2 uvrA
Replicates:	*One plate was used for each condition.*

*: Dosing solutions were adjusted to compensate the purity of the test substance by a correction factor of 67.4 %.

(f) Mutation assays:

Plate incorporation test ± S9 mix:	
Concentrations:	*100, 333, 1000, 3000 and 5000 µg/plate**
Tester strains:	TA 98, TA 100, TA 1535, TA 1537 and WP2 uvrA
Replicates:	*Triplicates*

*: Dosing solutions were adjusted to compensate the purity of the test substance by a correction factor of 67.4 %.

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 15 Mar – 04 May 2004
Finalisation date: 23 Dec 2004

2. Standard plate test (plate-incorporation test, SPT):
In tests with metabolic activation, an aliquot of 50 µL test solution, vehicle (deionized water) or positive control, an aliquot of 0.1 mL fresh bacterial culture and 0.5 mL S9 mix were added to 2 mL of molten top agar (supplemented with 0.5 mM histidine + 0.5 mM biotin or 0.5 mM tryptophan). In tests without metabolic action, 50 µL test solution, vehicle (deionized water) or positive control and 0.1 mL fresh bacterial culture were added to 2.5 mL of molten top agar. Afterwards, the mixture was vortexed and overlaid onto the surface of minimal bottom agar plates. After solidification, the plates were inverted and incubated for 52 ± 4 hours at 37 ± 2 °C. Each concentration and the controls were tested in triplicates. Following incubation, the bacterial background lawn was examined and the number of his^+ or trp^+ revertants colonies were counted.

3. Cytotoxicity
Toxicity was detected by a

- reduction in the number of spontaneous revertants
- clearing or diminution of the background lawn (= reduced his^- or trp^- background growth)

and recorded for all test groups both with and without S9 mix in all experiments.

4. Statistics

For all replicate platings, the mean revertants per plate and the standard deviations were calculated. No further statistical analysis was performed.

5. Acceptance criteria

The assay was considered valid if the following criteria were met:

- The tester strain cultures exhibited a characteristic number of spontaneous revertants per plate when treated with the solvent control. The acceptable ranges for the mean vehicle controls were 8 – 60 for TA 98, 60 – 240 for TA 100, 4 – 45 for TA 1535, 2 – 25 for TA 1537 and 5 – 40 for WP2 uvrA.
- The density of the tester strain cultures was ≥ 0.5 x 10^9 bacteria per mL and/ or had reached a target level of turbidity demonstrated to produce cultures with a density ≥ 0.5 x 10^9 bacteria per mL.
- The mean number of revertants induced by the positive controls were increased at least 3-fold over the mean value of the vehicle control for that strain.
- A minimum of three non-toxic concentrations was required to evaluate the assay data.

6. Evaluation criteria

A test item was considered positive (mutagenic) in the assay if the following criteria were met:

- There was an at least 2-fold increase in the mean number of revertant colonies per plate in at least one tester strain when compared to the appropriate vehicle control for tester strains TA 98, TA 100 and WP2 uvrA.
- There was an at least 3-fold increase in the mean number of revertant colonies per plate in at least one tester strain when compared to the appropriate vehicle control for tester strains TA 1535 and TA 1537.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Analytical determinations were not performed in the study.

B. CYTOTOXICITY

In the preliminary toxicity test, as well as in the main mutagenicity study, no cytotoxicity was observed with any tester strain up to the highest concentration of 5000 µg/plate, neither with nor without metabolic activation.

C. SOLUBILITY

Precipitation was not observed in any tester strain, at any concentration, neither with nor without S9 mix.

D. MUTATION ASSAY

In the first experiment, there was no increase in the number of his^+ or trp^+ revertant colonies observed for any strain at any concentration, neither in the presence nor in the absence of S9 mix. In the confirmatory experiment, there was a 3.3-fold increase in the number of revertant colonies for strain TA 1537 in the absence of S9 mix. The increase was not dose-related and did not meet the criteria for a positive evaluation, therefore the observation was considered to be incidental. In addition, the mean value of spontaneous revertants obtained for the vehicle control with strain TA 98 in the absence of S9 mix was not within the acceptable range specified in the protocol. Therefore, a third experiment was performed for strain TA 98 in the absence of S9 mix. In the repeat mutagenicity test, all data were acceptable and there was no positive increase in the mean number of revertants per plate. In addition, the number of spontaneous revertants induced by the solvent control was as expected for all other strains in both experiments.

The number of revertant colonies induced by the positive controls were within the range of the laboratories historical control data, demonstrating the functionality of the metabolic activation system and the sensitivity of the test.

Table 5.8.1-100: N-acetyl-glyphosate metabolite of glyphosate - mutagenicity results (Ames test) with and without metabolic activation ([redacted], 2004), first experiment

Experiment 1: Standard plate test (SPT)										
Strain	**TA 98**		**TA 100**		**TA 1535**		**TA 1537**		**WP2 uvrA**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Vehicle control										
Water mean	13	27	86	108	11	8	3	9	19	18
± *SD*	*± 3*	*± 5*	*± 13*	*± 10*	*± 7*	*± 3*	*± 2*	*± 2*	*± 2*	*± 2*
HCD[#] mean	15.9	26.4	85.4	91.9	15.8	12.9	7.7	9.5	16.4	17.2
± *SD*	*± 5.2*	*± 6.6*	*± 16.9*	*± 15.9*	*± 6.3*	*± 4.5*	*± 3.9*	*± 3.3*	*± 5.8*	*± 5.8*
[range]	*5 - 33*	*7 - 44*	*46 - 156*	*52 - 149*	*2 - 45*	*3 - 28*	*1 - 28*	*2 - 19*	*6 - 37*	*3 - 37*
Test item [µg/plate]										
100 mean	14	25	87	112	10	12	5	9	19	19
± *SD*	*± 3*	*± 10*	*± 6*	*± 6*	*± 4*	*± 2*	*± 2*	*± 1*	*± 4*	*± 2*
333 mean	10	22	94	108	10	12	4	11	18	19
± *SD*	*± 3*	*± 2*	*± 5*	*± 17*	*± 2*	*± 2*	*± 1*	*± 3*	*± 6*	*± 4*
1000 mean	12	26	88	115	8	9	6	9	17	17
± *SD*	*± 4*	*± 1*	*± 25*	*± 4*	*± 8*	*± 4*	*± 2*	*± 2*	*± 1*	*± 5*
3330 mean	15	30	94	107	10	15	7	7	17	18
± *SD*	*± 10*	*± 10*	*± 6*	*± 11*	*± 4*	*± 4*	*± 1*	*± 1*	*± 11*	*± 4*
5000 mean	16	24	98	114	9	14	4	5	15	17
± *SD*	*± 7*	*± 10*	*± 18*	*± 13*	*± 2*	*± 3*	*± 3*	*± 3*	*± 5*	*± 4*
Positive control										
[§] mean	290	249	1104	1335	985	249	581	121	161	406
± *SD*	*± 19*	*± 9*	*± 7*	*± 98*	*± 92*	*± 60*	*± 52*	*± 21*	*± 18*	*± 25*
HCD[#] mean	238.7	400.4	1054.9	706.4	749.8	143.4	835.6	118.6	242.5	595.0
± *SD*	*± 81.9*	*± 95.3*	*± 191.6*	*± 312.3*	*± 148.5*	*± 75.1*	*± 266.9*	*± 80.2*	*± 113.6*	*± 214.8*
[range]	*53 - 691*	*202 - 688*	*390 - 1515*	*111 - 2970*	*107 - 1291*	*68 - 691*	*97 - 1485*	*52 - 727*	*55 - 839*	*80 - 1098*

[§] = information on respective positive control is reported in Material and Method section I.A.2

[#] = Historical control data generated in the laboratory (time frame not specified)

Table 5.8.1-101: N-acetyl-glyphosate metabolite of glyphosate - mutagenicity results (Ames test) with and without metabolic activation ([redacted] 2004), second experiment

Experiment 2: Standard plate test (SPT)										
Strain	**TA 98**		**TA 100**		**TA 1535**		**TA 1537**		**WP2 uvrA**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
Vehicle control										
Water	5	19	69	91	13	10	3	11	25	21

Experiment 2: Standard plate test (SPT)										
Strain	**TA 98**		**TA 100**		**TA 1535**		**TA 1537**		**WP2 uvrA**	
Metabolic activation	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**	**- S9**	**+ S9**
mean										
± *SD*	± *2*	± *2*	± *2*	± *9*	± *1*	± *0*	± *2*	± *5*	± *5*	± *5*
HCD[#] mean	15.9	26.4	85.4	91.9	15.8	12.9	7.7	9.5	16.4	17.2
± *SD*	± *5.2*	± *6.6*	± *16.9*	± *15.9*	± *6.3*	± *4.5*	± *3.9*	± *3.3*	± *5.8*	± *5.8*
[range]	*5 - 33*	*7 - 44*	*46 - 156*	*52 - 149*	*2 - 45*	*3 - 28*	*1 - 28*	*2 - 19*	*6 - 37*	*3 - 37*
Test item										
100 mean	11	24	82	103	8	16	6	8	17	19
± *SD*	± *2*	± *3*	± *7*	± *0*	± *5*	± *5*	± *2*	± *2*	± *3*	± *4*
333 mean	11	23	76	114	12	16	10	11	18	24
± *SD*	± *4*	± *5*	± *3*	± *13*	± *2*	± *3*	± *3*	± *1*	± *5*	± *2*
1000 mean	14	19	85	102	10	8	8	10	18	18
± *SD*	± *1*	± *1*	± *8*	± *20*	± *4*	± *1*	± *1*	± *3*	± *5*	± *5*
3330 mean	14	25	78	99	12	16	4	10	19	24
± *SD*	± *4*	± *1*	± *6*	± *7*	± *5*	± *2*	± *2*	± *2*	± *3*	± *13*
5000 mean	12	19	76	109	16	16	6	11	19	24
± *SD*	± *3*	± *1*	± *1*	± *15*	± *7*	± *1*	± *3*	± *3*	± *2*	± *8*
Positive control										
[§] mean	343	113	1472	369	957	142	862	110	213	376
± *SD*	± *60*	± *16*	± *108*	± *41*	± *24*	± *16*	± *78*	± *15*	± *31*	± *25*
HCD[#] mean	238.7	400.4	1054.9	706.4	749.8	143.4	835.6	118.6	242.5	595.0
± *SD*	± *81.9*	± *95.3*	± *191.6*	± *312.3*	± *148.5*	± *75.1*	± *266.9*	± *80.2*	± *113.6*	± *214.8*
[range]	*53 - 691*	*202 - 688*	*390 - 1515*	*113 - 2970*	*107 - 1291*	*68 - 691*	*97 - 1485*	*52 - 727*	*55 - 839*	*80 - 1098*

[§] = information on respective positive control is reported in Material and Method section I.A.2

[#] = Historical control data generated in the laboratory (time frame not specified)

Table 5.8.1-102: N-acetyl-glyphosate metabolite of glyphosate - mutagenicity results (Ames test) with and without metabolic activation ([redacted], 2004), third experiment

Experiment 3: Standard plate test (SPT)	
Strain	**TA 98**
Metabolic activation	**- S9**
Vehicle control	
Water mean	9
± *SD*	± *5*
HCD[#] mean	15.9
± *SD*	± *5.2*
[range]	*5 - 33*
Test item	
100 mean	11

Experiment 3: Standard plate test (SPT)	
Strain	**TA 98**
Metabolic activation	**- S9**
± *SD*	± *2*
333 mean	11
± *SD*	± *7*
1000 mean	14
± *SD*	± *3*
3330 mean	12
± *SD*	± *3*
5000 mean	13
± *SD*	± *4*
Positive control	
§ mean	416
± *SD*	± *17*
HCD# mean	238.7
± *SD*	± *81.9*
[range]	*53 - 691*

§ = information on respective positive control is reported in Material and Method section I.A.2

= Historical control data generated in the laboratory (time frame not specified)

III. CONCLUSIONS

Under the conditions of the present study, N-acetyl glyphosate sodium salt, metabolite of glyphosate, was negative for gene mutation in the Ames standard plate test in the presence and absence of metabolic activation.

3. Assessment and conclusion

Assessment and conclusion by applicant:

In the present study, N-acetyl glyphosate sodium salt was negative for gene mutation in bacteria (*S. typhimurium* TA 98, TA 100, TA 1535, TA 1537 and *E. coli* WP2 uvrA) with and without metabolic activation.
The study was conducted in compliance with GLP and according to OECD guideline 471 (1997), The study is therefore considered valid.

Assessment and conclusion by RMS:

CA 5.8.1/042

1. Information on the study

Data point	CA 5.8.1/042
Report author	[redacted]
Report year	2004
Report title	Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells

Report No	7535-102
Document No	VER04-COV-02
Guidelines followed in study	OECD 473 (1997)
Deviations from current test guideline	Only 200 cells in metaphase were evaluated, whereas the currently valid OECD 473 (2016) recommends the evaluation of 300 metaphase cells per condition. In addition, cytotoxicity was evaluated based on mitotic indices, which is not recommended for cell lines. Evaluation criteria specified in the study report were inconsistent with those specified in the guideline.
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive summary

N-acetyl-glyphosate, sodium salt (batch: 123K5012, purity: 84.3 % as sodium salt; 67.4 % as free acid), metabolite of glyphosate, was investigated in a chromosome aberration test in Chinese hamster ovary (CHO) cells. Duplicate cultures were exposed to the test item, negative (medium), solvent (water) and positive controls (mitomycin C in the absence of S9 mix and cyclophosphamide in the presence of S9 mix) in the presence and absence of metabolic activation (Aroclor 1254-induced rat liver S9 fraction). Test item concentrations in the range of 10 – 2800 µg/mL were screened for cytotoxicity by visual inspection, prior to selection of concentration levels used for chromosome analysis in the range of 960 – 2800 µg/mL.

The cells were treated for 3 hours in the presence and absence of metabolic activation and for 20 hours in the absence of metabolic activation at 37 ± 2 °C. In the 3-hour treatment schedule, the cells were washed following exposure and re-incubated afterwards. All cultures were harvested 20 hours after start of exposure and chromosome preparations were made. A total of 200 metaphases per condition (100 metaphases per culture) were scored for structural chromosome aberrations. In addition, the percentage of polyploid and endoreduplicate cells was scored among 100 metaphases. Cytotoxicity was assessed as mitotic index and evaluated for 1000 cells per culture.

Precipitation of the test item in solvent or medium was not observed for any concentration, neither in the presence nor absence of S9 mix and independent of the treatment period. Cytotoxicity, evident as a slight reduction of the mitotic index, was observed after 3 hours of exposure at 1960 and 2800 µg/mL in the absence of S9 mix.

There was no statistically significant increase in the number of cells with chromosomal aberrations observed in any experiment at any concentration when compared to vehicle controls, neither in the presence nor in the absence of S9 mix. In addition, there was no increase in the frequency of cells in polyploidy and endoreduplication for any condition.

The frequency of aberrant metaphases in the negative, solvent and positive control cultures were within the range of the laboratory's historical control data, demonstrating the sensitivity of the test and the functionality of the S9 mix.

Based on the experimental results obtained in the present study, N-acetyl-glyphosate, sodium salt, metabolite of glyphosate, did not induce chromosomal aberrations in CHO cells *in vitro*, neither in the presence, nor in the absence of metabolic activation.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test N-acetyl-glyphosate sodium salt

material:

Identification:	Not specified
Description:	White powder
Lot/Batch #:	123K5012
Purity:	84.3 % (N-acetyl-glyphosate, sodium salt) and 67.4 % (N-acetyl-glyphosate as free acid)
Stability of test compound:	The stability of the test item under storage conditions (at room temperature with desiccant) was guaranteed until the expiry date 10 Feb 2006. The stability of the test item in solvent was at the responsibility of the sponsor.
Solvent (vehicle) used:	Water

2. Control Materials:

Negative control:	Culture medium
Solvent (vehicle) control:	Cell culture grade water (CCGW)
Positive control:	-S9 mix: Mitomycin C (MMC): 0.75 and 1.5 µg/mL for the 3-h treatment, 0.2 and 0.4 µg/mL for the 20-h treatment, respectively +S9 mix: Cyclophosphamide (CP): 7.5* and 12.5 µg/mL (* analysed for chromosomal aberrations)

3. Metabolic activation:

The metabolic activation system consisted of a rat liver post-mitochondrial fraction (S9 mix) and an energy producing system (NADP plus isocitric acid).
S9 mix was routinely prepared from the livers of rats, which received a single intraperitoneal injection of 500 mg/kg bw Aroclor 1254. Five days after treatment, the animals were sacrificed and the S9 homogenates were isolated. The metabolic activation system was prepared immediately before the experiment as follows:

S9 mix component	Concentration	Unit
Isocitric acid	10.5	mM
NADP, sodium salt	1.8	mM
S9	1.5	% (v/v)

4. Test organism:

Chinese hamster ovary (CHO) cells were used, established from an ovarian biopsy of a female Chinese hamster. The cell line was obtained from the laboratory of [redacted] and sub-cloned in the testing laboratory. Stock cultures were stored in liquid nitrogen. The cell line has an average cell cycle time of 12 – 14 hours and a modal chromosome number of 21. Stock cultures were maintained for up to 8 weeks after thawing. Twice during this period, the cells were screened for mycoplasma contamination.

5. Cell culture:

Medium: McCoy's 5a culture medium supplemented with 10 % heat-inactivated fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin G and 100 µg/mL streptomycin.

Incubation: *Cultures were incubated with loose caps in a humidified incubator at 37 ± 2 °C in an atmosphere of 5 ± 1.5 % CO_2 in the air.*

Cell culture establishment prior to exposure: *Cultures were initiated by seeding approx. 0.9 x 10^6 cells per 75 cm^2 flask 24 hours prior to treatment.*

6. Test concentrations and number of replicates:

Metabolic activation	Duration of exposure (Fixation time)	Concentrations§	Replicates
-S9 mix	3 h (20 h)	10.0, 19.0, 27.1, 38.8, 55.4, 79.1, 113, 161, 231, 329, 471, 672, 960*, 1370*, 1960* and 2800*# µg/mL	Duplicate
-S9 mix	20 h (20 h)	10.0, 19.0, 27.1, 38.8, 55.4, 79.1, 113, 161, 231, 329, 471, 672, 960*, 1370*, 1960* and 2800*# µg/mL	Duplicate
+S9 mix	3 h (20 h)	10.0, 19.0, 27.1, 38.8, 55.4, 79.1, 113, 161, 231, 329, 471, 672, 960*, 1370*, 1960* and 2800*# µg/mL	Duplicate

* Samples analysed for chromosomal aberrations; #: corresponding to > 10 mM of the free acid
§: Dosing solutions were adjusted to compensate the purity of the test substance by a correction factor of 67.4 %.

B. STUDY DESIGN AND METHODS

2. Dates of experimental work: 16 Mar – 22 Apr 2004
Finalisation date: 10 Sep 2004

3. Main cytogenicity test:

Treatment: Following cell culture establishment, duplicate cultures per condition were exposed to the test item, negative (culture medium), solvent (water) or positive control (mitomycin C in the absence of S9 mix and cyclophosphamide in the presence of S9 mix) in the presence and absence of metabolic activation.
The cells were treated for 3 hours with and without S9 mix and for 20 hours without S9 mix. After the 3-hour treatment period in the presence and absence of S9 mix, the cultures were washed with buffered saline, re-fed with culture medium and incubated for the remaining 17 hours until harvest. For the 20-hour treatment period in the absence of S9 mix, cells were harvested immediately following treatment.

Spindle inhibition: During the last 2 ± 0.5 hours of cultivation, cell division was arrested by addition of 0.1 µg/mL Colcemid to each culture.

Cell harvest: The cell cultures were trypsinised to collect mitotic and interphase cells. The

cells were swollen in 75 mM potassium chloride and fixed in methanol : glacial acetic acid (3 : 1 v/v) fixative.

Slide preparation: Fixed cells were dropped on glass slides and air-dried. The slides were stained with 5 % Giemsa solution, then air-dried and mounted permanently.

Metaphase analysis: A total number of 200 metaphase cells per condition (100 metaphase cells per culture) were examined by microscopy. Cells were selected for good morphology and only cells with the number of centromers equal to the model number 21 ± 2 (range 19 – 23) were evaluated. At least 25 cells were analysed from those cultures that had greater than 25 % of cells with one or more aberrations. The cells were analysed for different type of chromosome and chromatide aberrations, including gaps, breaks and complex exchanges. The percentage of polyploid and endoreduplicate cells was scored among 100 metaphases.

Cytotoxicity: Prior to harvest of the cultures, visual observations of cytotoxicity were made, including the assessment of confluency and the presence of mitotic (large, rounded cells) and dead, floating cells.
At scoring, the mitotic index of each culture was calculated based on the number of cells in metaphase observed per 1000 cells scored.

4. Statistics

The number and percentage of aberrant cells including and excluding gaps was analysed using a Cochran-Armitage test for linear trend and a Fisher's Exact test. The percentage of cells with aberrations in treated cells was compared to the results obtained for the vehicle control.
Statistical analysis was also performed for cells exhibiting polyploidy and/or endoreduplication in order to indicate significant events as indicators of possible induction of numerical aberrations; however, the test articles were evaluated only for structural aberrations and not for numerical aberrations in the protocol.

5. Acceptance criteria

The chromosome aberration test was considered acceptable if the following criteria were met:

- The vehicle control cultures contained less than approximately 5 % cells with aberrations.
- The positive control result was significantly higher than the vehicle control ($p \leq 0.01$).
- If the aberration results were negative and there was no significant reduction (≥ 50 %) in the mitotic index, the highest applicable dose (10 mM or 5 mg/mL) or a dose exceeding the solubility limit in culture medium must have been included.
- At least 3 dose levels for analysis of chromosome aberrations were included.

6. Evaluation criteria

A test substance was considered positive (clastogenic) in the chromosome aberration test if the following criteria were met:

- There was a significant increase in the number of cells with chromosomal aberrations observed at one or more concentrations.
- The linear trend evaluated the dose responsiveness. A dose-response was observed if a significant increase was seen at one or more concentrations.

A test substance was considered negative (not clastogenic) in the chromosome aberration test if no significant increase in the number of cells with chromosomal aberrations was observed at any

concentration.

II. RESULTS AND DISCUSSION

A. ANALYTCAL DETERMINATIONS

Analytical determinations of the test substance in the solvent were not perfomed.

B. CYTOTOXICITY

Visual inspection of the cultures prior to harvest revealed no indication of cytotoxic effects. Cytotoxicity, evident as a slight reduction of the mitotic index, was observed after 3 hours of exposure for the two highest concentrations in the absence of S9 mix. The mitotic indices were reduced by 20 and 15 % at 1960 and 2800 µg/mL, respectively. There was no cytotoxicity at any concentration after exposure for 3 hours in the presence of S9 mix or after 20 hours of exposure in the absence of S9 mix.

C. SOLUBILITY

Precipitation of the test item in vehicle or culture medium was not observed for any condition, up to the highest tested concentration, neither in the presence, nor in the absence of metabolic activation.

D. CYTOGENICITY

There was no statistically significant increase in the number of cells with chromosomal aberrations observed in any experiment at any concentration when compared to vehicle controls, neither in the presence nor in the absence of S9 mix. In addition, there was no increase in the frequency of cells in polyploidy and endoreduplication for any condition.

The frequency of aberrant metaphases in the negative, solvent and positive control cultures were within the range of the laboratory's historical control data, demonstrating the sensitivity of the test and the functionality of the S9 mix.

Table 5.8.1-103: Results of the cytogenicity test obtained with N-acetyl-glyphosate, sodium salt, metabolite of glyphosate, after 3 hours of exposure in the presence and absence of S9 mix and after 20 hours of exposure in the absence of S9 mix ([redacted] 2004)

Compound	Concentration [µg/mL]	Genotoxicity							Judge	Average % mitotic index	% Mitotic reduction
		No. of metaphases scored	no. structural aberrant cells[x]		% structural aberrant cells		% numerical aberrant cells				
			incl. gaps	excl. gaps	incl. gaps	excl. gaps	polyploid	endo-reduplicated			
Without metabolic activation; 3-hours treatment and 20-hours sampling											
Negative control											
Medium	0	200	0.0	**0.0**	0.0	**0.0**	**2.5**	**1.0**	negative	8.8	-
HCD[#] mean ± SD					1.4 ± 1.05	0.4 ± 0.56	1.3 ±1.44	0.0 ± 0.0			
range					0.5 - 3.5	0.0 - 1.5	0.0 - 4.0	0.0 ± 0.0			
Solvent											
CCGW	0	200	2.0	**0.0**	1.0	**0.0**	**1.5**	**0.5**	negative	11.00	0
HCD[#]					2.0 ±	0.7 ±	1.7 ±	0.3 ± 0.36			

Compound	Concentration [µg/mL]	No. of metaphases scored	Genotoxicity						Judge	Average % mitotic index	% Mitotic reduction
			no. structural aberrant cells[x]		% structural aberrant cells		% numerical aberrant cells				
			incl. gaps	excl. gaps	incl. gaps	excl. gaps	polyploid	endo-reduplicated			
mean ± SD					1.39	0.87	1.33				
range					0.0 - 4.0	0.0 - 2.5	0.0 - 3.0	0.0 - 1.0			
Test item											
	960	200	7.0	**1.0**	3.5	**0.5**	**2.5**	**0.5**	negative	nd	nd
	1370	200	4.0	**0.0**	2.0	**0.0**	**2.0**	**0.5**	negative	nd	nd
	1960	200	4.0	**0.0**	2.0	**0.0**	**3.0**	**0.5**	negative	8.8	20
	2800	200	2.0	**0.0**	1.0	**0.0**	**2.5**	**0.0**	negative	9.4	15
Positive control											
MMC	0.75	100	89	**87**	89	**87****	**2.5**	**0.5**	positive	nd	nd
HCD[#] mean ± SD					74.7 ± 10.20	72.2 ± 11.1 9	2.3 ± 1.91	0.0 ± 0.00			
range					55.0 - 88.0	52.0 - 88.0	0.0 - 5.0	0.0 - 0.0			
Without metabolic activation; 20-hours treatment and sampling											
Negative control											
Medium	0	200	2.0	**1.0**	1.0	**0.5**	**2.5**	**0.0**	negative	15.9	-
HCD[#] mean ± SD					2.5 ± 1.55	0.9 ± 1.17	2.7 ± 2.31	0.3 ± 0.90			
range					0.0 - 6.0	0.0 - 4.0	0.0 - 7.5	0.0 - 3.5			
Solvent											
CCGW	0	200	7.0	**0.0**	3.5	**0.0**	**3.0**	**0.0**	negative	16.4	0
HCD[#] mean ± SD					3.7 ± 2.66	1.30 ± 1.50	1.8 ± 1.47	0.3 ± 0.92			
range					0.0 - 9.5	0.0 - 5.0	0.0 - 5.0	0.0 - 4.0			
Test item											
	960	200	3.0	**0.0**	1.5	**0.0**	**2.0**	**0.0**	negative	nd	nd
	1370	200	1.0	**1.0**	0.5	**0.5**	**2.5**	**0.0**	negative	nd	nd
	1960	200	0.0	**0.0**	0.0	**0.0**	**3.0**	**0.0**	negative	nd	nd

Compound	Concentration [µg/mL]	No. of metaphases scored	Genotoxicity						Judge	Average % mitotic index	% Mitotic reduction
			no. structural aberrant cells[x]		% structural aberrant cells		% numerical aberrant cells				
			incl. gaps	**excl. gaps**	incl. gaps	**excl. gaps**	**polyploid**	**endo-reduplicated**			
	2800	200	2.0	**0.0**	1.0	**0.0**	**3.0**	**0.0**	negative	17.2	0
Positive control											
MMC	0.20	100	92	**89**	92	**89****	**3**	**0.5**	positive	nd	nd
HCD[#] mean ± SD					61.2 ± 16.20	58.4 ± 16.22	2.0 ± 1.76	0.2 ± 0.36			
range					36.0 - 93.0	36.0 - 90.0	0.0 - 5.0	0.0 - 1.0			
With metabolic activation; 3-hours treatment, 20-hours sampling time											
Negative control											
Medium	0	200	5.0	**0.0**	2.5	**0.0**	**4.0**	**4.5**	negative	11.9	-
HCD[#] mean ± SD					2.5 ± 1.43	0.9 ± 0.88	2.2 ± 1.93	0.6 ± 0.74			
range					0.5 - 6.5	0.0 - 3.0	0.0 - 8.5	0.0 - 2.0			
Solvent control											
CCGW	0	200	2.0	**0.0**	1.0	**0.0**	**3.0**	**1.5**	negative	13.3	0
HCD[#] mean ± SD					2.2 ± 1.37	0.7 ± 0.85	1.9 ± 1.58	0.4 ± 0.51			
range					0.0 - 5.0	0.0 - 3.0	0.0 - 5.0	0.0 - 2.0			
Test item											
	960	200	2.0	**0.0**	1.0	**0.0**	**3.5**	**2.5**	negative	nd	nd
	1370	200	3.0	**1.0**	1.5	**0.5**	**3.0**	**2.0**	negative	nd	nd
	1960	200	7.0	**2.0**	3.5	**1.0**	**3.5**	**2.5**	negative	nd	nd
	2800	200	4.0	**2.0**	2.0	**1.0**	**3.5**	**3.5**	negative	14.1	0
Positive control											
CP	7.50	100	62	**53**	62	**53****	**3.5**	**2.5**	positive	nd	nd
HCD[#] mean ± SD					57.5 ± 12.33	53.4 ± 12.93	3.3 ± 2.71	0.7 ± 1.05			
range					37.0 - 79.0	29.6 - 78.0	0.0 - 10.0	0.0 - 4.0			

Compound	Concentration [µg/mL]	No. of metaphases scored	Genotoxicity						Judge	Average % mitotic index	% Mitotic reduction
			no. structural aberrant cells[x]		% structural aberrant cells		% numerical aberrant cells				
			incl. gaps	excl. gaps	incl. gaps	excl. gaps	polyploid	endo-reduplicated			

HCD[#]: Historical control data from the laboratory's historical control range obtained in Jul - Dec 2003

MMC: Mytomycin C, positive control without S9 mix; CP:cyclophosphamide, positive control with S9 mix

CCGW: Cell culture grade water, solvent control

[**]Statistically significantly increased (- gaps) when compared to the vehicle control, $p \leq 0.01$

nd: Not determined

[x]: Total number of chromosome aberrations from 200 metaphases scored

III. CONCLUSIONS

Under the conditions of the test, N-acetyl-glyphosate, sodium salt, metabolite of glyphosate, has no clastogenic potential in Chinese hamster ovary (CHO) cells, neither in the presence nor in the absence of metabolic activation.

3. Assessment and conclusion

Assessment and conclusion by applicant:

In the present study, N-acetyl-glyphosate, sodium salt was negative for induction of chromosomal aberrations in Chinese hamster ovary (CHO) cells with and without metabolic activation.
The test was performed under GLP conditions and in accordance with OECD guideline 473 (1997). There were only minor deviations when compared to OECD 473 (2016). The number of metaphases investigated was only 200, which was the number to be investigated recommended by the previous OCED 473 (1997). The study is therefore considered valid.

Assessment and conclusion by RMS:

CA 5.8.1/043

1. Information on the study

Data point	CA 5.8.1/043
Report author	[redacted]
Report year	2006
Report title	In Vitro Mammalian Cell Gene Mutation Test (CHO/HGPRT)
Report No	DuPont-20155
Document No	-
Guidelines followed in study	OECD 476 (1997), US EPA OPPTS 870.5300, EC Commission Directive 2000/32/EC Annex 4E-B17 (2000), JMAFF (1985)
Deviations from current test guideline	The newly introduced cytotoxicity parameter RS (relative survival) in OECD 476 (2016) and the adjusted cloning efficiency were not re-calculated, since no data on the number of cells after treatment were provided. In the current study, cytotoxicity was evaluated based on cloning efficiency after treatment (CE_1, survival) and after selection (CE_2, viability), in accordance with the previous guideline version.

Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive summary

Disodium N-acetyl-N-(phosphonomethyl)glycine (IN-MCX20, batch: IN-MCX20-002, purity: 63 %) was tested *in vitro* for its ability to induce forward mutations in mammalian cells by assessing the mutation of the HPRT locus in Chinese hamster ovary (CHO K_1) cells. Duplicate cultures were exposed to the test item, solvent (water) and positive controls (ethylmethane sulfonate for cultures without S9 mix and benzo(a)pyrene for cultures with S9 mix) in the presence and absence of metabolic activation (Aroclor 1254-induced rat liver S9 fraction). Based on the results of a preliminary toxicity test, in which no substantial cytotoxicity was observed up to 2091 µg/mL (corresponding to 10 mM) with and without S9 mix, the concentrations for the main mutagenicity study were selected.

Test item concentrations in the range of 250 – 2091 µg/mL were used. After 5 hours of exposure with and without S9 mix, the cells were incubated for 7 days to allow expression of the mutant phenotype. The expression period was followed by a selection period, in which the cells were cultivated in medium supplemented with 6-thioguanidine for 9 days. Cytotoxicity was assessed as relative survival and relative viability after the expression and selection period, respectively.

There was no precipitation of the test item in solvent and in culture medium, neither in the presence nor absence of S9 mix. In addition, cytotoxicity, evident as a cloning efficiency ≤ 50 %) was not observed for any condition at any concentration. The relative cloning efficiency at the highest concentration of 2091 µg/mL was 115 % without S9 mix and 123 % with S9 mix. pH and osmolality were within an acceptable range.

Treatment with Disodium N-acetyl-N-(phosphonomethyl)glycine did not induce a significant increase in the number of mutant colonies at any concentration, neither in the presence nor in the absence of metabolic activation. For all conditions and at all dose levels, mutant frequencies were below the minimum value for a positive response.

Mutant frequencies of the control cultures remained within the range of the laboratory's historical control data. The positive control mutagens ethylmethane sulfonate and benzo(a)pyrene induced mutant frequencies that were at least 3-fold above those of vehicle controls, demonstrating the functionality of the S9 mix and the sensitivity of the test.

Under the conditions of the test, there was no evidence for gene mutation in CHO-K_1 cells *in vitro*, neither in the presence nor in the absence of metabolic activation.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	Disodium N-acetyl-N-(phosphonomethyl)glycine
Identification:	IN-MCX20
Description:	White solid
Lot/Batch number:	IN-MCX20-002
Purity:	63 %
Stability of test compound:	The stability of the test item at storage conditions (under nitrogen in a desiccator) was guaranteed until the expiry date 25 Apr 2009. The stability of the test substance in vehicle was confirmed by analytical methods.

2. Control material:

Negative control: The negative control was actually the solvent control.
Solvent (vehicle) control: Water
Positive control: - S9 mix: Ethylmethane sulfonate (EMS), 0.2 µL/mL in DMSO
+ S9 mix Benzo(a)pyrene (BaP), 4 µg/mL in DMSO

3. Metabolic activation:

S9 mix was purchased from [redacted] ([redacted]). The liver homogenate was produced from the livers of male Sprague-Dawley rats that were induced with Aroclor 1254. Immediately prior to use the S9 liver homogenate was thawed and mixed with co-factors as follows:

S9 mix component	Concentration	Unit
Sodium phosphate buffer (pH 7.4)	100	mM
KCl	33	mM
NADPH-generating system		
Glucose 6-phosphate	5	mM
NADP	4	mM
$MgCl_2$	8	mM
S9	10	% (v/v)

4. Test organism:

Chinese hamster ovary (CHO-K_1) cells were obtained from American Type Culture Collection (ATCC) in Manassas, Virginia, USA. The cell stocks were stored in liquid nitrogen and routinely tested for mycoplasma contamination. Cells used in the mutation assay were within three to four subpassages from cleansing in pre-treatment medium supplemented with hypoxanthine, aminopterin and thymidine (HAT) in order to assure karyotypic stability.

5. Cell culture media:

Pre-treatment medium (HAT medium): Ham's F12 medium supplemented with hypoxanthine, aminopterin and thymidine

Cultivation medium (F12FBS5-Hx): Ham's F12 medium, supplemented with 5 % dialysed fetal bovine serum, 100 U/mL penicillin and 100 µg/mL streptomycin

Treatment medium (±S9): *Cultivation medium (F12FBS5-Hx)*

Selection medium: Cultivation medium (F12FBS5-Hx) supplemented with 10 µM 6-thioguanidine (6TG)

Incubation: *At 37.5 ± 2 °C in a humidified atmosphere of 5 ± 2 % CO_2*

6. Locus examined: Hypoxanthin guanine phosphoribosyltransferase (HGPRT)

7. Test concentrations and number of replicates:

(a) Preliminary cytotoxicity and mutagenicity test:

Metabolic activation	Duration of exposure	Concentrations[§]	Replicates
± S9 mix	5 h	25, 50, 100, 250, 500, 750, 1000, 1500 and 2091[#] µg/mL	Triplicate plates from a single culture

[§]: Dosing solutions were adjusted to compensate the purity of the test substance by a correction factor of 1.59.
[#]: corresponding to a concentration of 10 mM

(b) Main gene mutation test:

Metabolic activation	Duration of exposure	Concentrations[§]	Replicates
± S9 mix	5 h	250, 500, 1000, 1500 and 2091[#] µg/mL	Triplicate plates from duplicate cultures

[§]: Dosing solutions were adjusted to compensate the purity of the test substance by a correction factor of 1.59.
[#]: corresponding to a concentration of 10 mM

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 22 Jun – 03 Aug 2006
Finalisation date: 13 Sep 2006

2. Preliminary cytotoxicity and mutagenicity test:
A preliminary test was performed to identify suitable dose levels for the main mutagenicity study. Cell cultures were established identically to the performance in the main mutagenicity test described below. The cells were exposed to nine test substance concentrations in the range of 25 - 2091 µg/mL for 5 hours in the presence and absence of S9 mix at 37 ± 2 °C. The highest concentration was equivalent to a 10 mM concentration. Following exposure, the cells were washed with Hank's Balanced Salt Solution (HBSS), re-incubated in fresh culture medium for approx. 20 hours, harvested and plated for determination of cell survival (cloning efficiency 1). After incubation for 7 days, the formed colonies were fixed, stained and counted.
Based on the results of the preliminary test, the test item concentrations for the main mutagenicity test were selected.

3. Main mutation assay:
Pre-treatment of cells:
For each test group, 5 x 10^5 cells were seeded into 25 cm^2 flasks and incubated for 16 - 24 hours prior to treatment.

Treatment:
On the day of treatment, the medium was exchanged. For treatment in the presence of S9 mix, 20 % of the medium were replaced by S9 mix. Duplicate cultures were exposed to the test item, vehicle (water) and positive controls (0.2 µL/mL ethylmethane sulfonate without S9 mix and 4 µg/mL benzo(a)pyrene with S9 mix) for 5 hours in the presence and absence of metabolic activation at 37 ± 2 °C. Test item concentrations ranged from 250 - 2091 µg/mL. Following exposure, the cells were washed with Hank's Buffered Salt Solution (HBSS) and the culturing was continued for another 18 – 24 hours. After the incubation period, the cells were trypsinised and counted. Triplicates of 100 cells/ 60 mm dish were seeded for the determination of survival (cloning efficiency 1) and duplicate cultures with ≤ 10^6 cells/ 100 mm dish were seeded for the expression of the mutant phenotype.

Expression period:
After treatment, duplicate cultures with ≤ 10^6 cells/ 100 mm dish were seeded and sub-cultured at 2-3 day intervals for a 7-day expression period. After the expression period, each culture was divided. Triplicates of 100 cells/ 60 mm dish were seeded for the determination of viability (cloning efficiency 2) and five culture dishes with 2 x 10^5 cells/ 100 mm dish were seeded for the selection of mutants.

Selection period:
For the selection of mutants, five culture dishes with 2 x 10^5 cells/ 100 mm dish were seeded in medium enriched with 10 µM 6-thioguanidine (selection medium). After an incubation period of 9 days, the colonies were fixed, stained and counted.

4. Cytotoxicity:

Cloning efficiency CE_1 (survival)
The survival (cloning efficiency 1) of test item-treated cells relative to solvent controls was determined in parallel to the mutagenicity test. At the end of the exposure period, a sample of each cell culture was collected to assess survival of the cells. Triplicates of 100 cells/ 60 mm dish were seeded and incubated for 8 days. Afterwards, the colonies were fixed, stained and counted.

Cloning efficiency CE_2 (viability)
The viability (cloning efficiency 2) was determined in parallel to the selection of mutants. After the expression period, triplicates of 100 cells/ 60 mm dish were seeded in medium without 6-thioguanine to assess cell viability. After an incubation period of 9 days, the developed colonies were fixed, stained and counted.

5. Evaluation:

Cytotoxicity (cloning efficiency CE)
The number of colonies divided by the number of cells plated was calculated for each sample. The absolute cloning efficiency was determined for each test group, as well as the relative cloning efficiency in comparison to the solvent control group.

CE_1 (survival)
The cytotoxicity of the test substance after the exposure period was determined for each test group and is indicated as absolute and relative cloning efficiency (CE_1 and RCE_1, respectively).

CE_2 (viability)
The cytotoxicity of the test substance at the end of the expression period was determined for each test group and is given as absolute and relative cloning efficiency (CE_2 and RCE_2, respectively).

The cloning efficiency (CE, %) was calculated for each test group as follows:

$$CE_{absolute} = \frac{\text{Total number of colonies}}{\text{Total number of cells plated}} \times 100$$

$$RCE_x = \frac{CE_{absolute} \text{ of the test group}}{CE_{absolute} \text{ of the vehicle control group}} \times 100$$

Mutant frequency (MF)
The cloning efficiency of mutant colonies in selective medium divided by the cloning efficiency in non-selective medium measured for the same culture at the time of selection was calculated for each sample.

Uncorrected mutant frequency:
The uncorrected mutant frequency (MF_{uncorr}) was calculated for each test group as follows:

$$\text{MF uncorrected} = \frac{\text{Total number of mutant colonies}}{\text{Number of seeded cells}} \times 10^6$$

Corrected mutant frequency

The corrected mutation frequency (MF_{corr}) was calculated regarding the values of CE_2:

$$\text{MF corrected} = \frac{\text{MF uncorrected}}{CE_2} \times 100$$

6. Statistics:
Statistical analysis was not performed.

7. Acceptance criteria:
The test was considered valid if the following criteria were met:

- The cloning efficiency of the vehicle control was > 50 % with a spontaneous mutant frequency within the range of the laboratories historical control data.
- The mutant frequency of the positive control was at least 3-fold as compared to the concurrent vehicle control and exceeded 40 mutants per 10^6 cells (minimum value for the positive control in the laboratories historical control data).
- At least 4 analysable concentrations showing mutants were required for evaluation.

8. 8. Evaluation criteria:
A test item was judged positive for gene mutation in mammalian cells if the following criteria were met:

- A mutant frequency of > 40 mutants per 10^6 cells (minimum value for the positive control in the laboratories historical control data) was observed at 2 or more consecutive concentrations of the test substance when compared to the solvent control.
- The observed increase in mutant frequency was accompanied by a concentration-related increase.
- A mutant frequency of > 40 mutants per 10^6 cells (minimum value for the positive control in the laboratories historical control data) was observed at the highest concentration only.

The test item was judged equivocal if a mutant frequency of > 40 mutants per 10^6 cells (minimum value for the positive control in the laboratories historical control data) was observed at any dose level other than the highest concentration.

The test item was judged negative for gene mutation in mammalian cells if a mutant frequency of > 40 mutants per 10^6 cells (minimum value for the positive control in the laboratories historical control data) was not observed at any dose level.

II. RESULTS AND DISCUSSION

1. ANALYTICAL DETERMINATIONS

Analytical determinations were performed for formulations 2.5, 10 and 20.91 mg/mL using a high performance liquid chromatography (HPLC) with MS/MS detection. The test item formulations were ± 15.6 % of the nominal concentrations, thus the test item was considered to be present at acceptable concentrations in the dosing solutions. In addition, the formulations were shown to be uniformely mixed (Coefficient of variation (CV) = 1, 2 and 4 % for 2.5, 10 and 20.91 mg/mL, respectively. There was no test substance in the 0 mg/mL sample. Stability of the test item in vehicle was confirmed for 5 hours at room temperature.

2. CYTOTOXICITY

In the preliminary cytotoxicity test, no cytotoxicity, evident as a clonig efficiency of ≤ 50 %, was observed. The relative cloning efficiency at the highest test item concentration (2091 µg/mL) was 78 % in the absence of S9 mix and 95 % in the presence of S9 mix. Osmolality and pH measurements of test

substance in medium at 2091 µg/mL were found to be within an acceptable range. Based on these findings, the concentrations for the main mutagenicity study were selected to be in the range of 250 – 2091 µg/mL, corresponding to 1.2 – 10 mM.
In the main mutagenicity experiment, there was as well no cytotoxicity observed at any dose level, neither in the presence nor absence of S9 mix. The relative cloning efficiency at the highest concentration of 2091 µg/mL was 115 % without S9 mix and 123 % with S9 mix. The pH in activated (+S9) and non-activated (-S9) cultures was 7.36 and 7.69 and comparable to the pH of solvent controls (7.45 and 8.04 in cultures with and without S9 mix, respectively). The observed changes in osmolality were ≤ 20 % and not considered significant.

3. SOLUBILITY

There was no precipitation observed up to the highest concentration tested, neither in the presence, nor absence of metabolic activation.

4. MUTANT FREQUENCY

Treatment with Disodium N-acetyl-N-(phsphonomethyl)glycine did not induce a significant increase in the number of mutant colonies at any concentration, neither in the presence nor in the absence of metabolic activation. For all conditions and at all dose levels, mutant frequencies were below 40 mutants per 10^6 cells (minimum value for the positive control in the laboratories historical control data) and no dose-response relationship was evident.
Mutant frequencies of the solvent control cultures remained within the range of the laboratories historical control data. The positive control mutagens ethylmethane sulfonate and benzo(a)pyrene induced mutant frequencies that were at least 3-fold above those of vehicle controls, demonstrating the functionality of the S9 mix and the sensitivity of the test.

Table 5.8.1-104: Results of the HGPRT gene mutation assay in mammalian cells with N-acetyl-glyphosate (IN-MCX20) ([redacted] 2006)

Test group	Total number of mutant colonies[$]	Mutant frequency (per 10^6 cells)	Cloning efficiency			
			CE_1 (survival, %)		CE_2 (viability, %)	
		corr.[#]	abs.	rel.	abs.	rel.[§]
Without metabolic activation; 5-hour exposure period						
Solvent						
Water	4	3.2	0.81	100	0.63	100
HCD mean ± SD		6.1 ± 6.6				
range		*0.0 - 24.0*				
Test item [µg/mL]						
250	6	5.5	0.82	101	0.54	86
500	10	7.6	0.83	102	0.66	105
1000	1	0.7	0.77	95	0.68	108
1500	7	4.8	0.74	91	0.73	116
2091	2	1.4	0.93	115	0.70	111
Positive control [µL/mL]						
EMS 0.2	470	352.5	0.57	70	0.67	106
HCD mean ± SD		216.0 ± 148.5				
range		*75.9 - 880.1*				
With metabolic activation; 5-hour exposure period						

Test group	Total number of mutant colonies[$]	Mutant frequency (per 10^6 cells)	Cloning efficiency			
			CE_1 (survival, %)		CE_2 (viability, %)	
		corr.[#]	abs.	rel.	abs.	rel.[§]
Solvent						
Water	17	12.3	0.58	100	0.69	100
HCD mean ± SD		5.5 ± 5.4				
range		*0.0 - 19.0*				
Test item [µg/mL]						
250	16	10.4	0.56	96	0.77	112
500	2	1.4	0.50	86	0.73	106
1000	12	8.0	0.52	90	0.75	109
1500	17	12.6	0.66	113	0.67	97
2091	7	4.2	0.72	123	0.83	120
Positive control [µg/mL]						
(B(a)P) 4.0	135	103.8	0.17	29	0.65	94
HCD mean ± SD		206.3 ± 118.5				
range		*89.7 - 379.8*				

[#] = correction on the basis of the absolute cloning efficiency 2 at the end of the expression period

[$]: number of mutant colonies 9 days after seeding 2 x 10^5 cells/100 mm dish, total of 5 cultures

[§]: calculated with total cloning efficiency data (solvent control = 100 %)

HCD: Historical control data, generated in the laboratory from 1989 - 2005

III. CONCLUSIONS

Based on the experimental findings and under the conditions of the test, Disodium N-acetyl-N-(phosphonomethyl)glycine did not induce gene mutations in the HGPRT locus of CHO-K_1 cells, neither in the presence nor in the absence of metabolic activation and is therefore considered negative for mutagenicity in mammalian cells *in vitro*.

3. Assessment and conclusion

Assessment and conclusion by applicant:

In the present study, Disodium N-acetyl-N-(phosphonomethyl)glycine was negative for mutagenicity at the HGPRT locus in CHO-K_1 cells with and without metabolic activation.
The study was conducted in compliance with GLP and according to OECD guideline 476 (1997). There were only minor deviations when compared to OECD 476 (2016), which were considered to not compromise the validity of the study. The study is therefore considered valid.

Assessment and conclusion by RMS:

CA 5.8.1/044

1. Information on the study

Data point	CA 5.8.1/044
Report author	[redacted]

Report year	2006
Report title	IN-MCX20: Mouse Bone Marrow Micronucleus Test
Report No	[redacted]-20154
Document No	-
Guidelines followed in study	OECD 474 (1997), US EPA OPPTS 870.5395 (1998), EEC Directive 2000/32/EC B12 (2000), JMAFF 12 Nousan (2000)
Deviations from current test guideline	According to the current guideline OECD 474 (2016), at least 4000 polychromatic erythrocytes per animal should be evaluated for the presence of micronuclei. However, in the present study only 2000 polychromatic erythrocytes were evaluated, since that was required in the previous OECD guideline (1997). Bone marrow exposure, indicated by a reduced polychromatic to normochromatic erythrocyte ratio, was not confirmed and there was no systemic toxicity observed, but dose levels included limit concentrations specified in the current guideline. In addition, evaluation and acceptance criteria specified in the study report were different from those recommended in the guideline.
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

Executive summary

Disodium N-acetyl-N-(phosphonomethyl)glycine (IN-MCX20, batch: IN-MCX20-002, purity: 63 %) was investigated in a micronucleus test in male and female Crl:CD1 (ICR) mice. Based on the results of a preliminary toxicity study, in which no toxicity was observed up to a limit dose of 2000 mg/kg bw, dose levels for micronucleus tests were selected. Groups of 5 mice per sex per dose level were administered a single dose of 500, 1000 or 2000 mg/kg bw by oral gavage. For the high dose group, 2 additional mice/sex/sampling time point were treated and served as backup (scoring of micronuclei was not performed). Similar constituted groups received the vehicle (Nanopure® water) or the positive control (30 mg/kg bw cyclophosphamide).

Body weights of the animals were collected before dosing, 24 hours after treatment and prior to scheduled necropsy. The animals were observed for clinical signs of toxicity and mortality 1, 3.5 and 24 hours after treatment and prior to scheduled necropsy. About 24 and 48 hours after dosing, the animals of the test item-treated and control groups were sacrificed and bone marrow smears were prepared. For animals of the positive control group, bone marrow was sampled 24 hours after dosing. For each animal, 2000 polychromatic erythrocytes (PCE) were scored for the presence of micronuclei. In addition, the ratio of PCE / normochromatic erythrocytes (NCE) was determined for 1000 erythrocytes.

Treatment with disodium N-acetyl-N-(phosphonomethyl)glycine induced no mortality in the animals and no clinical signs of toxicity were noted at any dose level. In addition, body weight and body weight gain were not affected in any dose group at any sampling time point. Based on the ratio of PCE / NCE, there was no evidence for bone marrow toxicity.

There was no statistically significant and no biologically relevant increase in the frequency of mPCE when compared to vehicle control animals at any dose level and for any sampling time.

Incidences of mPCE in solvent and positive control animals were as expected and fell within the range of the laboratory's historical control data, demonstrating the validity and sensitivity of the test system.

Based on the experimental findings of the present study and under the conditions of the test, disodium N-acetyl-N-(phosphonomethyl)glycine did not induce micronuclei in the bone marrow of male and female mice *in vivo*.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	Disodium N-acetyl-N-(phosphonomethyl)glycine
Identification:	IN-MCX20
Description:	White solid
Lot/Batch #:	IN-MCX20-002
Purity:	63 %
Stability of test compound:	The stability of the test item at storage conditions (under nitrogen in desiccator) was guaranteed until the expiry date 25 Apr 2009. The stability of the test substance in vehicle was confirmed by analytical methods.
Solvent (vehicle) used:	Nanopure® water
2. Control materials	
Solvent (vehicle) control:	Nanopure® water
Positive control:	Cyclophosphamide, 30 mg/kg bw in Nanopure® water
3. Test animals:	
Species:	Mouse
Strain:	Crl:CD1 (ICR)
Sex:	Male and female
Source:	[redacted]
Age at study initiation:	Approx. 7 weeks
Mean weight at dosing:	26.3 – 34.2 (males) and 20.6 – 27.1 (females)
Acclimation period:	At least 5 days
Diet/Food:	LLC Certified Rodent LabDiet® 5002 (PMI® Nutrition International), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually

4. Environmental conditions:

Temperature:	18 - 26 °C
Humidity:	30 - 70 %
Air changes:	Not specified
Photoperiod:	12-hour light and dark cycle

5. Test concentrations and treatment groups:

c) Preliminary toxicity study

Dose levels:	1500 and 2000 mg/kg bw
Concentrations:	Not specified
Dose volume:	Not specified
Number of animals:	3 males/group
Route of administration:	Oral gavage

d) Main micronucleus test

Dose levels:	500, 1000 and 2000 mg/kg bw[37]
Concentrations:	50, 100 and 200 mg/mL
Dose volume:	10 mL/kg bw
Number of animals:	5/ sex/group and additional 2/sex/sampling time point as backup for the high dose group (additional animals were not used for scoring of micronuclei)
Route of administration:	Oral gavage

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 24 May – 28 Jun 2006
Finalisation date: 28 Aug 2006

2. Animal assignment and treatment:

Preliminary toxicity study:
In a preliminary range-finding test, groups of 3 male mice were administered a single dose of 1500 or 2000 mg/kg bw by oral gavage. The animals were observed for clinical signs of toxicity and mortality immediately after dosing and daily for two days. Based on the results of the preliminary toxicity study, the dose levels for the micronucleus test were selected.

Main micronucleus test:
Groups of 5 mice per sex and dose level were administered a single dose of 500, 1000 or 2000 mg/kg bw. For the high dose group, 2 additional mice/sex/sampling time point were treated and served as backup (scoring of micronuclei was not performed). The test substance was dissolved in Nanopure® water and administered by oral gavage at a constant dosage volume of 10 mL/kg bw. Similar constituted groups of 5 mice/sex received the vehicle (Nanopure® water) or the positive control (30 mg/kg bw cyclophosphamide).
Individual body weights were collected before dosing and 24 and 48 hours after treatment. The animals were observed for clinical signs of toxicity and mortality 1, 3 - 5 and 24 hours after treatment and prior to scheduled necropsy. About 24 and 48 hours after dosing, the animals of the test item-treated and control groups were sacrificed by CO_2 asphyxiation and bone marrow smears were prepared. Animals of the positive control group were sacrificed 24 hours after administration.

3. Slide preparation:

After sacrifice, both femurs of the animals were removed, and marrow was aspirated with the aid of a syringe containing fetal bovine serum (FBS). After centrifugation, the pellet was re-suspended in FBS and a small drop was placed on pre-cleaned microscope slides. Smears were made using a Mini Prep® blood smearing instrument. At least 3 slides were prepared for each animal. The slides were air-dried, fixed in methanol and stained in acridine orange.

[37]: Dosing solutions were adjusted to compensate the purity of the test substance by a correction factor.

4. Slide evaluation:

Slides were coded and evaluated by fluorescent microscopy. For each animal, 2000 polychromatic erythrocytes (PCE) were scored for the presence of micronuclei. Cells containing more than one micronucleus were scored as single micronucleated PCE (mPCE). In addition, the proportion of PCE among 1000 erythrocytes, expressed as PCE / normochromatic erythrocytes (NCE) ratio, was determined.

If no increase in the incidence of mPCE was observed at the 24-hour sampling time point at any dose level, slide evaluation for the 48-hour sampling was performed for animals of the high dose and control group only.

5. Statistics:

Data for the amount of micronucleated polychromatic erythrocytes (mPCE) among 2000 erythrocytes and the ratio of PCE among normochromatic erythrocytes (NCE) were transformed prior to analysis using an arcsine square root or Freeman-Tukey function. Transformed data for PCE and MNPCE frequencies were analyzed separately for normality of distribution and equal variance using the Shapiro-Wilk and Levene's tests, respectively.

For those data that were normally distributed and had equal variance, parametric statistics (e.g. analysis of variace (ANOVA) and Dunnett's test were performed using the transformed data. For those data that were normally distributed but had unequal variance, a robust ANOVA and unequal-variance Dunnett test was done. For those data that were not normally distributed, nonparametric statistics (e.g., Kruskal-Wallis and Dunn's tests) utilizing non-transformed data was performed. The individual animal was considered the experimental unit. All data analyses was one-tailed and conducted at a significance level of 5 %.

6. Acceptance criteria:

The study was considered valid if the following criteria were met:

- In the vehicle control group, the mean frequency of micronucleated polychromatic erythrocytes (mPCE) was within the range of the laboratory's historical control range.
- The positive control induced a statistically significant increase in the frequency of mPCE as compared to the vehicle control group.

7. Evaluation criteria:

The test item was considered positive if the following criteria were met:

- The group mean number of micronucleated polychromatic erythrocytes (mPCE) was statistically significantly increased at one or more concentrations when compared to the concurrent vehicle control value.
- An accompanying statistically significant dose-response increase in mPCE was observed.

The test item was considered negative if the following criteria were met:

- There was no statistically significant, dose-related increase in the group mean mPCE above the concurrent vehicle control at any concentration.
- The mPCE values were within reasonable limits of the recent (past 3 years) laboratory historical control range.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The dosing formulations were analysed by high performance liquid chromatography (HPLC) and UV/Vis detection. The data indicated that the achieved concentration of test substance in vehicle was ± 14 % of the nominal concentration, which was considered acceptable. In addition, the formulations were shown to be uniformely mixed (Coefficient of variation (CV) = 6, 9 and 3 %) for 50, 100 and 200 mg/mL, respectively. There was no test substance in the 0 mg/mL sample. Stability of the test item in vehicle was confirmed for 5 hours at room temperature.

B. PRELIMINARY TOXICITY STUDY

In the range-finding experiment, no clinical signs of toxicity or mortality were observed.

C. MAIN MICRONUCLEUS TEST

Systemic toxicity:

Mortality:

No mortality occurred.

Clinical signs of toxicity:

There were no clinical signs of toxicity observed at any time point or at any dose level in male or female animals exposed to the test substance.

Body weight and body weight gain:

There were no significant changes in body weight or body weight gain in either male or female animals of any test group.

Evaluation of bone marrow slides:

The ratio of polychromatic (PCE) to normochromatic erythrocytes (NCE) was not affected upon treatment with the test substance at any dose level and sampling time point, indicating that no bone marrow toxicity occurred.

In addition, there was no statistically significant and no biologically relevant increase in the frequency of mPCE when compared to vehicle control animals at any dose level and for any sampling time.

Incidences of mPCE in solvent and positive control animals were as expected and fell within the range of the laboratory's historical control data, demonstrating the validity and sensitivity of the test system.

Table 5.8.1-105: Summary of genotoxicity data obtained with disodium N-acetyl-N-(phosphonomethyl)glycine (IN-MCX20) in the micronucleus test in mice ([redacted], 2006)

Treatment	Dose (mg/kg bw)	Sampling time	Males		Females	
			mPCE ± SD / 2000 PCE	PCE / NCE	mPCE ± SD / 2000 PCE	PCE / NCE
Solvent control						
Deionised water	/	24 h	1.2 ± 1.3	1.003 ± 0.460	0.2 ± 0.4	1.042 ± 0.457
	/	48 h	0.4 ± 0.6	1.868 ± 0.820	1.0 ± 1.7	1.420 ± 0.370
HCD mean ± SD	/	Not specified	3 ± 2	1.20 ± 0.43	3 ± 2	1.32 ± 0.49
range			*0 - 8*	*0.198 - 2.425*	*0 - 10*	*0.144 - 2.731*
Test item						
Test item	500	24 h	1.0 ± 0.7	0.833 ± 0.608	1.4 ± 1.1	1.320 ± 0.310
	1000	24 h	1.0 ± 1.2	1.360 ± 0.170	0.2 ± 0.4	0.972 ± 0.350
	2000	24 h	1.0 ± 1.0	1.220 ± 0.485	2.0 ± 2.3	1.635 ± 0.444
	2000	48 h	1.4 ± 1.5	1.433 ± 0.590	1.8 ± 1.3	1.610 ± 0.484
Positive control						
CPA	30	24 h	17.2 ± 4.3*	1.213 ± 0.239	17.4 ± 6.9*	1.187 ± 0.255
HCD mean ± SD	not specified	24 h	27 ± 15	1.29 ± 0.42	22 ± 11	1.38 ± 0.48
range	not specified		*8 - 81*	*0.364 - 2.623*	*4 - 67*	*0.143 -2.534*

PCE: polychromatic erythrocytes; mPCE: micronucleated polychromatic erythrocytes; NCE: normochromatic erythrocytes

HCD: Historical control data, generated in the laboratory in 16 male and 16 female studies, conducted from 2001 - 2005

Treatment	Dose (mg/kg bw)	Sampling time	Males		Females	
			mPCE ± SD / 2000 PCE	PCE / NCE	mPCE ± SD / 2000 PCE	PCE / NCE

CPA: cyclophosphamide; * statistically significant when compared to solvent control

III. CONCLUSIONS

Based on the results of the present study and under the conditions of the test, disodium N-acetyl-N-(phosphonomethyl)glycine did not induce micronuclei in bone marrow of male and female mice and is therefore considered negative for clastogenicity *in vivo*.

3. Assessment and conclusion

Assessment and conclusion by applicant:

In the present study, disodium N-acetyl-N-(phosphonomethyl)glycine was negative for clastogenic and aneugenic effects in the bone marrow of male and female Crl:CD1 (ICR) mice *in vivo*.
The study was conducted under GLP conditions and in accordance with OECD guideline 474 (1997). Only 2000 polychromatic erythrocytes were evaluated, since that was required in the previous OECD guideline (1997). Bone marrow toxicity, indicated by a reduced polychromatic to normochromatic erythrocyte ratio, or systemic toxicity were not observed. However, the test was performed at limit dose levels in line with the current guideline. The deviations were considered to be of minor degree and to not compromise the validity of the study. The study is therefore considered valid.

Assessment and conclusion by RMS:

CA 5.8.2 Supplementary studies on the active substance

In addition to the studies, which are mandatory to meet current data requirements, several further studies were performed with glyphosate and its salts, respectively.
A 28-day oral immunotoxicity study in female mice was performed according to US EPA OPPTS 870.7800 to assess the immunosupressive potential of glyphosate. Glyphosate did not suppress the humoral component of the immune system when evaluated using an antibody-forming cell (AFC) assay (CA 5.8.2/001) and thus did not show an immuntoxicological potential.
Two mechanistic studies were performed to assess the postulated relationship of alterations of the salivary gland observed in a number of repeated dose toxicity studies and the low pH-value of glyphosate acid-containing preparations. The comparative studies with citric and glyphosate acid on different strains of rats revealed that repeated application of diets at a low pH may result in higher parotid salivary gland weights and histological alterations. The observed findings in salivary glands are attributable to local irritation in the oral cavity and the subsequent adaptive response caused by the administration of glyphosate acid in the diet with a low pH. These adaptive responses can vary in severity between the different strains of rats (CA 5.8.2/002, CA 5.8.2/003), as does the strain-specific time for reversibility of these effects.
Additional pharmacological studies were performed in rats, guinea pigs, mice, and rabbits investigating the potential of glyphosate to affect parameters such as behaviour, haematology, cardiac and circulatory function, respiratory rate, or muscle contractibility *in vivo* and *ex vivo* (CA 5.8.2/004, CA 5.8.2/005, CA 5.8.2/006). Rats were dosed (oral gavage) with glyphosate technical at a dose level of 5000 mg/kg bw; control animals received vehicle only. One-hour post dosing control and treated animals were examined for haematological, electrocardiographic, or behavioural/functional changes. No treatment related effects were observed in treated animals. In addition, *ex vivo* studies on the isolated guinea pig ileum and isolated rat gastrocnemius muscle were performed using a saturated solution of glyphosate (12 mg/mL). This high

concentration of glyphosate caused a contraction of isolated guinea pig ileum, which could be antagonized with atropine indicating a parasympathomimetic activity of glyphosate *ex vivo* at high concentrations. No effect on muscle contraction of the rat gastrocnemius muscle when the sciatic nerve was stimulated was observed when glyphosate was injected (CA 5.8.2/004). The ammonium salt of glyphosate was applied to mice intraperitoneally and rabbits intravenously and clinical signs as well as respiratory parameters and blood pressure were monitored. Glyphosate was not tolerated at a dose level of 5000 mg/kg bw when injected intraperitoneally. Mice receiving a single i.p injection died within 30 min. Anaesthetised rabbits receiving a single intravenous injection of 500 mg/kg bw glyphosate ammonium salt died within a few minutes (unanesthetised rabbits did not die). Surviving animals and animals at lower dose levels of both species exhibited transient symptoms like decreased blood pressure, reduced activity and neuromuscular signs (CA 5.8.2/005). Similarly, the orally administered dose of 5000 mg/kg bw glyphosate, was not tolerated by rats, when the animals were previously anesthetised with chloralose-urethane mixture. Two hours post dosing, a marked decrease in arterial pressure was observed. All animals died within seven hours (CA 5.8.2/006).

Further, the potentiation of toxic effects by glyphosate when co-administered with other substances was investigated. The LD_{50} values of 2-4 D Na salt, isoproturon, or metolachlor decreased when co-administered with 5000 mg/kg bw glyphosate (CA 5.8.2/007). The effect was however, not pronounced.

The acute oral toxicity of glyphosate and the isopropylamine salt of glyphosate was assessed in female goats (CA 5.8.2/008, CA 5.8.2/009). The acute oral LD_{50} of glyphosate was calculated to be 3530 mg/kg bw in goats. Treated animals showed a decreased feed consumption, diarrhoea, and body weight loss (observation period 14 days) (CA 5.8.2/008). The acute oral LD_{50} of an aqueous solution of the isopropylamine salt of glyphosate was calculated to be 5700 mg/kg bw in goats. Considering the content of the glyphosate isopropylamine salt in the aqueous solution, both LD_{50} values are comparable (compare CA 5.8.2/008 and CA 5.8.2/009). Treated animals exhibited reduced feed consumption, diarrhoea, and body weight loss (observation period 14 days). In addition, several goats displayed various neurological signs (CA 5.8.2/009).

The subacute oral toxicity of the isopropylamine salt of glyphosate was investigated in female cattle. Glyphosate isopropylamine salt was administered in doses of 540, 830, 1290, and 2000 mg/kg bw/day on seven consecutive days by stomach tube administration to Brahman cross heifers. All top dose heifers died within 1.5 days after receiving the sixth or seventh dose. Clinical signs of general toxicity included a decrease in feed intake, body weight loss, diarrhoea and behavioural depression including signs of nervous dysfunction, and were noted at the two upper dose levels. Diarrhoea and decreased feed consumption were also seen in the mid dose group receiving 830 mg/kg bw/day (CA 5.8.2/010).

An *ex vivo* study examined the irritating effect of glyphosate isopropylamine salt in comparison with its product formulation or ingredients of the formulation on gastrointestinal mucosa of anesthetized dogs. Direct application of Roundup® herbicide, or surfactant caused mild mucosal damage in the stomach and intestine. These effects were more severe with the Roundup® formulation than with either the isopropylamine salt of glyphosate or the surfactant alone. The intestine appeared to be more affected than the stomach. The severity of the damage was equivalent to that caused be 0.25 N hydrochloric acid (CA 5.8.2/011).

Acute toxicity of glyphosate was also studied after intraperitoneal injection in rats (CA 5.8.2/012, CA 5.8.2/013). The acute intraperitoneal LD_{50} was calculated to be 919 mg/kg bw in female rats (CA 5.8.2/012) and 281 mg/kg bw and 467 mg/kg bw in male and female rats, respectively (CA 5.8.2/013). Based on these results, the acute toxicity of glyphosate was higher by intraperitoneal injection as compared to oral, dermal or inhalation exposure.

In the study from [redacted] (2012, CA 5.8.2/014), the *in vitro* percutaneous absorption of glyphosate acid through abraded rabbit skin was assessed following a 6-hour exposure period and a subsequent 18 hour monitoring period. The results of this *in vitro* study indicate the dermal absorption of glyphosate through abraded rabbit skin is slow. The vast majority of glyphosate will be washed off during normal washing procedures. The mean total amount absorbed after 24 hours was 2.42 % of the applied dose. The reported total potentially absorbable amount, represented by the mean absorbed dose together with the mean amount in the remaining dermis was 2.66 % of the applied dose.

A literature search for the active substance glyphosate resulted in two publications considered relevant and reliable for reporting. The publications are summariaed in the summary table presented above the first

publication in the respective summary section below.
Glyphosate was investigated for its potential to interfere with cellular function *in vitro* (CA 5.8.2/014, CA 5.8.2/015). Glyphosate was shown to reduce liver and cardiac organoid integrity and viability at doses from 250 µM to 2500 µM. When cardiac organoids were exposed to glyphosate a reduction of the beating rate was found (CA 5.8.2/014).
The transcriptome and metabolome profile of differentiated HepaRG cells was examined after exposure to 0.06, 6, and 600 µM glyphosate. Glyphosate was found to be only weakly toxic, inducing little change in transcriptome profiles when compared to other concurrent test substances. Metabolomics analysis of HepaRG cells exposed to glyphosate revealed a significant decrease in long chain fatty acid- and polyunsaturated fatty acid-levels (CA 5.8.2/015).

Table 5.8.2-1: Other/special studies on toxicological properties of glyphosate

Annex point	Study	Study type	Substance(s)	Reference list- related category§	Result
CA 5.8.2/001	[redacted] 2012	*in vivo:* 28-Day Oral (Dietary) Immunotoxicity Study in Female B6C3F1 Mice	Glyphosate (Batch: GLP-0807-19475-T, Purity: 85.2 % by weight including water, 95.11 % dried)	Valid, Category 2a	Treatment with glyphosate did not suppress the humoral component of the immune system when evaluated using the splenic antibody-forming cell (AFC) assay.
CA 5.8.2/002	[redacted] 2010	*in vivo:* Eight-week oral (diet and gavage) toxicity study of citric acid in male Crl:CD® (Sprague Dawley) rats	Citric acid, anhydrous (Batch: XR3050, Purity: 99.9 %)	Valid, Category 2a	Rats, dosed with acidic diet or acidic fluid, exhibited higher parotid salivary gland weights when compared to pH neutral, basal diet fed control animals. Histologic effects consisted of cytoplasmic alterations in the parotid salivary glands characterized by the presence of hypertrophied acinar cells with basophilic granular cytoplasm. The observed effects are considered adaptive responses to local irritation of the low pH diet in the oral cavity rather than adverse effects.
CA 5.8.2/003	[redacted] 1996	*in vivo:* 28-Day Oral (Dietary) Study. Comparison of salivary gland effects in male Alpk:AP_fSD (Wistar-derived) [AP], Sprague Dawley (Charles River CD) [CD], and Fischer 344 [F344] rats	Glyphosate acid (Batch: P24, Purity: 95.6 %)	Valid, Category 2a	Administration of diets containing 20000 ppm glyphosate acid to rats produced marked strain differences in the severity of effect in the parotid salivary gland. Microscopic examination showed the most pronounced effect occurred in the F344 strain (diffuse cytoplasmic basophilia and enlargement of the parotid acinar cells). Similar but slight effects

Table 5.8.2-1: Other/special studies on toxicological properties of glyphosate

Annex point	Study	Study type	Substance(s)	Reference list- related category§	Result
					involving small foci of cells occurred in the AP and CD strains. Complete recovery of salivary gland effects was apparent in AP and CD strains following a 4-week post-exposure period; significant, but not complete, recovery was evident in the F344 strain.
CA 5.8.2/004	[redacted] 1996	*in vivo:* Pharmacology screening study in male and female Sprague Dawley (Charles River CD) rats *ex vivo*: Pharmacology screening study on isolated guinea pig ileum (male Dunkin Hartley Albino guinea pigs) and isolated rat gastrocnemius muscle	Glyphosate technical (Batch: H95D161A, Purity: 95.3 %)	Valid, Category 2a	*in vivo:* Following oral gavage (5000 mg/kg bw), haematological parameters, electrocardiograms, and nervous system parameters (open field observations, response to various stimuli) were investigated. There were no differences in response between treated and control animals. *ex vivo*: Glyphosate caused a contractile response to isolated guinea pig ileum similar to that seen with acetylcholine.
CA 5.8.2/005	[redacted] 1992	*in vivo:* General pharmacological study (7 days) in male and female ICR mice (single i.p. injection) and male albino rabbits (single i.v. injection)	Glyphosate, ammonium salt (MON-8750) (Batch: RUD-9201-3544F, Purity: 94.78 %)	Supportive, Category 2a	Mice were treated with 0, 78.1, 313, 1250, and 5000 mg/kg bw glyphosate. All animals dosed with 5000 mg/kg bw died. Mice dosed with 1250 mg/kg bw exhibited clinical signs of toxicity affecting awareness, motor activity, central nervous system excitation, posture, motor incoordination, muscle tone, reflexes, and autonomic signs. In addition, decreased blood pressure was observed. Mice receiving doses ≤313 mg/kg bw exhibited no clinical signs of toxicity. Rabbits were dosed with 0, 7.81, 31.3, 125, and 500 mg/kg bw glyphosate. Half of the rabbits were clinically observed. The other half was used for digital monitoring of respiratory and circulatory parameters under urethane-anaesthesia. Animals dosed with 500 mg/kg

Table 5.8.2-1: Other/special studies on toxicological properties of glyphosate

Annex point	Study	Study type	Substance(s)	Reference list- related category§	Result
					bw without anaesthesia showed decreased spontaneous activity and increased respiratory rate. Animals dosed with 500 mg/kg bw under anaesthesia died within a few minutes after injection. These animals exhibited decreased heart rate, blood pressure and respiratory rate, and voltage of QRS complex in electrocardiogram. Rabbits dosed with 125 or 31.3 mg/kg bw exhibited transient symptoms like decreased blood pressure, reduced activity and neuromuscular signs. Animals dosed with 7.81 mg/kg bw exhibited no clinical signs of toxicity.
CA 5.8.2/006	[redacted], 1988	*in vivo*: Toxicodynamic study, oral, in male S/Wistar rats	Glyphosate, technical (Batch: 72390788, Purity: 96 %)	Supportive, Category 3a	Toxicodynamics were examined by the measurement of heart rate, ECG, and venous- and arterial blood pressure. At 5000 mg/kg bw glyphosate the animals narcotised with chloralose-urethane mixture died within 2 to 7 hours. After treatment, decreased pulse, respiratory rate, and arterial pressure were measured. ECG-II and venous pressure changes were incidental and insignificant. White spots were seen in the edges of livers of the dead animals.
CA 5.8.2/007	[redacted] 1988	*in vivo:* Single oral administration of glyphosate in combination with either 2-4 D Na salt, isoproturon, or metolachlor to male Wistar rats	Glyphosate, technical (Batch: not specified, Purity: not specified)	Supportive, Category 2a	LD_{50} values of 2-4 D Na salt, isoproturon and metolachlor were slightly lower when co-administered with glyphosate at 5000 mg/kg bw.
CA 5.8.2/008	[redacted], 1987	*in vivo:* Acute oral toxicity (gavage), female Spanish goat	Glyphosate (Batch: XHJ-64, NBP1494248, Purity: 98.7 %)	Supportive, Category 2a	LD_{50} = 3530 mg/kg bw
CA 5.8.2/009	[redacted], 1987	*in vivo:* Acute oral toxicity (gavage), female Spanish goat	Glyphosate, isopropylamine salt (MON-0139) (Batch: LURT 08020, Purity: 62.5 %)	Supportive, Category 2a	LD_{50} = 5700 mg/kg bw

Table 5.8.2-1: Other/special studies on toxicological properties of glyphosate

Annex point	Study	Study type	Substance(s)	Reference list- related category§	Result
CA 5.8.2/010	[redacted] 1987	*in vivo:* Subacute toxicity, oral (gavage) exposure for seven days, in female cattle (Braham cross heifers)	Glyphosate, isopropylamine salt (MON-0139) (Batch: LBRT 08023, Purity: 62.4 %)	Supportive, Category 2a	Animals received doses of 540, 830, 1290, or 2000 mg/kg bw/day. Clinical signs of toxicity occurred at doses ≥830 mg/kg bw/day, including loss of appetite and diarrhoea. Mortality occurred at doses of ≥1290 mg/kg bw/day. Nervous system effects (head tremors, convulsions, ataxia, etc.) were observed at 2000 mg/kg bw/day prior to death.
CA 5.8.2/011	[redacted] 1987	*in vivo/ex vivo:* Irritating effect of glyphosate, surfactant, and roundup on exposed stomach and small intestine mucosa in anesthetized Beagle dogs	- Glyphosate, isopropylamine salt (MON139) (Batch: LIRT09023, Purity: 63.2 %) - Surfactant (MON-0818) (Batch: not specified, Purity: not specified) - Roundup (Batch: Oda B6E20 88.10, Purity/ Composition: 41 % isopropylamine salt of glyphosate, 15 % surfactant, water) - Hydrochloric acid, 6 N; diluted to 0.25 N with water	Supportive, Category 2a	Direct application of Roundup® herbicide, or surfactant caused mild mucosal damage in the stomach and intestine. These effects were more severe with the Roundup formulation than with either the IPA salt of glyphosate or the surfactant alone. The intestine appeared to be more affected than the stomach. The severity of the damage was equivalent to that caused be 0.25 N hydrochloric acid.
CA 5.8.2/012	[redacted] 1980	*in vivo:* Acute intraperitoneal toxicity, female Wistar rat	Glyphosate (Batch: not specified, Purity: not specified)	Supportive, Category 4a	LD_{50} = 919 mg/kg bw
CA 5.8.2/013	[redacted] 1978	*in vivo:* Acute intraperitoneal toxicity, male and female Wistar rat	Glyphosate (Batch: not specified, Purity: 98.4 %)	Supportive, Category 3a	Males: LD_{50} = 281 mg/kg bw Females: LD_{50} = 467 mg/kg bw
CA 5.8.2/014	[redacted] 2012	*in vitro:* Dermal absorption through rabbit skin	Glyphosate (Batch: GLP-0810-1915-A, 95.93 %); [^{14}C]-glyphosate (Lot: 4675JJN002-1, 96.7 %)	Valid, Category 2a	2.66 % glyphosate systemically available via dermal absorption. Translates to 133 mg/kg bw/day systemic NOAEL in repeated dose dermal toxicity in rabbits by Johnston (CA 5.3.3/008)

§: The category describes the acceptability/reliability of the study within the AIR 5 submission. This category does not describe hazard classification. (for details please refer to the Doc ID: 110054-B-GRG_Jun_2020)

CA 5.8.2/001

1. Information on the study

Data point:	CA 5.8.2
Report author	[redacted]
Report year	2012
Report title	Glyphosate – A 28-Day Oral (Dietary) Immunotoxicity Study in Female B6C3F1 Mice
Report No	[redacted]-50393
Document No	M-651162-01-1
Guidelines followed in study	US EPA OPPTS 870.7800 (1998)
Deviations from current test guideline (No equivalent EU or OECD Test Guideline. US EPA specific guideline developed under FIFRA)	The study was performed according to EPA OPPTS 870.7800 to meet and evaluate US EPA specific endpoint for immunotoxicity under the Federal Insecticide, Fungicide, and Rodenticide Act. No equivalent OECD study guideline exists.
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes, conducted under GLP
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The potential immunotoxicity of glyphosate was evaluated after repeated dietary administration to B6C3F1 mice. Four groups of 10 female mice were offered diets containing glyphosate concentrations of 0, 500, 1500 or 5000 ppm (equivalent to 0, 150, 449, and 1448 mg/kg bw/day) and for 28 consecutive days. A further group of 10 females were used as positive immunosuppressive control group. These mice received basal diet for 28 days and were treated with an intraperitoneal (i.p.) injection of 50 mg/kg bw/day cyclophosphamide monohydrate (CPS) once daily for four consecutive days (study days 24 - 27).
The animals were checked twice daily for mortality and moribundity, and once daily for clinical signs. Detailed clinical examinations were performed once per week. Body weights were recorded twice weekly. Food consumption was recorded in weekly intervals, and food intake was calculated for the corresponding body weight intervals. Blood samples for IgM antibody analysis were collected from all mice at scheduled necropsy. At termination, the animals were sacrificed and subjected to a full macroscopic post-mortem examination. Spleens and thymus were weighed and specified tissues preserved.
There were no test substance-related effects on survival, clinical observations, body weight, food consumption, as well as any gross pathological changes. There were no test substance-related effects on spleen or thymus weights (absolute or relative to final body weight), spleen cellularity, or the T-cell dependent antibody response (TDAR), as measured by the splenic antibody-forming cell (AFC) IgM Specific Activity (AFC/10^6 spleen cells) and Total Spleen Activity (AFC/spleen), at any dosage level tested.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Identification:	Glyphosate
Description:	White powder
Lot/Batch #:	GLP-0807-19475-T
Purity:	95.11 % (dried)
Stability of test compound:	Expiry date: 2011-06-10
Vehicle and/ or positive control:	Basal diet Cyclophosphamide monohydrate
Test animals:	
Species:	Mouse
Strain:	B6C3F1/Crl
Source:	[redacted]
Age:	Approx. 37 days (on arrival)
Sex:	Female
Weight at dosing:	16.5 – 20.0 g
Acclimation period:	14 days
Diet/Food:	Certified Rodent LabDiet® # 5002 (meal) (PMI Nutrition International, LCC.), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in stainless steel, wire-mesh cages suspended above cage-board.
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 50 ± 20 % Air changes: 10/hour Photocycle: 12 hours light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2010-10-05 to 2010-11-16

Animal assignment and treatment

In a 28-day oral immunotoxicity study groups of 10 female B6C3F1/Crl mice received daily dietary doses of 0, 500, 1500 and 5000 ppm glyphosate (equivalent to 0, 150, 449 and 1448 mg/kg bw/day).

A further group of 10 females were used as positive immunosuppressive control group. These mice received basal diet for 28 days and were treated with an intraperitoneal (IP) injection of 50 mg/kg bw/day CPS once daily for four consecutive days (study days 24 - 27).

Test diets were prepared weekly and stored at room temperature. For the vehicle and positive control groups an appropriate amount of basal diet was weighed into a plastic storage bag. For the test substance groups 500 g of basal diet was weighed (pre-mixture). An appropriate amount of glyphosate was weighted into a mortar, mixed with a small amount of the pre-mixture basal diet, and ground until uniform. This admixture was transferred to a Hobart mixer and mixed with the remainder of the pre-mixture basal diet for five minutes. The resultant mixture was then transferred to a V-blender with a sufficient amount of basal diet to achieve the correct diet concentration and mixed for an additional 10 minutes using an intensifier bar during the first and last three minutes of mixing to ensure a homogeneous mixture. The test diets were prepared from the lowest to highest concentration. The stability and homogeneity of the test substance in the diet was determined in an in-house stability study at 450 and 5500 ppm. Analyses for achieved concentrations

on the test diets were done during study weeks 0 and 3.

Mortality
Each animal was checked for mortality or signs of morbidity twice a day during the treatment period.

Clinical observations
A check for clinical signs of toxicity was made once daily on all animals. In addition, a detailed clinical examination was performed once a week during the study period, beginning one week prior to randomisation, and on the day of scheduled necropsy.

Body weight
Individual body weights were recorded twice weekly, beginning approximately one week prior to randomization, at the time of animal selection for randomization, on study day 0, and just prior to the scheduled necropsy. Mean body weights and mean body weight changes were calculated for the corresponding intervals.

Food consumption and test substance intake
The quantity of food consumed was recorded for each animal weekly, beginning approximately one week prior to randomization, and just prior to the scheduled necropsy. Food intake was calculated as g/animal/day for the corresponding body weight intervals. The mean amounts of glyphosate consumed (mg/kg bw/day) per dose group were calculated from the mean food consumed (g/kg of body weight/day) and the appropriate target concentration of glyphosate in the food (mg/kg of diet).

Serum collection for possible IgM antibody analysis
All animals were immunised with an intravenous injection of sRBC on study day 24. For determination of the possible extent of the suppression of IgM antibody production blood samples were collected from all animals at scheduled necropsy and processed to serum. Following euthanasia by carbon dioxide inhalation, approximately 0.75 mL of blood was collected from the inferior vena cava of each mouse into a tube containing no anticoagulant and allowed to clot. Serum was obtained and aliquots of approximately 150 µL (including any remainder serum) were transferred to cryovials and stored frozen (approximately -70 °C).

Sacrifice and pathology
A complete necropsy was conducted on all animals at scheduled termination or on animals that died or were sacrificed during the study period. Any macroscopic findings were recorded. The following organ weights were determined from all animals surviving to scheduled termination: spleen and thymus. The ratio of organ weights to final body weights were calculated.
Tissue samples were taken from the spleen and thymus. Spleen samples were placed in EBSS/HEPES buffer. Thymus samples were preserved in 10 % neutral-buffered formalin.

Spleen processing for immunotoxicological evaluation
For the determination of the number of specific IgM antibody-forming cells directed towards sRBC an AFC assay, as a modification of the Jerne plaque assay (Jerne *et al.*, 1963, 1974) was conducted.

Spleens were collected from all animals at the scheduled necropsy (study day 28) immediately following blood collection. Individual spleens were placed into individual tared tubes containing EBSS with 15 mM HEPES, supplemented with gentamicin as a bacteriostat, and maintained on ice. Each tube was then weighed to provide a "wet" weight for each spleen. Spleen samples from Groups 1 - 4 animals were randomized and coded for antibody-forming cell (AFC) analysis. Spleen samples from Group 5 were labelled as positive control samples for analysis. The spleen samples were placed on crushed ice until procession for AFC analysis.
The spleen samples were processed into single-cell suspensions. The cell suspensions were then centrifuged and resuspended in EBSS with HEPES. Spleen cell counts were performed using a Model Z1™ Coulter Counter®. Viability of splenocytes was determined using propidium iodide and the Coulter® EPICS® XL-MCL™ Flow Cytometer.

Statistics
Body weight, body weight change, and food consumption data were subjected to a parametric one-way ANOVA (Snedecor and Cochran, 1980) to determine intergroup differences. If the ANOVA revealed statistically significant ($p<0.05$) intergroup variance, Dunnett's test (Dunnett, 1955, 1964) was used to compare the test substance treated groups to the control group.
The positive control data were evaluated using the Student's t-Test (Sokal and Rohlf, 1981) and compared to the basal diet control group.

Organ weight (wet spleen and thymus), final body weight, and AFC data obtained were first tested for homogeneity of variances using the Bartlett's Chi Square test (Bartlett, 1937). Homogeneous data were evaluated using a parametric one-way ANOVA (Kruskal and Wallis, 1952). When significant differences occurred, the treatment groups were compared to the basal diet control group using Dunnett's test (Dunnett, 1955, 1964). Non-homogeneous data were evaluated using a non-parametric ANOVA (Wilson, 1956). When significant differences occurred, the treatment groups were compared to the basal diet control group using the Gehan-Wilcoxon test when appropriate (Gross & Clark, 1975). The Jonckheere's test (Hollander & Wolfe, 1973) was used to test for dose-related trends across the basal diet control and test substance treated groups. The positive control data were evaluated using the Student's t Test (Sokal & Rohlf, 1981) and compared to the basal diet control group. The criteria for accepting the results of the positive control group included a statistically significant ($p\leq0.05$) decrease in the response when compared to the response of the basal diet control group.
The AFC data were expressed as Specific Activity, IgM antibody forming cells per million spleen cells (AFC/106 spleen cells), and as IgM Total Spleen Activity (AFC/spleen).

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS
The achieved concentrations of glyphosate in the dietary preparation were in the range of 85.6 – 97.5 % of nominal, and therefore within the acceptable range of 85 – 115 %. The diet formulations were homogeneous and stable for 10 days when stored at room temperature with the following exception. During homogeneity/concentration acceptability testing, the 450 ppm diet formulation was 83.1 % of target. The 5500-ppm diet formulation was within acceptable range (90.8 %) but was considered low, therefore, calibration standards were prepared as matrix-based samples and a cross-validation was conducted. The diet formulations were reanalysed using matrix-based calibration standards and met the testing facilities SOP acceptance criteria for homogeneity and concentration acceptability. Based on these results, the protocol-specified doses of test substance were offered to the animals. The test substance was not detected in the basal diet that was offered to the basal diet control (Group 1) and positive control (Group 5) groups.

B. MORTALITY AND CLINICAL SIGNS
There were no mortalities observed during the study period.

C. CLINICAL OBSERVATIONS
There were no test substance-related clinical findings.

D. BODY WEIGHT
There were no test substance related effects on body weights in any of the dose groups.

E. FOOD CONSUMPTION AND COMPOUND INTAKE
There were no test substance-related effects on food consumption noted.

The group mean achieved doses are summarised below.

Table 5.8.2-2: Glyphosate – A 28-Day Oral (Dietary) Immunotoxicity Study in Female B6C3F1 Mice ([redacted] 2012): Group mean achieved dose levels of glyphosate

Dose group	Dietary concentration (ppm)	Mean achieved dose level (mg/kg bw/day)
1 (vehicle control)*	0	0
2 (low)	500	150.1
3 (mid)	1500	449.1
4 (high)	5000	1447.5
5 (positive control)	50 mg/kg CPS**	0

* basal diet group
** CPS = cyclophosphamide

F. NECROPSY

Gross pathology

There were no test substance-related macroscopic effects.

Treatment with the positive control CPS produced a small thymus in three of the 10 animals. These changes were consistent with the known effects of CPS in female B6C3F1 mice.

Organ weights

There were no test substance-related effects on terminal body weights or on spleen or thymus weights (absolute or relative to final body weight) when the test substance-treated groups were compared to the basal diet control group.

Treatment with the positive control CPS produced statistically significantly lower spleen and thymus weights (absolute and relative to final body weight) when compared to the basal diet control group. These changes were consistent with the known effects of CPS in female B6C3F1 mice.

The results of final body and organ weight determinations are presented in the table below.

Table 5.8.2-3: Glyphosate – A 28-Day Oral (Dietary) Immunotoxicity Study in Female B6C3F1 Mice ([redacted] 2012): Final body weight and organ weight data

Dose group	Body weight (g)[#]	Spleen weight		Thymus	
		Absolut	Relative	Absolut	Relative
		(mg) [#]	(% of body weight) [#]	(mg) [#]	(% of body weight) [#]
1 (vehicle control)*	20.9 ± 0.3	85.3 ± 3.5	0.41 ± 0.02	44.3 ± 3.5	0.21 ± 0.02
2 (low)	20.6 ± 0.2	82.3 ± 4.6	0.40 ± 0.02	41.5 ± 1.9	0.20 ± 0.01
3 (mid)	21.6 ± 0.3	91.6 ± 6.5	0.42 ± 0.03	45.9 ± 2.7	0.21 ± 0.01
4 (high)	21.3 ± 0.2	86.0 ± 3.6	0.40 ± 0.02	42.0 ± 2.6	0.20 ± 0.01
5 (positive control)	21.5 ± 0.3	50.2 ± 3.2**	0.23 ± 0.02**	13.3 ± 0.8**	0.06 0.01**

[#] Values presented the mean ± SD derived from the number of animals evaluated per dose group
* basal diet group
** Statistically significant from vehicle control at p ≤0.01

G. AFC ASSAY RESULTS

There were no test substance-related effects on spleen cell numbers, and in the functional evaluation of the IgM antibody-forming cell (AFC) response, treatment with glyphosate did not result in a statistically significant suppression of the humoral immune response when evaluated as either Specific Activity (AFC/106 spleen cells) or Total Spleen Activity (AFC/spleen). There were no statistically significant differences nor any dose-related trends noted when the basal diet control and test substance-treated groups were compared.

Statistically significantly lower spleen cell numbers, mean specific activity, and mean total spleen activity values were noted in the positive control (CPS treated) group when compared to the basal diet control group. These effects were consistent with the known immunosuppressant effects of CPS and validated the appropriateness of the AFC assay.
The results of the AFC assay are summarised in the table below.

Table 5.8.2-4: Glyphosate – A 28-Day Oral (Dietary) Immunotoxicity Study in Female B6C3F1 Mice ([redacted] 2012): Results of AFC assay

Dose group	Spleen cells (x 10^7)#	IgM AFC / 10^6 spleen cells#	IgM AFC/spleen (x 10^3)#
1 (vehicle control)*	11.29 ± 0.65	1160 ± 131	127 ± 11
2 (low)	11.45 ± 0.64	1273 ± 123	144 ± 16
3 (mid)	13.45 ± 1.24	1368 ± 163	190 ± 37
4 (high)	12.51 ± 0.66	1514 ± 204	195 ± 32
5 (positive control)	5.18 ± 0.53**	0 ± 0**	0 ± 0**

Values presented the mean ± SD derived from the number of animals evaluated per dose group
* basal diet group
** Statistically significant from vehicle control at p ≤0.01

Study conclusion
Repeated dietary administration of glyphosate to females B6C3F1 mice did not suppress the humoral component of the immune system. The no-observed-effect level (NOEL) for suppression of the humoral immune response in female B6C3F1 mice offered glyphosate in the diet for 28 days was considered to be 5000 ppm (equivalent to 1448 mg/kg bw/day), the highest dietary concentration.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:
Treatment of female B6C3F1 mice for 28 days with glyphosate did not suppress the humoral component of the immune system when evaluated using the AFC assay. The no-observed-effect level (NOEL) was considered to be 5000 ppm (equivalent to 1448 mg/kg of body weight/day) in female mice, the highest dietary concentration. The study was performed according to EPA OPPTS 870.7800 and no deviations were apparent. The study is considered informative and acceptable.

Assessment and conclusion by RMS:

CA 5.8.2/002

I. Information on the study

Data point:	CA 5.8.2
Report author	[redacted]

Report year	2010
Report title	An 8-Week Oral (Diet and Gavage) Toxicity Study of Citric Acid in Male Rats
Report No	[redacted]-50361
Document No	Not reported.
Guidelines followed in study	Guideline does not exist. Proof of concept study for salivary gland alterations due to treatment with acidic diet.
Deviations from current test guideline	Not applicable.
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes, conducted under GLP.
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

A number of repeat dose studies in rodents with glyphosate technical acid have identified alterations of the salivary glands, described as increased basophilic staining and enlargement of cytoplasm, especially in the parotid salivary glands. The toxicological significance of these observations were considered not relevant, by some reviewers and unknown by others. In the 2004 JMPR review of glyphosate, a hypothesis was proposed that the low pH of glyphosate technical acid in the diet caused local irritation in the oral cavity leading to the observed salivary gland effects. The objective of this study was to evaluate the potential effects of low pH diet on the parotid salivary glands. Citric acid was selected as an appropriate surrogate for glyphosate, having both a similar pH-dilution curve and low toxicity. Citric acid was presented in the diet (14000 ppm) and compared with a typical pH basal diet control group. A higher pH diet group fed basal diet with trisodium citrate dihydrate (21400 ppm, an equivalent citrate ion concentration to the citric acid group) was also compared with the typical pH basal diet control group. In addition, low pH aqueous citric acid was administered by gavage and compared to a control deionised water gavage group to evaluate potential systemic effects of the citrate ion on the parotid salivary glands. These five test groups, each consisting of 10 male rats, were dosed for eight weeks (minimum of 56 days).
Clinical signs, bodyweight and food consumption were monitored during the study. All animals were subjected to a gross necropsy examination and a comprehensive histopathological evaluation of tissues was performed. The findings are summarised as follows:
There were no test substance-related clinical signs of toxicity, as well as no test substance-related effects on body weight, and food consumption.

Test substance-related effects on organ weights consisted of statistically significantly higher parotid salivary gland weights in the low pH diet group only (citric acid) when compared to the respective control group. Non-statistically significantly higher parotid salivary gland weights were noted in the gavage citric acid and high pH dietary (trisodium citrate dihydrate) groups when compared to their respective control group. There were no statistically significant test substance-related effects on the fused mandibular/sublingual salivary gland weights when the respective control and test substance-treated groups were compared; however, a non-statistically significantly higher fused mandibular/sublingual salivary gland weight was noted in the low pH diet group (14000 ppm citric acid).

Histological effects consisted of cytoplasmic alterations in the parotid salivary glands characterized by the presence of hypertrophied acinar cells with basophilic granular cytoplasm. Although the overall incidence of affected animals was similar in all control and citric acid or trisodium citrate dihydrate-treated groups, these effects were clearly most severe in the low pH diet group (14000 ppm citric acid in basal diet). With

the absence of microscopic findings such as cytotoxicity and hyperplasia, the observed effects are considered to be an adaptive response to local irritation of the low pH diet in the oral cavity rather than an adverse effect.

Citric acid administered orally via gavage or diet and trisodium citrate dihydrate administered via the diet to Sprague Dawley rats for 56 days resulted in higher parotid salivary gland weights and a generally correlative increase in severity of background cytoplasmic alterations in the parotid salivary glands in all dose groups (gavage citric acid, diet citric acid, and diet trisodium citrate dihydrate). The magnitude of change in parotid gland weight and severity of the cytoplasmic alteration in the parotid salivary glands was most severe in the low pH diet citric acid group.

I. MATERIALS AND METHODS

A. MATERIALS

Test materials:

Identification:	Anhydrous Citric Acid
Description:	White powder
Lot/Batch #:	XR3050
Purity:	99.9 %
Stability of test compound:	Stable at room temperature until 2010-01-06
Identification:	Trisodium Citrate Dihydrate (TCD)
Description:	White crystalline solid
Lot/Batch #:	1387609
Purity:	99.3 %
Stability of test compound:	Stable at room temperature until 2011-03-01
Vehicle and/ or positive control:	Gavage: deionised water, Diet: plain diet
Test animals:	
Species:	Rats
Strain:	Sprague-Dawley (CD)
Source:	[redacted]
Age:	Approx. 6 weeks upon beginning of treatment
Sex:	Males
Weight at dosing:	177 - 227 g
Acclimation period:	14 days
Diet/Food:	Certified Rodent LabDiet #5002 (PMI Nutrition International, LLC), *ad libitum* except for fasting period prior to necropsy
Water:	Tap water, *ad libitum*
Housing:	Upon arrival, animals were housed three per cage for approximately 3 days. Thereafter, all animals were housed individually in clean, stainless steel, wire-mesh cages suspended above cage-board.
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 50 ± 20 % Air changes: at least 10/hour Photocycle: 12 hours light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2009-02-10 to 2009-04-21

Animal assignment and treatment
In an 8 week gavage and feeding study groups of 10 male Sprague Dawley rats received the respective vehicles or test substances for 56 consecutive days via oral gavage (Groups 1 and 3) or in the diet (Groups 2, 4 and 5; see table below). A low pH diet containing 14000 ppm of citric acid in basal diet was offered continuously to Group 4. A high pH diet containing 21400 ppm of trisodium citrate dihydrate in basal diet (at an equivalent citrate ion concentration to Group 4) was offered continuously to Group 5. A concurrent control group (Group 2) received the basal diet on a comparable regimen. Citric acid in the vehicle, deionised water, was administered orally by gavage at a dose level of 791-1316 mg/kg bw/day to Group 3. Concentrations of the Group 3 formulations were calculated and adjusted weekly, based on the average food consumption and body weights of the Group 4 animals from the previous week of dosing in order to maintain approximately equivalent citric acid dose levels to Group 4. A concurrent gavage control group (Group 1) received the vehicle on a comparable regimen.

Table 5.8.2-5: An 8-Week Oral (Diet and Gavage) Toxicity Study of Citric Acid in Male Rats ([redacted] 2010): Study group assignment

Group number	Test substance application	Dose level (mg/kg bw/day or ppm)	Dose volume (mL/kg)	Number of animals
1	Gavage Vehicle	0	10	10
2	Basal Diet	0	na	10
3	Gavage Citric Acid (low pH)	791-1316	10	10
4	Diet Citric Acid (low pH)	14000*	na	10
5	Diet Trisodium Citrate (high pH)	21400*	na	10

na - not applicable, * stoichiometrically equivalent for citrate ion concentration

Observations
All animals were observed twice daily for mortality and moribundity. Clinical examinations were performed daily, and detailed physical examinations were performed weekly.

Body weight
Individual body weights were recorded weekly.

Food consumption and compound intake
Food consumption was recorded weekly.

Sacrifice and pathology
All animals sacrificed at scheduled termination were subjected to a gross pathological examination. Any macroscopic findings were recorded.
The following organ weights were determined: parotid salivary glands, mandibular salivary glands and sublingual salivary glands. The mandibular and sublingual salivary glands were weighed together as one organ since they were fused and could not be adequately separated for weighing.
Tissue samples were taken from the following organs and preserved in buffered formalin: adrenals, aorta, bone & bone marrow (sternum and femur (incl. joint)), brain (cerebrum at two levels; cerebellum with medulla/pons), caecum, colon, duodenum, epididymides, eyes with optic nerves, gross lesions, harderian glands, heart, ileum, jejunum, kidneys, lacrimal gland (exorbital), liver, lungs (incl. bronchi), mammary gland, lymph nodes (mandibular, mesenteric and axillary), nasal cavity, oesophagus, pancreas, Peyer's patches, pituitary, prostrate, rectum, salivary glands (mandibular, parotid, sublingual), sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord (cervical, thoracic, lumbar), spleen, stomach, testes,

thymus, thyroid/parathyroid, tongue, trachea and urinary bladder.
Microscopic examination was performed on the parotid salivary glands and gross lesions from all animals at the scheduled necropsy.

Statistics
All statistical tests were performed using the [redacted] Toxicology Data Management System (WTDMS™). Analyses were conducted using two-tailed tests (except as noted otherwise) for minimum significance levels of 1 % and 5 %, comparing each test substance-treated group to its respective control group.
Body weight, body weight change, food consumption, and organ weight data were subjected to a parametric one-way analysis of variance (ANOVA) to determine intergroup differences. If the ANOVA identified statistically significant ($p<0.05$) intergroup variance, Dunnett's test was used to compare each of the test substance-treated groups to the respective control group (Group 1 to Group 3 and Group 2 to Groups 4 and 5). Group 1 was also compared to Group 2.
Statistical analysis of the severity of histological changes was conducted. Individual animals were assigned severity scores based on parotid salivary gland changes (0=without histological change, 1=minimal change, 2=mild change, and 3=moderate change). The severity scores were then compared statistically using the Mann-Whitney U-test by comparing Group 1 to Group 3 and Group 2 to Groups 4 and 5.

II. RESULTS AND DISCUSSION

A. MORTALITY
No deaths occurred during the study.

B. CLINICAL OBSERVATIONS
All clinical findings in the test substance-treated groups were noted with similar incidence in the control groups, were limited to single animals, and/or were common findings for laboratory rats of this age and strain.

C. BODY WEIGHT
There were no statistically significant differences when the respective control and test substance-treated groups were compared.

D. FOOD AND TEST SUBSTANCE CONSUMPTION
Food consumption was unaffected by citric acid or trisodium citrate dihydrate administration. A statistically significant decrease in food consumption of the gavage citric acid group (Group 3, Week 7/8) was probably due to biological variability and not considered related to test substance administration.

E. PATHOLOGY
Organ weights
Test substance-related effects on organ weights consisted of statistically significant higher absolute and relative parotid salivary gland weights in the low pH diet group (14000 ppm citric acid) when compared to the dietary control group; the magnitude of change was >40 % (see table below).
Higher absolute and relative parotid salivary gland weights were also observed in the low pH gavage group (791-1316 mg/kg bw/day citric acid) and in the high pH diet group (21400 ppm TCD) when compared to their respective control groups. However, the parotid salivary gland weight differences in the low pH gavage and high pH diet groups were not statistically significant and were of much lesser magnitude of change.
There were no other statistically significant test substance-related effects on the fused mandibular/ sublingual or parotid salivary gland weights when the control groups and test substance-treated groups were compared.

Table 5.8.2-6: An 8-Week Oral (Diet and Gavage) Toxicity Study of Citric Acid in Male Rats ([redacted] 2010): Mean absolute and relative organ weights (mean ± SD)

	Gavage administration		Dietary administration		
	Aqueous control	**791-1316 mg/kg bw/day citric acid**	**Basal diet control**	**Low pH diet, 14000 ppm citric acid**	**High pH diet, 21400 ppm trisodium citrate dihydrate**
Absolute mandibular / sublingual fused glands weight (g)	0.7625 ± 0.05446	0.7873 ± 0.08397	0.7682 ± 0.08670	0.8872 ± 0.16548	0.7869 ± 0.07028
Relative mandibular / sublingual fused glands weight (g/100 g bw)	0.179 ± 0.0105	0.180 ± 0.0178	0.173 ± 0.0221	0.199 ± 0.0339	0.183 ± 0.0201
Absolute parotid gland weight (g)	0.3500 ± 0.12450	0.4082 ± 0.11990	0.2758 ± 0.08514	0.3905* ± 0.10920	0.3502 ± 0.08986
Relative parotid gland weight (g/100 g bw)	0.083 ± 0.0299	0.095 0.0304	0.062 ± 0.0194	0.088* ± 0.0236	0.082 0.0220

* statistically significantly different from relevant control group ($p<0.05$) using Dunnett's test

Necropsy
All macroscopic findings noted were considered spontaneous and/or incidental in nature and unrelated to test substance administration.

Histopathology
Test substance-related histological effects consisted of a higher severity of cytoplasmic alterations in the parotid salivary glands of the citric acid and trisodium citrate dihydrate-treated groups when compared to their respective control groups (see table below). The severity of cytoplasmic alteration was increased in all dose groups; however, the cytoplasmic alteration was clearly most severe in the low pH diet group (Group 4; 14000 ppm citric acid). Furthermore, statistical significance for the grade of severity was only achieved in the low pH diet group, while the severity in the other dose groups was not statistically significantly different from the respective control group.
Cytoplasmic alteration in the parotid salivary glands was characterized by the presence of hypertrophied acinar cells with basophilic granular cytoplasm. The severity grades varied from minimal to moderate, displayed by increasing numbers of affected acinar cells and more pronounced hypertrophy of acinar cells with increasing severity grade.
Cytotoxicity and hyperplasia were not observed and consequently, the observed changes were considered adaptive responses rather than adverse effects. There were no other test substance-related histological changes.

Table 5.8.2-7: An 8-Week Oral (Diet and Gavage) Toxicity Study of Citric Acid in Male Rats ([redacted] 2010): Histological findings

	Gavage administration		Dietary administration		
	Aqueous control	**791-1316 mg/kg bw/day citric acid**	**Basal diet control**	**Low pH diet, 14,000 ppm citric acid**	**High pH diet, 21,400 ppm trisodium citrate dihydrate**
Parotid salivary glands [a]	9	10	10	10	10
Incidence (%)	*100*	*100*	*70*	*100*	*90*

minimal	8	6	5	0	4
mild	1	3	2	6	5
moderate	0	1	0	4	0
Average severity [b]	**1.1**	**1.5**	**0.9**	**2.4****	**1.4**

[a] number of tissues examined from each group
[b] 1= minimal, 2= mild and 3= moderate; animals without a histological change were assigned a severity score of 0
** significantly different from relevant control group ($p<0.01$) using the Mann-Whitney U-Test

Study conclusion
Citric acid administered orally via gavage or diet and trisodium citrate dihydrate administered via the diet to Sprague Dawley rats for 56 days resulted in higher parotid salivary gland weights and a generally correlative increase in severity of background cytoplasmic alterations in the parotid salivary glands at all dose levels with equivalent citrate ion doses (791-1316 mg/kg bw/day gavage citric acid, 14000 ppm diet citric acid, and 21400 ppm diet trisodium citrate dihydrate). These effects were noted as most severe in the low pH dietary test group. In the absence of cytotoxicity and hyperplasia the noted effects are considered an adaptive response rather than an adverse effect and are consistent with the hypothesis that low pH diets result in adaptive cellular responses within the salivary glands.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:
Higher parotid salivary gland weights and histological alterations were noted as statistically significant in the low pH citric acid diet administered to Sprague Dawley rats. This proof of concept study is considered supportive evidence that low pH diets result in adaptive salivary gland alterations, as noted in some glyphosate acid repeat dose dietary studies.

Assessment and conclusion by RMS:

CA 5.8.2/003

1. Information on the study

Data point:	CA 5.8.2
Report author	[redacted]
Report year	1996
Report title	Glyphosate Acid: Comparison of Salivary Gland Effects in Three Strains of Rat
Report No	[redacted]/P/5160
Document No	Not reported.
Guidelines followed in study	No guideline exists for this kind of study.

Deviations from current test guideline	Not applicable.
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes, conducted under GLP.
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The purpose of this study was to investigate the rat strain susceptibility of the effects of glyphosate acid on the salivary gland after 4 weeks administration in these strains of rat. In studies with F344 rats, glyphosate acid has been shown to cause effects on the salivary gland (NTP, 1992[38]). In contrast, there was no evidence of microscopic changes in the salivary gland in a previously conducted 28 day feeding study with glyphosate acid (20000 ppm in the diet) in Alpk:AP$_f$SD rats, although there was an effect on gland weight ([redacted] 1995)[39].

Study groups of 24 male Alpk:AP$_f$SD (Wistar-derived; AP), Sprague-Dawley (Charles River CD; CD) and Fischer 344 (F344) rats received 0 or 20000 ppm glyphosate acid. Eight animals from each group were killed on Day 29 and the remaining animals were retained without treatment for a further 4 (8 rats/group) or 13 weeks (8 rats/group). Clinical observations, bodyweights and food consumption were measured and at the end of the scheduled periods, the animals were killed and subjected to a necropsy. Salivary glands were weighed and taken for subsequent histopathology examination.

Treatment with 20000 ppm glyphosate acid produced significant reductions in bodyweight and minor reductions in food consumption in AP and CD rats but no effects on bodyweight or food consumption were seen in the F344 rat. In contrast, salivary gland weight was unaffected in the CD rat but was increased in both AP and F344 rats at the end of the administration period. Microscopic examination of the salivary glands showed the most pronounced effect occurred in the F344 strain where there was diffuse cytoplasmic basophilia and enlargement of the parotid acinar cells. Similar but slight effects involving small foci of cells only occurred in the AP and CD strains.
Recovery of effects was apparent in all strains during the recovery periods. Bodyweight and food consumption returned to control values in both AP and CD strains. After four weeks on control diet, significant recovery of the salivary gland changes, in terms of both weight and micropathology, was evident in the F344 strain and the AP and CD rats were indistinguishable from their corresponding controls. After 13 weeks on control diet slightly more treated F344 rats showed minor focal changes in the salivary gland compared to the contemporaneous controls and group mean salivary gland weights were increased slightly.
Administration of diets containing 20000 ppm glyphosate acid to male rats for 4 weeks produced marked strain differences in the severity of effect in the parotid salivary gland. Microscopic examination of the salivary glands showed the most pronounced effect in the F344 strain. Similar but slighter effects occurred in the AP and CD strains.
Complete recovery of effects were apparent in AP and CD strains following the 4-week recovery period and significant recovery had occurred in the F344 strain. It is not clear whether the slightly higher incidence of minor focal changes in the salivary glands of the F344 strain after 13-week recovery was a residual effect of treatment or represented the random variation in the background incidence in this strain.

[38] NTP (1992). Technical Report on Toxicity Studies of Glyphosate Administered in Dosed Feed to F344/N Rats and B6C3F1 Mice. United States Department of Health and Human Services, National Toxicology Program Toxicity Reports Series Number 16

[39] [redacted] (1995) 28day dietary toxicity study in the rat. [redacted]/L/6624

I. MATERIALS AND METHODS

A. MATERIALS

Test materials:

Identification:	Glyphosate acid
Description:	White solid
Lot/Batch #:	P24
Purity:	95.6 %
Stability of test compound:	No data given in the report
Vehicle and/ or positive control:	Plain diet
Test animals:	
Species:	Rats
Strain 1:	Alpk:AP$_f$SD
Source:	[redacted]
Weight at dosing:	175.0 – 176.1 g
Strain 2:	Sprague-Dawley
Source:	[redacted]
Weight at dosing:	179.6 – 181.5 g
Strain 3:	Fischer 344
Source:	[redacted]
Weight at dosing:	107.4 – 108.9 g
Age:	approx. 28-30 days (on delivery)
Sex:	Males
Acclimation period:	11-13 days
Diet/Food:	CT1 diet (supplied by Special Diet Services Limited, Witham, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Animals were housed by strain and four per cage.
Environmental conditions:	Temperature: 21 ± 3 °C Humidity: 50 ± 20 % Air changes: at least 15/hour Photocycle:12 hours light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 1996-01-15 to 1996-05-14

Animal assignment and treatment

In a 28 days feeding study groups of 24 male Alpk:AP$_f$SD (Wistar-derived; AP), Sprague-Dawley (Charles River CD; CD) and Fischer 344 (F344) rats received 0 or 20000 ppm glyphosate acid. Eight animals from each group were killed on Day 29 and the remaining animals were retained without treatment for a further 4 (8 rats/group) or 13 weeks (8 rats/group).

Two test diet batches were prepared prior to start of treatment by mixing 1255 g test substance to 58.745 kg diet and blending. Samples of both preparations were analysed to verify the achieved concentration.

Clinical observations
Cage-side observations were performed daily. A detailed physical examination was performed prior to administration and weekly thereafter. Detailed clinical examinations were performed at the same time as body weights were recorded.

Body weight
Individual body weights were recorded on start of administration and weekly thereafter.

Food consumption and compound intake
Food consumption was recorded continuously throughout the study for each cage of rats and calculated as a weekly mean (g food/rat/day) for each cage.

Sacrifice and pathology
All animals were killed by halothane anaesthesia and subjected to a gross examination of the salivary glands. The salivary glands were weighed (left and right separately) and microscopically examined (left) in animals surviving until the end of the study.

Statistics
All data were evaluated using analysis of variance and/or covariance by the GLM procedure in SAS (1989). Least-squares means for each group were calculated using the LSMEAN option in SAS PROC GLM. Unbiased estimates of differences from control were provided by the difference between each treatment group least-squares mean and the control group least-squares mean. Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean using a two-sided Student's t-test, based in the error mean square in the analysis.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS
The mean achieved concentration of glyphosate acid in both batches of diet was within 2 % of the target concentration (see table below).

Table 5.8.2-8: Glyphosate Acid: Comparison of Salivary Gland Effects in Three Strains of Rat ([redacted] 1996): Achieved concentrations of glyphosate acid in the diet

	Nominal concentration (ppm)	Mean analysed concentration (ppm)	% of nominal concentration
Batch 1	20,000	19,985	99.9
Batch 2	20,000	20,355	101.8

B. MORTALITY
There were no treatment-related deaths. One treated AP rat was killed in Week 7 following accidental damage to its snout.

C. CLINICAL OBSERVATIONS
There were no treatment-related findings in any of the groups noted during the study period.

D. BODY WEIGHT
AP rats
During the administration period significantly lower group mean bodyweight than for control was seen. At the end of the administration period the difference was approximately 7 %. The reduction in bodyweight was maintained during the 4-week recovery period (approximately 7 % at the end of Week 9) but no

differences in bodyweight were apparent by the end of the 13-week recovery period.

CD rats
Group mean bodyweights for treated animals were significantly reduced during the administration period in comparison to controls. The reduction in bodyweight was approximately 7 % (after adjusting for initial bodyweight) at the end of the administration period. However, bodyweights quickly recovered and were approximately 4 % lower (not statistically significant, after adjusting for initial body weight), compared with control, after the 4-week recovery period, and 5 % higher than controls (after adjusting for initial body weight) by the end of the 13-week recovery period.

F344 rats
No treatment related effects were observed.

E. FOOD CONSUMPTION
AP rats
Overall, food consumption in the treated group tended to be slightly lower than the control during the administration period although this achieved statistical significance only in Week 7. No effects were seen at the end of the recovery period.

CD rats
Group mean food consumption for treated animals was generally lower than controls during the administration period, reaching statistical significance in Week 2 and 4. Food consumption for the recovery animals returned to control levels by Week 8.

F344 rats
There was no evidence of any treatment related effects.

F. NECROPSY
Organ weights
There was no evidence of any effects of glyphosate acid on the salivary gland weight at any time point in CD rats. On the contrary, salivary gland weights were increased in the treated AP and F344 rats at the end of the administration period in comparison to control. While no effects were noted in the 4- or 13-week recovery AP animals, in F344 rats the salivary gland weights were still increased at these time points, although there was clear evidence of recovery.

Table 5.8.2-9: Glyphosate Acid: Comparison of Salivary Gland Effects in Three Strains of Rat ([redacted]., 1996): Mean salivary gland weights at necropsy

Organ	AP		CD		F344	
	0	20000 ppm	0	20000 ppm	0	20000 ppm
Terminal weight (g)						
Left salivary gland	0.652	0.740*	0.715	0.695	0.461	0.666**
Right salivary gland	0.523	0.659*	0.623	0.626	0.422	0.577*
Weight after 4 week recovery (g)						
Left salivary gland	0.748	0.703	0.844	0.742	0.488	0.555
Right salivary gland	0.639	0.623	0.701	0.637	0.428	0.505*
Weight after 13 week recovery (g)						
Left salivary gland	0.750	0.760	0.790	0.819	0.623	0.612
Right salivary gland	0.669	0.681	0.668	0.705	0.495	0.528

* Statistically significant difference from the control group mean at $p<0.05$(Student's t-test, two-sided).
** Statistically significant difference from the control group mean at $p<0.01$ (Student's t-test, two-sided).
n = 8 animals/study group

Macroscopic findings
No macroscopic abnormalities were seen in salivary glands in any rat, either at the end of the administration period or after the 4- or 13-week recovery periods.

Microscopic findings
Treatment-related findings were confined to the parotid salivary gland and comprised alteration in the staining of the cytoplasm of the acinar cells. The affected cells appeared strongly basophilic and enlarged (recorded as basophilia of parotid acinar cells).
At the end of the four-week administration period, this change was most prominent in F344 rats. All rats showed marked cytoplasmic basophilia that was diffuse, involving the whole of the parotid gland. However, no evidence of cell degeneration or necrosis was seen. Most of the control F344 rats also showed a minor degree of basophilia involving occasional acinar cells only.
The other two strains, AP and CD, both showed the same effect in the parotid gland after four weeks of treatment, but at a much reduced severity compared to the F344. In addition, the distribution was different in that only small focal groups of acinar cells were affected in the AP and CD rats in contrast to the diffuse involvement seen in the F344. The effect was weakest in the CD rat.

The incidence data at the end of the administration period indicate that the background change varies in control rats in the three strains. None was seen in the AP controls, there was a single CD control rat with a minimal focal change, whereas 7 out of 8 F344 controls showed minor changes.
After four weeks recovery in the F344 strain the severity of the parotid basophilia was reduced to minimal or slight and affected small foci of acinar cells only. No changes were seen in the CD rats and only a single AP rat showed a minimal change. As an AP control rat showed changes at this time point this is considered not to be related to treatment.
After 13 weeks recovery no treatment-related changes were seen in the AP and CD strains. Slightly more of the F344 rats showed minor focal changes compared to the corresponding control group but this may reflect variations in the background spontaneous change rather than a residual effect of treatment.

Table 5.8.2-10: Glyphosate Acid: Comparison of Salivary Gland Effects in Three Strains of Rat ([redacted] 1996): Histopathological findings in salivary glands

Finding	AP		CD		F344	
	0	20000 ppm	0	20000 ppm	0	20000 ppm
Termination*						
Atrophy (marked)	0 / 8	0 / 8	1/8	0 / 8	0 / 8	0 / 8
Interstitial fibrosis (marked)	0 / 8	0 / 8	1/8	0 / 8	0 / 8	0 / 8
Basophilia of parotid acinar cells (minimal/slight/moderate/marked)	0 / 8	8 / 8 (1/6/1/0)	1/8 (1/0/0/0)	7 / 8 (4/1/2/0)	7 / 8 (6/1/0/0)	8 / 8 (0/0/0/8)
Microscopic findings after 4 week recovery*						
Mononuclear cell infiltration (minimal)	0 / 8	1 / 8	0 / 8	0 / 8	1 / 8	0 / 8
Basophilia of parotid acinar cells	1 / 8	1 / 8	0 / 8	0 / 8	0 / 8	6 / 8
Mucous metaplasia of parotid (slight)	0 / 8	1 / 8	0 / 8	0 / 8	0 / 8	0 / 8
Microscopic findings after 13 week recovery*						
Mononuclear cell infiltration (minimal)	0 / 8	0 / 7	0 / 8	0 / 8	1 / 8	1 / 8
Atrophy (minimal)	0 / 8	0 / 7	0 / 8	0 / 8	1 / 8	0 / 8
Basophilia of parotid acinar cells	1 / 8	1 / 7	1 / 8	1 / 8	1 / 8	5 / 8

*number of animals affected / total number of animals examined

Study conclusion
Administration of diets containing 20000 ppm glyphosate acid to male rats for 4 weeks produced marked strain differences in the severity of effect in the parotid salivary gland. Microscopic examination of the

salivary glands showed the most pronounced effect occurred in the F344 strain where there was diffuse cytoplasmic basophilia and enlargement of the parotid acinar cells. Similar but slighter effects occurred in the AP and CD strains involving small foci of cells only.
Complete recovery of effects was apparent in AP and CD strains following the 4-week recovery period and significant recovery had occurred in the F344 strain. It is not clear whether the slightly higher incidence of minor focal changes in the salivary glands of the F344 strain after 13-week recovery was a residual effect of treatment or represented the random variation in the background incidence in this strain.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:
Administration of diets containing 20000 ppm glyphosate acid to male rats for 4 weeks produced marked strain differences in the severity of effect in the parotid salivary gland. Microscopic examination of the salivary glands showed the most pronounced effect occurred in the F344 strain where there was diffuse cytoplasmic basophilia and enlargement of the parotid acinar cells. Similar but slighter effects occurred in the AP and CD strains involving small foci of cells only. A complete recovery from the effects on the salivary gland was observed in the AP and CD strains following a 4-week recovery period. A significant, though incomplete, recovery had occurred in the F344 strain, supporting the hypothesis that low pH diets induce a non-adverse adaptive response in salivary glands. Following a 13-week recovery period a slightly higher incidence of minor focal changes in the salivary glands of the F344 strain treatment group was noted, compared with the F344 strain control group and compared with the AP and CD strain. The study is considered acceptable.

Assessment and conclusion by RMS:

CA 5.8.2/004

1. Information on the study

Data point:	CA 5.8.2
Report author	[redacted]
Report year	1996
Report title	Glyphosate Technical: Pharmacology Screening Study in the Rat
Report No	434/021
Document No	Not reported.
Guidelines followed in study	JMAFF, 59 Nohsan No. 4200 (1985)
Deviations from current test guideline	Not applicable.
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes, conducted under GLP.

Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The test material was evaluated for evidence of pharmacological activity using a series of *in vivo* and *ex vivo* screening methods. For *in vivo* studies five male and five female rats were dosed with glyphosate technical at a dose level of 5000 mg/kg with similar sized control groups receiving vehicle only. Approximately one hour after dosing control and treated animals were examined for either haematological changes, electrocardiographic changes or behavioural/functional changes. *Ex vivo* studies on the isolated guinea pig ileum and isolated rat gastrocnemius muscle were performed using saturated solutions of the test material.

***In vivo* studies**
There were no differences in response between treated and control animals.

***Ex vivo* studies**
Glyphosate Technical (12 mg/mL) caused a contractile response to isolated guinea pig ileum similar to that seen with acetylcholine. The effect seen was abolished when the ileum was pre-incubated with atropine sulphate.
Injection of tubocurarine resulted in a significant diminution of the contractile response of the rat gastrocnemius muscle when the sciatic nerve was stimulated. On the contrast, there was no effect on muscle contraction when either glyphosate technical or physiological saline was injected.

Conclusion
At a maximum oral gavage dose level of 5000 mg glyphosate technical/kg bw there were no effects seen from the *in vivo* screens performed. When administered to the isolated guinea pig ileum glyphosate technical caused a contractile response similar to that seen with known parasympathomimetic agents. Evaluation of innervated muscle response showed that glyphosate technical, when administered at the maximum solubility concentration in physiological saline, did not cause any neuromuscular blocking activity.

I. MATERIALS AND METHODS

A. MATERIALS

Test materials:

Identification:	Glyphosate Technical	
Description:	White powder	
Lot/Batch #:	H95D161A	
Purity:	95.3 %	
Stability of test compound:	No data given in the report.	
Vehicle and/ or positive control:	*in-vivo*	1 % carboxymethyl cellulose
	ex-vivo (guinea pig)	distilled water, krebs physiological buffer solution,
	ex-vivo (guinea pig)	physiological saline

Test animals:

in-vivo	Species:	Rats

Strain:	Sprague-Dawley (CD)
Source:	[redacted]
Age:	no data
Sex:	Males and females
Weight at dosing:	176 - 200 g
Acclimation period:	At least 6 days
Diet/Food:	SQC Rat and Mouse Diet No.1 Expanded (Special Diets Services Ltd., Witham Essex, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	By sex in groups of five in polypropylene cages with stainless steel grid floors.
Environmental conditions:	Temperature: 19 – 25 °C Humidity: 40 – 75 % Air changes: at least 15/hour Photocycle: 12 hours light/dark cycle
ex-vivo Species:	Guinea pig
Strain:	Dunkin Hartley
Source:	[redacted]
Age:	no data
Sex:	Males
Weight at dosing:	250 - 300 g
Acclimation period:	no data
Diet/Food:	Guinea Pig FDI Diet (Special Diets Services Ltd., Witham Essex, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	By sex in groups of up to three in polypropylene cages with solid floors and sawdust bedding.
Environmental conditions:	Temperature: 17 – 23 °C Humidity: 30 – 70 % Air changes: at least 15/hour 12 hours light/dark cycle
in-vivo Species:	Rats
Strain:	Sprague-Dawley (CD)
Source:	[redacted]
Age:	no data
Sex:	Males and females
Weight at dosing:	110 - 125 g
Acclimation period:	no data
Diet/Food:	SQC Rat and Mouse Diet No.1 Expanded (Special Diets Services Ltd., Witham Essex, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	By sex in groups of five in polypropylene cages with stainless steel grid floors.

Environmental conditions: Temperature: 19 – 25 °C
Humidity: 30 – 70 %
Air changes: at least 15/hour
Photocycle: 12 hours light/dark cycle

B. STUDY DESIGN AND METHODS

Test material preparation

In vivo

The test material was suspended daily in 1 % carboxymethyl cellulose (vehicle) by weighing an amount into a suitable container and adding vehicle to make the appropriate final volume. Homogeneity was assured by mixing the formulations with a Silverson mixer/homogeniser.

Ex vivo – Guinea pig ileum

The test material was dissolved initially in distilled water and subsequently in freshly prepared Krebs physiological buffer solution. A saturated solution was used. The test material/buffer solution was shaken until the test material was dissolved.

Ex vivo – Rat gastrocnemius muscle

The test material was dissolved in sterile physiological saline. A saturated solution was used. The test material/vehicle was shaken until the test material was dissolved.

The test material was dissolved at the maximum solubility of 12 g/L for all *ex vivo* studies.

Methods for *in vivo* studies

Dose rationale

The selected dose level (5000 mg/kg bw) was based on the results of an acute oral toxicity study in rats previously conducted by the laboratory.

Group allocation and dosing

Animals were dosed by oral gavage administration once only on the day allocated for each dose group as determined by the type of evaluation specified.

Table 5.8.2-11: Glyphosate Technical: Pharmacology Screening Study in the Rat ([redacted], 1996): Study design

Group	Males	Females	Evaluation	Dose (mg/kg bw)	Dose volume (mL/kg bw)
1	5	5	Haematology	0*	10
2	5	5		5000	10
3	5	5	Electrocardiography	0*	10
4	5	5		5000	10
5	5	5	Behaviour/functional tests	0*	10
6	5	5		5000	10

*Control animals were treated with vehicle only.

Haematology

Approximately one hour after dosing blood samples were taken via a marginal tail vein from all animals from Groups 1 and 2. The blood was collected into tubes containing Potassium EDTA anticoagulant. For measurement of clotting (prothrombin) time samples were collected into tubes containing sodium citrate (0.11 mol/L).

The following parameters were evaluated: Haemoglobin (Hb), Total erythrocyte count (RBC), Haematocrit

(Hct), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC), Total Leucocyte Count (WBC), Platelet Count (PIT), Clotting (Prothrombin) time (CT).

Electrocardiography (ECG)
Approximately one hour after dosing animals from Groups 3 and 4 were anaesthetised using halothane anaesthesia and assessed for cardiac activity using an electrocardiograph. A limb lead was attached to each limb and connected to the electrocardiogram. The equipment was set to lead II measurement at a sensitivity of either 10 mm/mvolt or 5 mm/mvolt and a chart speed of 25 mm/second. Alternative control and treated animals were evaluated.

The following parameters are measured: Heart rate, P-R interval, QRS interval, Q-T interval, P-amplitude, R-amplitude, and T-amplitude.

Behavioural/functional tests
Approximately one hour after dosing animals from Groups 5 and 6 were placed individually in a purpose built arena and assessed for behaviour and response to various stimuli using a modified Irwin Screen.

The following observations were performed: salivation, hypo/hyperthermia, skin colour, respiration, lacrimation, palpebral closure, piloerection, exophthalmia, gait, twitches, tremors, convulsions, abnormal behaviour, tail elevation, transfer arousal, urination count, defaecation count and vocalisation count.
The following manipulative tests were performed: Finger Approach, Touch Escape, Tail Pinch, Toe Pinch, Grasp Response, Auditory Startle Response, Pupil Response to Light and Palpebral Reflex.

Evaluation of isolated guinea pig ileum
Sections of ileum were dissected from previously untreated guinea pigs killed by cervical dislocation. The sections of ileum were transferred to a purpose-built isolated organ bath containing Krebs buffer solution. The isolated ileum was connected to the lever arm of an isotonic transducer by a cotton ligature. The transducer was connected to a chart recorder. Contractions of the isolated ileum could then be recorded. Standard solutions of acetylcholine, a known agonist, were prepared and added to the volume of buffer solution used to bathe the isolated ileum. A maximum volume of 2 mL was used for all experiments to ensure the integrity of the tissue in the medium. The contraction response of isolated ileum was recorded for each concentration of acetylcholine to produce a standard curve. Between the addition of each new concentration of acetylcholine, the buffer in the organ bath was flushed out and replaced by fresh buffer. The test material, dissolved in buffer, was added and its response compared with standards.
Following initial results, an antagonist (atropine) to the effects of acetylcholine was added together with the agonist. The results were then compared with the effects of only an antagonist added to the test material.

The following were recorded: response to acetylcholine (agonist, sympathomimetic), response to test material, response to atropine (antagonist) and acetylcholine (agonist), and response to atropine (antagonist) and test material.

Evaluation of rat gastrocnemius muscle
Previously untreated rats were killed by cervical dislocation. The abdomen was immediately dissected open and the dorsal aorta exposed. A butterfly needle was inserted into the dorsal aorta, near to the bifurcation in a posterior direction.
A volume of 0.3 mL of lithium heparin at a concentration of 10 mg/mL in sterile saline was injected into the dorsal aorta followed by 0.5 mL of sterile saline. The gastrocnemius muscle of the hind limb was exposed with the sciatic nerve intact. The gastrocnemius muscle was detached from the ankle joint and this area was ligated with cotton, which was then attached to the lever arm of a transducer. The limb was held in place by a series of pins. An electrical stimulus of 12 volts was applied to the sciatic nerve and the muscle response was recorded. This action was repeated at approximately twelve—second intervals until sufficient responses had been recorded.
The experiment was repeated on separate animals with doses of tubocurarine (positive control) injected into

the dorsal aorta instead of sterile saline. The experiment was also repeated on a separate animal with the test material dissolved in sterile saline.

The following were recorded: Response to injection of sterile saline, response to injection of tubocurarine, and response to injection of test material.

Evaluation of data

In vivo

Data were processed to give group mean values and standard deviations where appropriate.

Evaluation of electrocardiographic outputs

All measurements were performed using a vernier calliper with digital readout. All measurements were recorded twice in order to minimise operator bias.

Isolated guinea pig ileum

The final concentration of test material in the organ bath was based on the serial dilution value plus the additional dilution in the total volume of the organ bath (50 mL).

II. RESULTS AND DISCUSSION

In vivo studies

Evaluation of blood parameters

There were no biologically significant differences, among the parameters measured, between treated and control animals.

Table 5.8.2-12: Glyphosate Technical: Pharmacology Screening Study in the Rat ([redacted], 1996): Group mean haematology values

Parameter	0 mg/kg bw		5000 mg/kg bw	
	Male (n=5)	Female (n=5)	Male (n=5)	Female (n=5)
WBC	14.2 ±3.2	11.0 ±2.2	12.8 ±3.9	10.6 ±2.6
RBC	6.8 ±0.3	6.8 ±0.3	6.6 ±0.3	6.8 ±0.3
HB	14.2 ±0.3	14.1 ±0.4	14.8 ±0.6	14.6 ±0.4
HCT	41.9 ±1.0	41.6 ±0.9	42.5 ±1.1	42.1 ±1.9
MCV	61.8 ±2.4	61.6 ±2.7	64.0 ±2.5	61.5 ±1.4
MCH	20.9 ±0.9	20.8 ±1.0	22.2 ±0.8	21.4 ±0.6
MCHC	33.9 ±0.5	33.8 ±0.9	34.8 ±0.6	34.7 ±0.6
PLT	1079.2 ±169.9	1020.0 ±55.7	1024.8 ±89.5	1164.0 ±160.9
Clotting Time (seconds)	28.8 ±1.7	26.2 ±1.3	27.3 ±1.2	26.3 ±2.2

Evaluation of cardiovascular system

One female of the control group had cardiac arrhythmia characterised by extra systoles. There were no biologically significant differences in the mean values recorded during electrocardiography (ECG) for wave amplitudes, wave intervals or heart rate.

Table 5.8.2-13: Glyphosate Technical: Pharmacology Screening Study in the Rat ([redacted], 1996): Group mean ECG values

Group Sex	Dose		Amplitude (mv)			Interval (msec)			Heart rate (bpm)
			P	R	T	P-R	QRS	Q-T	
3 male	0 mg/kg bw	**Mean**	**0.09**	**0.70**	**0.08**	**43.20**	**16.60**	**32.68**	**408.0**
		SD	0.02	0.23	0.09	6.32	7.77	10.37	22.6
		N	5	5	5	5	5	5	5

3 female		**Mean**	**0.09**	**0.59**	**0.06**	**49.20**	**15.00**	**35.70**	**441.0**
		SD	0.03	0.13	0.02	4.41	5.91	12.28	34.4
		N	4	4	4	4	4	4	4
4 male	5000 mg/kg bw	**Mean**	**0.12**	**0.68**	**0.11**	**37.20**	**17.84**	**31.00**	**441.6**
		SD	0.05	0.19	0.06	8.62	7.50	11.95	20.2
		N	5	5	5	5	5	5	5
4 female		**Mean**	**0.10**	**0.75**	**0.09**	**37.08**	**17.00**	**35.36**	**415.2**
		SD	0.02	0.13	0.03	9.22	2.33	5.16	34.5
		N	5	5	5	5	5	5	5

bpm = beats per minute, SD = standard deviation

Behavioural and functional tests

There were no biologically significant differences in behaviour and response to manipulative tests. The intergroup variances in response were considered to be within normal variation for this model.

Table 5.8.2-14: Glyphosate Technical: Pharmacology Screening Study in the Rat ([redacted], 1996): Group mean summary of behavioural assessments*

Parameter	0 mg/kg bw														5000 mg/kg bw													
	Male (n=5)							Female (n=5)							Male (n=5)							Female (n=5)						
	Number of animals classified as (score)																											
	0	1	2	3	4	5	6	0	1	2	3	4	5	6	0	1	2	3	4	5	6	0	1	2	3	4	5	6
Transfer arousal					5							5					1		4							4		
Tail elevation										5															4	1		
Finger approach					4		1					5					2		3							4		1
Touch escape			1		1		3					1		4					4		1					3		2
Tail pinch			1		4												2		3									
Grasp response			4		1			2		1		2			1		2		2			2		2		1		
Vocalisation	4		1					2		2			1		4			1				2	2	1				
Toe pinch					2		3													1	4							

Comment of assessor: only parameters with values differing between treated and control groups listed: no differences between treated and control groups were seen for all other behavioural/functional parameters that had been assessed. Scores 0-6 were not clearly explained in study report, however higher scores were indicative for higher grade of activity.

Evaluation of isolated guinea pig ileum

The addition of acetylcholine to the medium containing the isolated guinea pig ileum resulted in contraction of the tissue in a concentration—related response. Incubation with atropine sulphate immediately prior to addition of acetylcholine reduced or stopped the contraction response in a concentration related manner.
The addition of Glyphosate Technical at the maximum solubility in buffer also resulted in contraction of the ileum. The force of contraction was increased by an increasing volume of the test material in solution. Incubation with atropine sulphate prior to addition of Glyphosate Technical also resulted in the lack of a contractile response.

Table 5.8.2-15: Glyphosate Technical: Pharmacology Screening Study in the Rat ([redacted], 1996): Response of guinea pig ileum to a known agonist and glyphosate

Agonist		Height of response (mm)	% of maximum response
Test material	**Final concentration**		
Acetylcholine bromide	2.2×10^{-6} m	39	100
	1.8×10^{-6} m	33.5	85.9
	1.5×10^{-6} m	25.5	65.4
	1.0×10^{-6} m	15	38.5
Glyphosate (in distilled water)	4.26×10^{-4} m	7	17.9
	7.1×10^{-4} m	19	48.7

Glyphosate (in Krebs buffer)	7.1 x 10^{-4} m	12	30.8
	1.42 x 10^{-3} m	11	28.2
	2.84 x 10^{-3} m	24.5	62.8

Table 5.8.2-16: Glyphosate Technical: Pharmacology Screening Study in the Rat ([redacted], 1996): Response of guinea pig ileum to a known agonist and glyphosate in the presence of a known antagonist

Agonist		Antagonist		% of maximum response
Test material	Final concentration (M)	Test material	Final concentration (M)	
Acetylcholine bromide	3.0 x 10^{-6}	---		118.8
				108.2
				100
	1.5 x 10^{-6}			54.1
	1.0 x 10^{-6}			47.1
	4.0 x 10^{-7}			12.9
	2.0 x 10^{-7}			8.2
	3.0 x 10^{-6}	Atropine sulphate	1.0 x 10^{-8}	0
			5.0 x 10^{-9}	18.8
			2.0 x 10^{-9}	76.5
	2.0 x 10^{-6}		2.0 x 10^{-9}	30.6
Glyphosate	2.84 x 10^{-3}	---		62.4
				41.2
	2.84 x 10^{-3}	Atropine sulphate	4.0 x 10^{-9}	0
				0

Table 5.8.2-17: Glyphosate Technical: Pharmacology Screening Study in the Rat ([redacted] 1996): Response of guinea pig ileum to a known agonist and glyphosate in the presence of a known antagonist

Agonist		Antagonist		% of maximum response
Test material	Final concentration (M)	Test material	Final concentration (M)	
Acetylcholine bromide	2.0 x 10^{-6}	---		62.1
	3.0 x 10^{-6}			63.8
				70.0
				79.3
				100
	3.0 x 10^{-6}	Atropine sulphate	4.0 x 10^{-9}	0
			2.0 x 10^{-9}	0
Glyphosate	1.42 x 10^{-3}	---		13.8
	2.84 x 10^{-3}			44.8
	2.84 x 10^{-3}	Atropine sulphate	2.0 x 10^{-9}	3.4
				0
			4.0 x 10^{-9}	0

Evaluation of rat gastrocnemius muscle

Injection of tubocurarine, a known neuromuscular blocking agent, at a concentration of 25 mg/mL resulted in a significant reduction of the contractile response of the rat gastrocnemius muscle when the sciatic nerve was stimulated. There was no effect on muscle contraction when either Glyphosate Technical (12 mg/mL) or physiological saline was injected. The difference in force of response seen with Glyphosate Technical and physiological saline can be attributed to individual animal variation.

Table 5.8.2-18: Glyphosate Technical: Pharmacology Screening Study in the Rat ([redacted] 1996): Comparison of force of contraction of innervated skeletal muscle (gastrocnemius)

Test material	Concentration	Range of response in mm (n = 5)	Average response (mm)	% of control
Physiological saline		21 - 28	24.8	100
Tubocurarine	25 mg/mL	3 - 6	4.6	18.5
Glyphosate Technical	12 mg/mL	23 - 38	32.4	130.6

Study conclusion
At a maximum dose level of 5000 mg/kg bw glyphosate technical no effects were seen in the *in vivo* screens performed. When administered to the isolated guinea pig ileum *ex vivo*, glyphosate technical concentrations at millimolar (mM) levels caused a contractile response similar to that seen with a known parasympathomimetic agent at micromolar (µM) levels.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:
In vivo
At a maximum dose level of 5000 mg glyphosate technical/kg bw no biologically relevant effects on the treated animals were seen in the *in vivo* screens performed. During extensive clinical observations parameters indicative for a disturbance of the animal's health status, such as hypo/hyperthermia, skin colour, lacrimation, respiration, palpebral closure, and salivation were similar between vehicle control and treated rats. This confirmed that the selected dose level had no toxic effect on the rats. The intergroup variances in response to manipulative tests can be considered as normal biological variation.
Similarly, there were no relevant differences between treated and control animals in the haematological and cardiac parameters measured.

Ex vivo: Muscle contractibility
Evaluation of innervated rat gastrocnemius muscle response showed that glyphosate, when administered at the maximum solubility concentration in physiological saline, did not cause any neuromuscular blocking activity. The effect of glyphosate technical was directly compared to that of a known neuromuscular blocking agent (atropine sulphate), which caused a significant reduction in the muscle response to electrical stimulation of the sciatic nerve.

Ex vivo: Intestinal contractibility
Addition of acetylcholine as a positive control substance to isolated guinea pig ileum resulted in contraction in a concentration-dependent response at micromolar (µM) concentrations. Atropine sulphate immediately prior to addition of acetylcholine reduced or stopped the contraction response in a concentration related manner. This confirmed the reliability of the methodology. Glyphosate caused a contractile response of isolated guinea pig ileum at much higher millimolar (mM) concentrations, which could be antagonized with atropine. Thus, a parasympathomimetic activity of glyphosate was observed in this *ex vivo* model at high concentrations.

The study used appropriate negative control animals, as well as appropriate positive control substances (agonists and antagonists). The age of study animals was not stated. The study is considered acceptable.

Assessment and conclusion by RMS:

CA 5.8.2/005

1. Information on the study

Data point:	CA 5.8.2
Report author	[redacted]
Report year	1992
Report title	Ammonium Salt of Glyphosate (Mon-8750): General Pharmacological Study
Report No	[redacted] 90-0149/[redacted]-92-15
Document No	Not reported.
Guidelines followed in study	None stated.
Deviations from current test guideline	Not applicable.
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes.
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Effects of a single intraperitoneal (mice) or single intravenous (rabbits) administration of the ammonium salt of glyphosate (purity: 94.78 %) on clinical appearance, and nervous, respiratory and circulatory system were assessed. Groups of three male and female ICR mice (15 male and 15 female in total) received 78, 313, 1250, or 5000 mg glyphosate/kg bw intraperitoneally. Groups of three male rabbits (30 male in total) received 7.81, 31.3, 125 or 500 mg glyphosate/kg bw intravenously. Mice and half of the male rabbits were clinically observed at frequent intervals after dosing for up to seven days. The other half of male rabbits was used for digital monitoring of respiratory and circulatory parameters under urethane-anaesthesia.

At the top dose levels, all mice died within 0.5 hours and all anesthetized rabbits within a few minutes after injection. Non-anesthetized rabbits survived intravenous application of 500 mg/kg bw (dose resulting from highest water soluble concentration) although animals showed decrease in spontaneous activity and increase in respiratory rate. The rabbits completely recovered within 3 hours. In rabbits, which died, heart rate, blood pressure and respiratory rate decreased and voltage of QRS complex in electrocardiogram were noted. At the next lower dose levels (1250 mg/kg bw in mice or 125 mg/kg bw and 31.3 mg/kg bw in rabbits, respectively), transient symptoms like a decrease in blood pressure, reduced activity and neuromuscular signs were observed but cleared to normal values or behaviour within some hours at the latest. No abnormalities were observed in mice dosed at 313 mg/kg bw or less and in rabbits dosed at 7.81 mg/kg bw.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:	Ammonium salt of glyphosate (MON-8750)
Identification:	Not stated
Description:	Not stated
Manufacturer	Not stated
Lot/Batch #:	RUD-9201-3544F
Purity:	94.78 %
Storage conditions:	Sealed container in a refrigerator
Stability of test compound:	Not stated
Vehicle:	Physiological saline (0.9 % NaCl), pH 5.0

Test animals:

Species:	Mouse
Strain:	ICR (SPF)
Source:	[redacted]
Age:	7 weeks
Sex:	Male/female
Weight at dosing:	Males: 32 - 41 g, females : 26 - 33 g
Acclimation period:	At least 7 days
Diet/Food:	Certified pelleted diet MF (Oriental Yeast Co., Ltd., Tokyo), *ad libitum*
Water:	Water from a private well passed through precipitating and sedimentary procedures and sterilized by ultraviolet light and chlorine, *ad libitum*
Housing:	Three mice per aluminium cage
Environmental conditions:	Temperature: 22 ± 1 °C Humidity: 55 ± 5 % Air changes: 15/hour (all fresh air system) Photocycle: 12 hours light/dark cycle

Test animals:

Species:	Rabbit
Strain:	Japanese white strain (SPF)
Source:	[redacted]
Age:	10 weeks
Sex:	Males
Weight at dosing:	2.3 - 2.8 kg (clinical observation group) 2.4 - 3.0 kg (respiration, blood pressure, ECG group)
Acclimation period:	At least 7 days
Diet/Food:	Certified pelleted diet RC4 (Oriental Yeast Co., Ltd., Tokyo), *ad libitum*
Water:	Same as for mice
Housing:	Individually in aluminium cage
Environmental conditions:	Same as for mice

B. STUDY DESIGN

Table 5.8.2-19: Ammonium Salt of Glyphosate (Mon-8750): General Pharmacological Study ([redacted], 1992): Animal assignment and treatment

Parameter investigated	Species	Sex	Route	Volume (mL/kg bw)	Number of animals per dose				
					Intraperitoneal dose (mg/kg bw)				
					0	78	313	1250	5000
Clinical observation	Mouse	Male	Intraperitoneal	20	3	3	3	3	3
		Female			3	3	3	3	3
					Intravenous dose (mg/kg bw)				
					0	7.81	31.3	125	500 *
Clinical observation	Rabbit	Male	Intravenous	2	3	3	3	3	3
Respiration, blood pressure, ECG					3	3	3	3	3

ECG = electrocardiogram
Control groups received physiological saline at the same volume of administration
* highest soluble concentration for intravenous route

The test substance was dissolved in physiological saline (0.9 % NaCl) adjusting the pH to ca 5.0 by NaOH solution. Intraperitoneal and intravenous routes were chosen to achieve complete absorption of the test substance.

Clinical Observation in mice
Neuropharmacological changes of male and female mice were examined according to Irwin's method (Irwin 1967, in Animal and Clinical Pharmacologic Techniques in Drug Evaluation, Nodine and Siegler). Animals were observed prior to, and 5 min., 0.5, 1, 3, and 6 hours after dosing and then once a day for a week.

Clinical Observation in rabbits
Detailed observations were conducted according to the information/reference provided in the study report. Animals were observed prior to, and 5 min., 0.5, 1, 3, and 6 hours after dosing and then once a day for a week.

Monitoring of respiratory and circulatory parameters in rabbits
Three rabbits per dose were anesthetized with urethane (ca. 1.5 g/kg bw subcutaneous). Rabbits were hold in dorsal position. All parameters were recorded on a polygraph (RM-6000, Nihon Koden Inc., Tokyo). A homeothermic blanket (KN-474, Natsume Seisakusho Co., Ltd.) was used to keep the body temperature constant. The test substance was administered to a rabbit after steady recordings were obtained. Recordings were continued for 4 hours after dosing.

A thermistor type respiration sensor (TR-612T, Nihon Koden Inc.) was fixed to a cannula inserted into a cut opening of the trachea. Respiratory rate was measured by a digital counter (AT-601G, Nihon Koden Inc.). Blood pressure was measured via a cannula inserted into the left femoral artery connected to a high pressure transducer (TP-200T, Nihon Koden Inc.). Systolic, diastolic and mean blood pressures were measured by a digital counter (AP-611G, Nihon Kohden Inc.). ECG was recorded via electrodes fixed to the extremities by Lead II. Heart rate was measured from the pulse of blood pressure by a digital counter (AT-601G, Nihon Kohden Inc.)

Statistics
Student's t-test was used to compare a dosed group with the corresponding control group. Differences

between groups were considered to be statistically significant at the p≤0.05 level.

II. RESULTS AND DISCUSSION

A. CLINICAL OBSERVATIONS IN MICE

All mice of the top dose died within 0.5 h after application. Clinical signs observed after application consisted of manifestation of passivity, a decrease in spontaneous activity, reactivity, and pain response, clonic and tonic convulsions, a decrease in righting reflex, limb tone, body tone, abdominal tone, grip strength, corneal reflex and ipsilateral flexor reflex (IFR). In addition a decrease in alertness, abnormal posture (prone position) and abnormal skin color (cyanosis) was observed in male mice. Staggering gait was observed for females of the high dose.

At the mid dose group of 1250 mg/kg bw abnormal behaviour was observed in male and female mice for awareness, motoractivity, central nervous system (CNS), excitation, posture, motor incoordination, muscle tone, reflexes, and autonomic signs. Animals recovered within1 hour after administration.

No clinical signs were observed at the doses of 313 mg/kg bw or less.

Table 5.8.2-20: Ammonium Salt of Glyphosate (Mon-8750): General Pharmacological Study ([redacted] 1992): Summary of in vivo experiments in mice

Test			Intraperitoneal dose* (mg/kg bw)			
			78.1	313	1250	5000
Clinical observation	Male	Dead/dosed	0/3	0/3	0/3	3/3
		Abnormal signs	–	–	+	++
	Female	Dead/dosed	0/3	0/3	0/3	3/3
		Abnormal signs	–	±	+	++

* Three mice per sex and per dose were treated with a single intraperitoneal injection)
Rating: (–) no, (±) possible, (+) slight, (++) moderate, (+++) severe effect

B. CLINICAL OBSERVATIONS IN RABBITS

Rabbits in the 500 mg/kg bw dose group showed a decrease in spontaneous activity and an increase in respiratory rate within 0.5 hours after injection and completely recovered within 3 hours. No distinct abnormalities were observed at the doses of 125 mg/kg bw or less.

C. RESPIRATORY AND CIRCULATION SYSTEM IN RABBITS

A transient decrease in blood pressure during the injection was observed in rabbits treated with 31.3, 125 and 500 mg/kg bw. The decrease in blood pressure fully recovered to the control level within a few minutes after the injection at 31.3 and 125 mg/kg bw. Rabbits of the high dose groups showed a decrease in blood pressure, respiratory rate, heart rate, and a voltage of QRS complex in ECG, and died within a few minutes after the injection. The tidal volume (amplitude of respiration) was augmented after the injection. No treatment related effects were observed at 7.81 mg/kg bw.

Table 5.8.2-21: Ammonium Salt of Glyphosate (Mon-8750): General Pharmacological Study ([redacted], 1992): Summary of in vivo experiments in rabbits

Test				Intravenous dose* (mg/kg bw)			
				7.81	31.3	125	500
Clinical observation	Male	Non-anesthetized	Dead/dosed	0/3	0/3	0/3	0/3
			Abnormal signs	–	–	±	+
Respiration, Blood pressure and ECG	Male	Anesthetized	Dead/dosed	0/3	0/3	0/3	3/3
			Respiratory rate	–	–	–	+ (decrease)
			Blood	–	+	+	++ (decrease

	pressure		(decrease)	(decrease)	
	Heart rate	–	–	–	+ (decrease)
	ECG	–	–	–	+ (decrease in QRS complex)

* Three male rabbits were dosed for each test group by single intravenous injection)
Rating: (–) no, (±) possible, (+) slight, (++) moderate, (+++) severe effect

Study conclusion
Intravenous injection of the test substance produced no death in conscious rabbits at the dose of 500 mg/kg bw, which is a practically maximum dose based on its water solubility. These facts suggest that human risk after an incidental exposure of ammonium salt of glyphosate would be low in the field. It is suggested that the acute toxicity of the test substance is low. Serious hazards could not be expected in humans after an acute exposure of the test substance except for the case receiving an extraordinary large amount of the test substance.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:
A pharmacological study on the toxicity of the ammonium salt of glyphosate was conducted in mice and male rabbits. The test substance was dissolved in physiological saline and adjusted to pH 5 with sodium hydroxide. Different doses up to a top dose level of 5000 mg/kg bw was administered to mice by single intraperitoneal injection. In rabbits the top dose level administered by single intravenous injection was 500 mg/kg bw, which reflected the highest soluble concentration.

At the top dose levels, all mice died within 0.5 hours and all anesthetized rabbits within a few minutes after injection. Non-anesthetized rabbits survived intravenous application of 500 mg/kg bw. They showed transient clinical abnormalities, but fully recovered within 3 hours. At the next lower dose levels transient symptoms like a decrease in blood pressure, reduced activity and neuromuscular signs were observed but cleared to normal values or behaviour within some hours at the latest. At doses up to 125 mg/kg bw in mice and 7.8 mg/kg bw in rabbits no abnormalities were noted.

Based on the available data it cannot be assessed if there was an interaction with urethane used for anaesthesia and the test substance resulting in the lethality of rabbits dosed at the top dose or whether the surgery together with the high dose resulted in the mortality. The study is however not relevant for classification purposes and does also not need to be considered in human risk assessments.

Assessment and conclusion by RMS:

CA 5.8.2/006

1. Information on the study

Data point:	CA 5.8.2

Report author	[redacted]
Report year	1988
Report title	Toxicodinamic study of glyphosate in rat
Report No	Not stated
Document No	Not reported.
Guidelines followed in study	None stated.
Deviations from current test guideline	Not applicable.
Previous evaluation	Not accepted in RAR (2015).
GLP/Officially recognised testing facilities	No. (GLP was not compulsory at the time the study was performed; non-guideline, investigative study).
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

The toxicodynamics of glyphosate were examined in rats by the measurement of heart rate, ECG, venous- and arterial blood pressure and body temperature.

The administered dose of 5000 mg/kg bw glyphosate, a dose close to the oral LD_{50} in rats according to the literature, killed the animals narcotised with chloralose-urethane mixture within 2 to 7 hours. After treatment, decreased pulse and respiratory rate was observed along with decreased systemic arterial pressure. ECG-II and venous pressure changes were incidental and insignificant. The mean median mercury pressure of the artery carotis was 102 mm Hg before treatment, 73 mm Hg and 54—55 mm Hg one hour and 2-3 hours after treatment, respectively. Body temperature was unchanged due to the applied experimental procedure. White spots were seen in the edges of livers of the dead animals. Microscopically, the spots were free of erythrocytes. When blood samples were haemolysed with distilled water and analysed spectrophotometrically, an absorption maximum of 567 nm was found. The haematin hydrochloride peak was recorded at 680 nm.

I. MATERIALS AND METHODS

A. MATERIALS

Non-labelled test material: Glyphosate

Identification:	Technical grade glyphosate
Description:	White crystalline substance appearance
Lot/Batch #:	72390788
Purity:	96 %
Stability of test compound:	Not stated.

Test animals:

Species:	Rat
Strain:	S/Wistar (SPF breeding stock of [redacted])
Source:	Not stated.
Age:	Not stated.

Sex:	Males	
Weight at dosing:	>400 g	
Acclimation period:	Not stated.	
Diet/Food:	Not stated.	
Water:	Not stated.	
Housing:	Shoebox-type macrolone cages	
Environmental conditions:	Temperature:	Not stated.
	Humidity:	Not stated.
	Air changes:	Not stated.

B. STUDY DESIGN AND METHODS

The reported acute oral lethal dose of glyphosate is 4900 mg/kg bw in rats. For the present study 5000 mg/kg bw was selected as the dose so that the dose would terminate the animals within a day, and allow sufficient time for observations before death.

Anaesthesia was carried out by IV injection of an aqueous solution containing chloralose-urethane in a 1:5 ratio. Injection volume depended on the degree of anaesthesia. The trachea was prepared by a medium incision on the neck and cannulated. To record the respiratory rate and volume the cannula was connected to a Hellige pulsotachometer.

Blood pressure in the left articular carotis was recorded by an 8-channel Hellige polygraph, supplemented with a Statham transducer-electromanometer, with a selector for continuous systolic diastolic and medium pressure. Venous pressure of the right ventricular jugular at the thoracic level was also recorded by the Statham head electromanometer polygraph. Heparin was administered into the cannula only. Lead ECG-II was recorded continuously by means of electrodes positioned into the paw of the animals. Body temperature was measured by a rectally inserted thermistor tube connected to the polygraph.

After half an hour to allow for stabilisation of the above parameters, animals were treated with 5000 mg/kg bw test compound suspended in an aqueous solution of mucilage hydroxyacetyl cellulose. Recordings on the Hellige polygraph were measured in the following sequence: heart rate, ECG, respiration, venous-, arterial pressure, body temperature. Recordings were made with 0.05 and 50 mm/sec paper speed. However, in case of faster recordings, values corresponding to different time intervals were indicated on the paper by hand.

II. RESULTS AND DISCUSSION

Heart rate
At study initiation the mean heart rate (derived from 10 animals) was 462 ± 40 min^{-1} (a level acceptable in anaesthetised rats). One hour after administration of 5000 mg/kg bw glyphosate the mean pulse rate decreased by about 5 %. The mean pulse rate consisted of both increased and decreased individual values, which were, perhaps, without toxicological significance, as no similar changes were observed at later time intervals.

ECG
No specific lead-II alterations were observed, although elevated ST and R waves, increased QRS, wide and spiked T wave were occasionally seen. No test compound-related, specific abnormalities were detected even before the observed reduction in systemic pressure.

Respiration rate
The pre-treatment value (58/min) was slightly decreased 1 hour after treatment (55/min). Further decreases

were seen at later time points: pulse rate at the end of the 2nd hour was 54/min (i.e. 7 % lower than at study initiation).

Venous pressure
Most values were at baseline level (around 0 mmHg), the maximum/minimum alterations were within 5 mmHg.

Arterial pressure
Values at study initiation (mean value 102 mmHg; a range of 80- 130 mmHg) decreased about 30 % after 1 hour (mean value 73 mm Hg). The mean value after 2 hours was 54 mm Hg (i.e. 50 % of study initiation value).

Gross pathology
Dosing was performed faultless in each case. Thoracic and abdominal organs were examined in all animals. Urinary vesicles were full of urine, due to the chloralose anaesthesia. No macroscopic changes were observed in animals surviving for 23 hours. In animals who survived for up to 4 to 5 hours, disseminated white spots of 2 to 5 mm size were seen, the origin of which were not in the gastric wall or intestines. Following microscopic examination, it was evident that the isolated spots were free of erythrocytes.

***In vitro* studies with blood**
Blood was brown when dissolved in physiological saline. When crystallised glyphosate was added to untreated rat blood at room temperature, a discolouration was observed which started after 2 to 3 minutes and resulted in a stable brown colour after 5 minutes. Based on visual assessment, the brown solution was unchanged and without sediment after 24 hours, and recorded a maximum absorption at 657 nm spectrophotometrically. The absorption maximum was 680 nm in the same photometer.

Table 5.8.2-22: Toxicodinamic study of glyphosate in rat ([redacted], 1988): Effects of a 5000 mg/kg bw per oral dose of glyphosate on Wistar rats No. 1 to 5

Parameter	Hour 0	Hour 1	Hour 2	Hour 3
Rat No. 1				
Pulse rate (min^{-1})	440	450	450	EXIT
ECG II	Normal	Normal	S-T elevation	-
Respiratory rate (min^{-1})	50	40 – 50	65	-
Venous pressure (mmHg)	0-1	0	0	-
Arterial pressure (mmHg)	105	75	50	-
Body temperature (°C)	37	37	37	-
Rat No. 2				
Pulse rate (min^{-1})	440	460	470	EXIT
ECG II	Normal	Normal	QRS increased	-
Respiratory rate (min^{-1})	70	60	Not measured	-
Venous pressure (mmHg)	-2 - 0	-5 - 0	0	-
Arterial pressure (mmHg)	100	55	30	-
Body temperature (°C)	37	37	37	-
Rat No. 3				
Pulse rate (min^{-1})	460	450	370	EXIT
ECG II	Confused	Normal	Normal	-
Respiratory rate (min^{-1})	40	50	55	-
Venous pressure (mmHg)	-	-	-	-
Arterial pressure (mmHg)	110	70	70	-
Body temperature (°C)	36	37	37	-
Rat No. 4				
Pulse rate (min^{-1})	430	460	EXIT	-
ECG II	Normal	Wedged	-	-
Respiratory rate (min^{-1})	52	63	-	-

Venous pressure (mmHg)	0 - 1	-1 - 0	-	-
Arterial pressure (mmHg)	105	105	-	-
Body temperature (°C)	36	36	-	-
Rat No. 5				
Pulse rate (min^{-1})	510	450	450	450
ECG II	Normal	Normal	Normal	Normal
Respiratory rate (min^{-1})	65	40	30	40
Venous pressure (mmHg)	2	2	2	2
Arterial pressure (mmHg)	100	70	50	40
Body temperature (°C)	37	36	37	37

Table 5.8.2-23: Toxicodinamic study of glyphosate in rat ([redacted], 1988): Effects of a 5000 mg/kg bw per oral dose of glyphosate on Wistar rat No. 6

Parameter	Hour 0	Hour 1	Hour 2.5	Hour 4.5	Hour 7
Pulse rate (min^{-1})	480	450	430	450	450
ECG II	Normal	Normal	Normal	Normal	Normal
Respiratory rate (min^{-1})	50	60	40	65	67
Venous pressure (mmHg)	1	7	3	2	0
Arterial pressure (mmHg)	110	90	50	100	60
Body temperature (°C)	36	36	38	36	36

Table 5.8.2-24: Toxicodinamic study of glyphosate in rat ([redacted], 1988): Effects of a 5000 mg/kg bw per oral dose of glyphosate on Wistar rats No. 7 to 10

Parameter	Hour 0	Hour 1	Hour 2	Hour 3	Hour 4
Rat Nº 7					
Pulse rate (min^{-1})	420	390	420	EXIT	
ECG II	Normal	Normal	R high top flat	-	-
Respiratory rate (min^{-1})	65	50	75	-	-
Venous pressure (mmHg)	1	1	1	-	-
Arterial pressure (mmHg)	75	60	55	-	-
Body temperature (°C)	35	35	36	-	-
Rat No. 8					
Pulse rate (min^{-1})	420	360	450	EXIT	-
ECG II	Normal	Normal	Normal	-	-
Respiratory rate (min^{-1})	56	50	75	-	-
Venous pressure (mmHg)	0 - 1	-1 - 0	-1 - 0	-	
Arterial pressure (mmHg)	130	75	50	-	
Body temperature (°C)	36	37	37	-	
Rat No. 9					
Pulse rate (min^{-1})	540	480	-	480	EXIT
ECG II	Normal	Normal	-	Normal	-
Respiratory rate (min^{-1})	70	55	-	55	-
Venous pressure (mmHg)	1	-1 - 0	-	-1 - 0	-
Arterial pressure (mmHg)	80	45	-	45	-
Body temperature (°C)	36	35	-	35	-
Rat No. 10					
Pulse rate (min^{-1})	480	480	480	EXIT	-
ECG II	Normal	Normal	Normal	-	-
Respiratory rate (min^{-1})	60	60	55	-	-
Venous pressure (mmHg)	0 - 1	0	0	-	-
Arterial pressure (mmHg)	105	80	70	-	-

Body temperature (°C)	36	36	36	-	-

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:
Half an hour after anesthetising with a chloralose-urethane mixture, male Wistar rats were administered a single dose of 5000 mg glyphosate/kg bw by oral gavage. All animals died within 2 to 7 hours. Treatment was followed by a marked decrease in arterial pressure by approximately 50 % two hours after treatment as compared to the initial values. There was no clear impact on heart rate and respiratory rate. ECG and venous pressure changes, if occurring in some animals, were considered incidental and body temperature was not affected.

While this study does provide some useful information, there are serious reporting deficiencies and errors within the report. Therefore, the study is only considered supportive.

Assessment and conclusion by RMS:

CA 5.8.2/007

1. Information on the study

Data point:	CA 5.8.2
Report author	[redacted]
Report year	1988
Report title	Synergism and potentiation in rats of glyphosate (technical) of Excel Industries Ltd., Bombay
Report No	Not stated
Document No	Not stated
Guidelines followed in study	None stated.
Deviations from current test guideline	Not applicable. No purity or batch number of test materials given. Total dosing volume for combined tests unclear.
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities (GLP was not compulsory at the time the study was performed; non-guideline, investigative study).
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Groups of 10 male Wistar rats were administered 5000 mg/kg bw of glyphosate as a single oral dose at a constant dose volume of 10 mL/kg bw in corn oil. Three compounds were administered in combination with 5000 mg glyphosate per kg bw: a) 2,4-D sodium salt (at six dose intervals between 380 and 1200 mg/kg bw), b) isoproturon (at five dose intervals between 3200 and 5000 mg/kg bw), and c) metolachlor (at six dose intervals between 2100 and 3800 mg/kg bw). After simultaneous dosing, the rats were observed for 14 days for toxic symptoms and mortality.

Prior to simultaneous dosing, each of the three compounds were administered to groups of rats individually to measure toxic symptoms and mortality.

LD_{50} values determined for the three substances, 2,4-D sodium salt, isoproturon and metalochlor were 750, 3900, and 2700 mg/kg bw, respectively. When administered after previous administration of 5000 mg glyphosate/kg bw, LD_{50} values of the three substances were reduced to 585, 3600, and 2450 mg/kg bw, respectively.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Identification:	Glyphosate (technical)
Description:	White powder
Lot/Batch #:	Not stated
Purity:	Not stated
Stability of test compound:	Not stated
Other materials	a) 2-4 D, sodium salt (white powder) b) Isoproturon (white powder) c) Metolachlor (liquid)
Vehicle:	Corn oil (dose volume: 10.0 mL/kg bw)

Test animals:

Species:	Albino rat
Strain:	Wistar
Source:	[redacted]
Age:	Not stated
Sex:	Male
Weight at dosing:	130 to 160 g
Acclimation period:	Not stated
Diet/Food:	Pelleted feed (supplied by Lipton India Ltd., Bangalore), overnight fasting.
Water:	Water, *ad libitum*
Housing:	Five per cage (poly propylene cages) with husk bedding
Environmental conditions:	Temperature: 19–25 °C Humidity: 25—75 % Air changes: not reported Photocycle: 12 hours light/dark cycle

B. STUDY DESIGN AND METHODS

The test material in different doses was administered orally by gavage to groups of 10 male rats and the animals were observed for 14 days for toxic symptoms and mortality. LD_{50} was calculated by the method of Litchfield & Wilcoxon (1949). LD_{50} was determined for each of the compounds; 2-4 D, sodium salt, isoproturon and metolachlor.

After determining the LD_{50} of each test material groups of 10 rats received a single administration of glyphosate followed by 2-4 D, sodium salt, isoproturon and metolachlor. The post administration observation period was 14 days.

II. RESULTS AND DISCUSSION

A. GLYPHOSATE IN COMBINATION WITH 2-4 D, SODIUM SALT

Prior to administration in combination with glyphosate, the LD_{50} of 2-4 D, sodium salt was determined. Six groups, all containing 10 rats, were administered 2-4 D, sodium salt (380, 470, 600, 750, 940, and 1200 mg/kg bw). Reported symptoms included; salivation, ataxia, and loss of righting reflex. The LD_{50} with fiducial limits was calculated: 750 (656 to 858) mg/kg bw.

Table 5.8.2-25: Synergism and potentiation in rats of glyphosate (technical) of Excel Industries Ltd., Bombay ([redacted] 1988): Mortality chart of 2-4 D, sodium salt

Number of rats	Dose (mg/kg bw)	Animals died on day														Mortality (%)
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	
10	380	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	470	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	600	0	2	0	0	0	0	0	0	0	0	0	0	0	0	20
10	750	1	4	0	0	0	0	0	0	0	0	0	0	0	0	50
10	940	3	3	2	0	0	0	0	0	0	0	0	0	0	0	80
10	1200	4	6	-	-	-	-	-	-	-	-	-	-	-	-	100

Glyphosate (5000 mg/kg bw) was then administered in combination with 2-4 D, sodium salt (380, 470, 600, 750, 940, and 1200 mg/kg bw) to six groups all containing 10 rats. Mortalities are tabulated below. Reported symptoms included; salivation, ataxia, and loss of righting reflex. The LD_{50} of 2-4 D, sodium salt in the presence of glyphosate was calculated: 585 mg/kg bw.

Table 5.8.2-26: Synergism and potentiation in rats of glyphosate (technical) of Excel Industries Ltd., Bombay ([redacted], 1988): Mortality chart for different doses of 2-4 D, sodium salt combined with glyphosate

Number of rats	Glyphosate + 2-4 D, sodium salt (mg/kg bw)	Animals died on day														Mortality (%)
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	
10	5000 + 380	0	1	0	0	0	0	0	0	0	0	0	0	0	0	10
10	5000 + 470	1	2	0	0	0	0	0	0	0	0	0	0	0	0	30
10	5000 + 600	2	3	0	0	0	0	0	0	0	0	0	0	0	0	50
10	5000 + 750	4	4	0	0	0	0	0	0	0	0	0	0	0	0	80
10	5000 + 940	5	4	1	-	-	-	-	-	-	-	-	-	-	-	100
10	5000 + 1200	7	3	-	-	-	-	-	-	-	-	-	-	-	-	100

B. GLYPHOSATE IN COMBINATION WITH ISOPROTURON

Prior to administration in combination with glyphosate, the LD_{50} of isoproturon was determined. Five groups, all containing 10 rats, were administered isoproturon (3200, 3600, 4000, 4500, and 5000 mg/kg bw). No symptoms were recorded. The LD_{50} with fiducial limits was calculated: 3900 (3582 to 4247) mg/kg bw.

Table 5.8.2-27: Synergism and potentiation in rats of glyphosate (technical) of Excel Industries Ltd., Bombay ([redacted], 1988): Mortality chart for isoproturon

Number of rats	Dose (mg/kg bw)	Animals died on day														Mortality (%)
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	
10	3200	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	3600	0	2	1	0	0	0	0	0	0	0	0	0	0	0	30
10	4000	2	3	1	0	0	0	0	0	0	0	0	0	0	0	60
10	4500	2	3	3	0	0	0	0	0	0	0	0	0	0	0	80
10	5000	5	4	1	-	-	-	-	-	-	-	-	-	-	-	100

Glyphosate (5000 mg/kg bw) was then administered in combination with isoproturon (3200, 3600, 4000, 4500, and 5000 mg/kg bw) to five groups all containing 10 rats. Mortalities are tabulated below. Reported symptoms included; profuse salivation, urination and loose motion, ataxia, chromodacryorrhea, muscle paralysis. The LD_{50} of isoproturon in the presence of glyphosate was calculated: 3600 mg/kg bw.

Table 5.8.2-28: Synergism and potentiation in rats of glyphosate (technical) of Excel Industries Ltd., Bombay ([redacted], 1988): Mortality chart for different doses of isoproturon combined with glyphosate

Number of rats	Glyphosate + isoproturon (mg/kg bw)	Animals died on day														Mortality (%)
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	
10	5000 + 3200	0	2	0	0	0	0	0	0	0	0	0	0	0	0	20
10	5000 + 3600	2	2	1	0	0	0	0	0	0	0	0	0	0	0	50
10	5000 + 4000	3	5	0	0	0	0	0	0	0	0	0	0	0	0	80
10	5000 + 4500	3	6	1	-	-	-	-	-	-	-	-	-	-	-	100
10	5000 + 5000	5	5	-	-	-	-	-	-	-	-	-	-	-	-	100

C. GLYPHOSATE IN COMBINATION WITH METOLACHLOR

Prior to administration in combination with glyphosate, the LD_{50} of metolachlor was determined. Six groups, all containing 10 rats, were administered Metolachlor (2100, 2400, 2700, 3000, 3400, and 3800 mg/kg bw). Reported symptoms included; sluggishness and hunched back. The LD_{50} with fiducial limits was calculated: 2700 (2437 to 2992) mg/kg bw.

Table 5.8.2-29: Synergism and potentiation in rats of glyphosate (technical) of Excel Industries Ltd., Bombay ([redacted] 1988): Mortality chart for metolachlor

Number of rats	Dose (mg/kg bw)	Animals died on day														Mortality (%)
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	
10	2100	0	1	0	0	0	0	0	0	0	0	0	0	0	0	10
10	2400	2	1	0	0	0	0	0	0	0	0	0	0	0	0	30
10	2700	2	2	1	0	0	0	0	0	0	0	0	0	0	0	50
10	3000	1	5	1	0	0	0	0	0	0	0	0	0	0	0	70
10	3400	4	4	1	0	0	0	0	0	0	0	0	0	0	0	90

10	3800	4	6	-	-	-	-	-	-	-	-	-	-	-	-	100

Glyphosate (5000 mg/kg bw) was then administered in combination with metolachlor (2100, 2400, 2700, 3000, 3400, and 3800 mg/kg bw) to six groups all containing 10 rats. Mortalities are tabulated below. Reported symptoms included; sluggishness and hunched back. The LD_{50} of metolachlor in the presence of glyphosate was calculated: 2450 mg/kg bw.

Table 5.8.2-30: Synergism and potentiation in rats of glyphosate (technical) of Excel Industries Ltd., Bombay ([redacted], 1988): Mortality chart for different doses of metolachlor combined with glyphosate

Number of rats	Glyphosate + metolachlor (mg/kg bw)	Animals died on day														Mortality (%)
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	
10	5000 + 2100	1	1	0	0	0	0	0	0	0	0	0	0	0	0	20
10	5000 + 2400	1	2	1	0	0	0	0	0	0	0	0	0	0	0	40
10	5000 + 2700	3	4	0	0	0	0	0	0	0	0	0	0	0	0	70
10	5000 + 3000	4	4	1	0	0	0	0	0	0	0	0	0	0	0	90
10	5000 + 3400	4	6	-	-	-	-	-	-	-	-	-	-	-	-	100
10	5000 + 3800	3	7	-	-	-	-	-	-	-	-	-	-	-	-	100

Table 5.8.2-31: Synergism and potentiation in rats of glyphosate (technical) of Excel Industries Ltd., Bombay ([redacted], 1988): Summary of determined LD_{50} values in male Wistar rats

	2,4-D, Na salt	5000 mg/kg bw glyphosate + 2,4-D, Na salt	Isoproturon	5000 mg/kg bw glyphosate + isoproturon	Metalochlor	5000 mg/kg bw glyphosate + metolachlor
LD_{50} (mg/kg bw)	750	585	3900	3600	2700	2450

Study conclusion
A single oral administration of glyphosate in combination with 2,4-D, sodium salt, isoproturon, or metolachlor caused no potentiation in albino rats.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:
Groups of 10 male Wistar rats were administered 5000 mg/kg bw of glyphosate as a single oral dose at a constant dose volume of 10 mL/kg bw in corn oil, followed by administration of different doses of three other substances, 2,4-D sodium salt, isoproturon, or metolachlor. The exact total dosing volume of combined administration was not reported.

The LD_{50} values of these three substances decreased when co-administered with glyphosate.

The value of this study is questionable, however, information on combined acute effects might be used as supplementary data.

Assessment and conclusion by RMS:

CA 5.8.2/008

1. Information on the study

Data point:	CA 5.8.2
Report author	[redacted]
Report year	1987
Report title	The acute toxicity of glyphosate in female goats
Report No	80006
Document No	[redacted]-80-450
Guidelines followed in study	None stated.
Deviations from current test guideline	Not applicable, non-guideline study.
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes.
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

Four groups consisting of five female Spanish goats were administered single doses of glyphosate acid via stomach tube at doses of 1980, 3090, 4620, and 10000 mg/kg bw. Water was used as a vehicle to give a constant dosing volume of 500 mL per animal. Four control groups of five goats each were administered water only.

All animals treated with 10000 and 4620 mg/kg bw and one animal treated with 3090 mg/kg bw died. The acute oral LD_{50} (14 days) was calculated to be 3530 mg/kg bw.

Clinical signs observed in goats that died included cessation of feeding activity, loss in body weight, abdominal distress, depression, ataxia, mild diarrhoea, and, shortly prior to death, recumbency. Pulmonary oedema to some degree was noted in several animals and was judged a terminal event.

Clinical signs observed in surviving goats included decreased feed consumption, diarrhoea, and body weight loss. Clinical signs noted in goats treated with the minimum dose included decreased feed consumption and diarrhoea. All clinical signs were absent at the end of the experiment (14–15 days).

Of the animals that survived until terminal sacrifice, those in the 3090 mg/kg bw group had mean body weights less than their respective control group. Goats in the 1980 mg/kg bw group had mean body weights similar to their respective control group. Feed consumption was not measured, but cessation of feeding activity was observed in all treatment groups.

No gross lesions that could be attributed to treatment were seen.
Histological examination was performed only on heart, liver, kidney, spleen and other tissues with grossly visible lesions from animals given glyphosate at 4620 and 3090 mg/kg bw, and of 4 sacrificed control animals. Mild to severe tubular nephrosis was the most consistent histopathological lesion observed in goats that died. 4/5 of the goats that died during the observation period had mild fatty change in the liver. Animals in the 3090 mg/kg bw group that survived lacked such lesions.

Changes of blood urea nitrogen concentration, serum creatinine concentration, and in the number of circulating segmental neutrophils were the most consistent laboratory findings at all dose levels. However, clinical chemistry parameters for surviving treated animals were similar to those of the control animals towards the end of the study.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Identification:	N-(phosphonomethyl)glycine (glyphosate)
Description:	White crystalline solid
Lot/Batch #:	XHJ-64, NBP1494248
Purity:	98.7 %
Stability of test compound:	>1 year at 29.5 °C
Vehicle:	Water was used to suspend the test article and to wash any residue from the container holding the dose and from the stomach tube. Total volume of water used was approximately 500 mL/goat

Test animals:

Species:	Goat
Description:	Spanish goats
Source:	[redacted]
Age:	Approximately 8 months to 4 years of age
Sex:	Females
Weight at dosing:	13.37 - 44 kg (mean bodyweight range 24.4 - 29.5 kg)
Acclimation period:	30 days
Diet/Feed:	During the initial acclimatization (outdoor): bermuda grass hay and commercial goat meal containing not less than 16 % crude protein [Purina® Goat Chow® (Coarse) (Ralston Purina Company, Gonzales, TX, USA)]. After the initial acclimatisation (indoor) the goats were fed bermuda grass only.
Water:	Fresh water and mineral salt blocks were available at all times.
Housing:	During acclimatisation: Outdoor covered pens After dosing: Indoor pens
Study initiation:	22 Dec 1980
Study termination	05 May 1981

Environmental conditions: Not reported

B. STUDY DESIGN AND METHODS

Animal assignment and treatment
Groups of female Spanish goats received the test item, glyphosate, at dose levels of 10000, 4620, 3090, and 1980 mg/kg bw by oral gavage as a single dose in a sequential manner. Initially, the test item was given to a group receiving 10000 mg/kg bw and subsequent doses were selected based on the observed responses. Control animals were treated with tap water. All goats were penned by treatment group and observed daily during pre-treatment and at least twice daily following treatment. Surviving animals were observed for 14 – 15 days after treatment. Only goats without visible evidence of active disease and weighing more than 13 kg were used in this study.

Concomitant treatments
During the initial holding period, all goats were vaccinated for enterotoxaemia, treated for internal parasites and observed for signs of disease.

Randomization
All goats available for experimental use were ranked according to increasing bodyweight. The resulting ranking was divided into 5 nearly equal groups. Treatment and control groups of 5 goats each were formed by randomly selecting 1 goat from each of the 5 divisions of the ranking in order that the resulting groups would h representative with respect to bodyweight of the pool from which they were selected.

Body weight
Body weights were determined prior to dosing and for surviving animals on post-dosing days 7 and 14.

Sampling time points
Blood samples were collected on 3 separate days during pre-treatment and after dosing (designated as day 0) on days 1, 3, 7, and 14. Additional samples were then taken at unscheduled times when dictated by clinical signs or evidence of impending death.

Clinical biochemistry
Blood was collected from the external jugular vein into evacuated glass tubes (Vacutainer tubes, Becton) and allowed to clot at ambient temperature; the serum was separated by centrifugation. Serum lactic dehydrogenase activity, creatine phosphokinase activity, and glucose concentration was determined as soon as possible after sample collection.
An atomic absorption spectrophotometer (Perkin Elmer Model 403, Perkin-Elmer Corp.) was used to determine serum levels of calcium, magnesium, potassium, and sodium as outlined in the manufacturer's manual. Serum levels of glucose, urea nitrogen, total protein, alkaline phosphatase, lactic dehydrogenase, and glutamic oxaloacetic transaminase were determined on automated equipment (Gilford model 3500 computer directed analyser, Gilford Instruments, Inc.) according to manufacturer's procedures. Four other serum components were determined with the same automated equipment; however, different procedures and reagents were used for these determinations, i.e., inorganic phosphorous (Inorganic Phosphorous Reagent, Worthington Diagnostics), cholinesterase (Reagent Set Kit, Biodynamics/BMC Div), gamma-glutamyl transferase (Reagent Set Kit, Biodynamics/BMC Div), and creatine phosphokinase (CK-NAC Reagent Set Kit, Biodynamics/BMC Div.). Serum creatinine and serum uric acid were determined on automated equipment according to manufacturer's procedures (Technicon Auto Analyser, Technicon Corporation).

Haematology
Blood was collected from the external jugular vein into evacuated glass tubes (Vacutainer tubes, Becton) containing disodium ethylenediamine-tetraacetate. Blood smears for differential leukocyte counts were made immediately from blood containing no anticoagulant and stained with Wright's stain (Hema-Tek

Slide Stainer, Ames Company). One hundred leukocytes were classified. Haemoglobin concentration was measured as cyanmethaemoglobin with a haemoglobinometer (Coulter Hemoglobinometer, Coulter Electronics). Erythrocyte count, mean corpuscular volume, haematocrit and total leukocyte count were determined with an electronic particle counter (Coulter Model ZBI with MCV/Hemat Computer and Channelyzer). Mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration values were calculated.

Gross pathology and histopathology

All goats given glyphosate and one randomly selected goat from each control group were subjected to post-mortem examination at the end of the post-treatment observation period (days 14-15). All surviving goats to be necropsied were killed by the intravenous administration of a commercial euthanasia solution (T-61, National Laboratories Corp., Somerville, NJ 08876).

Specimens of heart, liver, kidney and spleen were collected from all necropsied animals for histopathological examination. Additional specimens were collected when grossly visible lesions were observed. Slides for histologic examination were prepared from heart, liver, kidney, spleen and other tissues with grossly visible lesions from all animals given glyphosate at 4620 and 3090 mg/kg body weight, and from each of the four untreated control goats that were sacrificed. Tissues were fixed in neutral buffered 10 % formalin and embedded in paraffin. Sections were stained with haematoxylin and eosin and examined microscopically.

Statistics

The LD_{50} was calculate by the moving average method. Group median, minimum and maximum values were calculated for each post-treatment sampling time for all clinical biochemical and haematological parameters. Control values (principals with concurrent vehicle controls) plus all post-treatment values from the concurrent vehicle control goats and calculating for this pool the following statistics: median, minimum value, maximum value, 5^{th} quantile, and 95^{th} quantile. Study-wide minimum and maximum control values were obtained by pooling control data from all 4 dose levels. Medians for post-treatment determinations that fell above or below the 95^{th} or 5^{th} quantiles respectively were considered significantly greater or less than the control median ($P < 0.05$). Single values for individual animals were considered to have changed significantly if they fell outside the absolute range of control values. The Mann-Whitney test was used for comparison of median percent change in body weight between goats that received glyphosate and their concurrent controls.

II. RESULTS AND DISCUSSION

A. MORTALITY, SURVIVAL TIME, AND LD_{50}

All animals treated with 10000 and 4620 mg/kg bw glyphosate died. 1/5 animals treated with 3090 mg/kg bw also died. The median lethal dose (LD_{50}) was calculated to be LD_{50}, goat, oral = 3530 mg/kg bw. The mortality, survival times and median LD_{50} are given in the table below.

Table 5.8.2-32: The acute toxicity of glyphosate in female goats ([redacted] 1987): Mortality, survival time and median[1] LD_{50} in female goats

Group No. (N=5)	Glyphosate dose (mg/kg bw)	Mortality rate (%)	Survival time (hours)		
			Mean	Min.	Max.
13	10000	100	13.7	3.9	19.2
15	4650	100	16.5	2.4	32.2
16	3090	20	-	71.7	71.7
14	1980	0	-	-	-
LD_{50} = 3530 (2950-4220)[2] mg/kg bw					

[1] Glyphosate doses of 4820 and 7520 mg/kg bw were used in calculating the LD_{50} with the assumption that the mortality for both these levels would be the same as at 4620 mg/kg bw (100 %)
[2] 95 % confidence limits for LD_{50}

B. CLINICAL OBSERVATIONS

One control goat gave birth to a single live kid approximately one hour before the time of treatment. Another control goat gave birth to one normal and one small and very weak kid on day 4 of the experiment. One goat of the 4620 mg/kg bw-treated animals gave birth to two full-term kids during the night before dosing. Abnormal clinical signs observed across all treatment groups are tabulated below.

Table 5.8.2-33: The acute toxicity of glyphosate in female goats ([redacted] 1987): Clinical findings in female goats

Group No.	Dose (mg/kg bw)	Observations
13	10000	CFA (5)[1], apparent colic (2), depressed demeanour (2), slight ataxia (3), recumbency (4), death (5)
15	4620	CFA (5), apparent colic (2), depressed demeanour (2), ataxia (2), laboured breathing (2), recumbency (4), diarrhoea (1), death (5)
16	3090	Animal that died (1): CFA, diarrhoea, apparent colic, subdued demeanour, thirst, ataxia, recumbency, nystagmus, death All surviving animals: DFC (4), apparent colic (2) and diarrhea (4)
14	1980	DFC (5), diarrhoea (3)

[1] Number of goats affected; CFA = cessation of feeding activity; DFC = decreased feed consumption

C. BODY WEIGHT CHANGES

Immediately prior to death most animals exhibited a loss in body weight. Of the animals that survived until terminal sacrifice, those in the 3090 mg/kg bw group had mean body weights significantly less than their respective control group. Control animals and goats in the 1980 mg/kg bw group had similar mean body weights. The feed consumption was not measured, but cessation of feeding activity was observed in all treatment groups.

Table 5.8.2-34: The acute toxicity of glyphosate in female goats ([redacted] 1987): Body weight changes in female goats

Glyphosate dose (mg/kg bw)	Post-treatment day weighted	Number of goats weighed	Percent change from initial weight		
			Median	Minimum	Maximum
Goats weighed immediately prior to death					
10000 - 4620	0	6[1]	-1.8	-3.9	3.8
4620	1	1	0	0	0
3090	3	1	-8.2	-8.2	-8.2
Surviving goats weighed at scheduled times					
3090	7	4	-12.7[3]	-18.1	-5.1
Control	7	4[2]	4.7	0	19.3
3090	14	4	-4.7	-14.7	7.0
Control	14	4[2]	-2.7	-6.6	14.8
1980	7	5	-6.9	-11.7	-3.7
Control	7	5	-2.8	-13.3	1.6
1980	14	5	-5.6	-6.2	-1.9
Control	14	5	-4.2	-7.2	0.8

[1] Carcass weights of 3 other goats that died on day 0 were not determined
[2] Data for one control goat omitted because of changes in body weight brought about by parturition
[3] Significantly different (P<0.01) from control median
Control = concurrent control goats for preceding group

D. POST-MORTEM GROSS PATHOLOGICAL OBSERVATIONS

No gross lesions that could be attributed to treatment were seen.
All results of the post-mortem gross pathology observations are tabulated below.

Table 5.8.2-35: The acute toxicity of glyphosate in female goats ([redacted] 1987): Summary of gross pathological findings in treated and control goats

Dose (mg/kg bw)	D/S	Body as a whole	Respiratory	Cardio-vascular	Haemic and lymphatic	Gastro-intestinal	Urogenital
10000	5/0	NGPD	Pulmonary oedema (4/5)[1] Pneumonia (1/5)	NGPD	NGPD	NGPD	NGPD
4620	5/0	Serous atrophy of fat (1/5)	Pulmonary oedema (4/5)	NGPD	NGPD	Hepatic atrophy (1/5)	Macerated foetus (1/5)
3090	1/4	Serous atrophy of fat (1/5)	Pneumonia (1/5)	Pericarditis (1/5)	NGPD	Fatty liver (1/5) Rumen haemorrhage (1/5)	Pallor, kidneys (4/5) Renal hypertrophy (1/5) Endometritis (2/5) Foetal Death (1/5)
1980	0/5	NGPD	Pulmonary oedema (3/5) Pneumonia (1/5)	NGPD	NGPD	Fatty liver (1/5) Chronic hepatitis (1/5)	Renal atrophy (1/5)
Controls[2]	0/4	Minimal fat stores (1/4)	Pulmonary oedema (2/4)	NGPD	NGPD	NGPD	Pallor, kidneys (2/4) Cystitis (1/4) Metritis (1/4) Endometritis (1/4)

D/S = Died/Sacrificed; NGPD = No gross pathological diagnosis; (number of animlas with finding /number of animals)
[1] Animal affected/total animals in group
[2] only one randomly selected goat from each of the 4 control groups subjected to post mortem examination

E. HISTOPATHOLOGICAL OBSERVATIONS

Histological examination was performed only on heart, liver, kidney, spleen and other tissues with grossly visible lesions from animals given glyphosate at 4620 and 3090 mg/kg bw and of 4 sacrificed control animals. The most consistent finding in animals that died during the observation period was mild to severe tubular nephrosis. 3090 mg/kg bw-treated animals that survived lacked such lesions. 4/5 goats that died during the observation period had mild fatty change in the liver.

Table 5.8.2-36: The acute toxicity of glyphosate in female goats ([redacted] 1987): Major histopathological diagnoses in selected female goats given glyphosate and control goats

Dose (mg/kg bw)	Group No.	Goat No.	Control for Group Number	Organ or Organ System				
				Heart	Spleen	Liver and Gallbladder	Urogenital	Other
4620	15	470[1]	-	NMD	NMD	Mild fatty change	Moderate to severe tubular nephrosis	N/A
		502[1]	-	Sarco-cystosis	NMD	Mild fatty change	Moderate to severe tubular nephrosis	N/A
		533[1]	-	NMD	NMD	Mild fatty change	Degeneration of placenta	N/A
		536[1]	-	NMD	NMD	Mild fatty change	Moderate to severe tubular nephrosis	N/A
		539[1]	-	NMD	NMD	NMD	Moderate to severe tubular nephrosis	N/A
0	28	528	15	NMD	NMD	Mild fatty change	NMD	N/A
3090	16	509[1]	-	Epi-carditis	NMD	Hepatic micro-abscesses	Severe tubular nephrosis, inflammation of placenta	Rumen necrosis and ulceration, pleuritis, broncho-pneumonia
		317	-	NMD	NMD	NMD	NMD	N/A
		424	-	NMD	NMD	Moderate fatty change	NMD	N/A
		543	-	NMD	NMD	NMD	NMD	N/A
		548	-	NMD	NMD	NMD	Endo-metritis	N/A
0	23	511	16	Multiple scars	NMD	NMD	Endometritis and retention of foetal membrane	N/A
0	19	494	13	NMD	NMD	NMD	NMD	N/A
0	21	461	14	NMD	NMD	NMD	NMD	N/A

[1] Fatally poisoned goats; all other goats shown were euthanized at the end of the study
NMD = No Morphologic Diagnosis.
N/A = Organ or system not examined histologically.

F. CLINICAL BIOCHEMISTRY AND HAEMATOLOGY

Elevation of blood urea nitrogen concentration, serum creatinine concentration, and numbers of circulating segmental neutrophils were the most consistent laboratory findings in goats given glyphosate. These changes were observed at all dose levels used in this study.

Clinical laboratory findings were almost universally within or near reference range for surviving glyphosate-treated goats at the end of the experiment (day 14).

Biochemical and haematological measurements, where one or more values fall outside of reference range, are tabulated below.

Table 5.8.2-37: The acute toxicity of glyphosate in female goats ([redacted] 1987): Blood parameter findings in goats

Paramter (unit)	Ref. value*	Control[1]	Death in <4 h (1 goat)[2]	Death in 15-20 h (3 goats)[3]	Death in 32-72 h (2 goats)[4]	Death in 72 h (1 goat)[5]	Sign of intoxication observed (4 goats)[6]		No signs of intoxication observed (5 goats)[7]	
BUN (mg/dL)	10.0-20.0	2.3-21.3	12.7	16.2-25.0^{++}	16.7-35.2^{++}	55.5^{++}	15.1-45.7^{++}	3.1-26.4^{++}	5.2-54.5^{++}	7.0-31.3^{++}
SC (mg/dL)	1.0-1.8	0.7-1.7	1.5	2.7-3.3^{++}	4.0-6.0^{++}	14.1^{++}	1.8-5.3^{++}	0.8-1.6	0.8-5.0^{++}	0.8-1.3
GLU (mg/Dl)	50.0-75.0	43-80	407^{++}	48-57	35-38	268^{++}	36-87	51-87	55-100	59-98
Na (mg/dL)	327-356	311-354	303^{++}	333-335	313-321	327	317-331	301-332	317-347^{++}	319-350^{++}
K (mg/dL)	13.6-26.1	12.7-21.2	44.8^{++}	21.8-31.5	15.2-20.5	12.9	6.7-18.1	9.4-17.0	12.3-18.3	11.9-18.2
Ca (mg/dL)	8.9-11.7	8.0-15.7	18.9^{++}	15.2-18.6^{++}	8.7-10.3	6.3^{++}	6.2-10.3^{++}	7.9-10.2	6.1-10.1	8.5-11.4
P (mg/dL)	2.9-7.3	3.5-11.3	16.0^{++}	5.4-9.5	8.8-17.00^{++}	21.6^{++}	4.4-22.4^{++}	4.1-8.0	3.7-9.0	5.3-8.7
Mg (mg/dL)	2.8-3.6	1.30-3.20	4.01^{++}	2.87-3.61^{++}	1.98-3.07	2.38	1.53-2.48	1.53-2.38	1.98-3.22	1.29-2.38
SGOT (IU/L)	167-513	31-151	78	49-126	60-85	129	44-69	47-229^{++}	40-91	46-106
LDH (IU/L)	123-392	108-385	295	172-543^{++}	268-288	694^{++}	100-233	127-415^{++}	145-276	144-226
CPK (IU/L)	20-42	44-878	213	124-184	59-173	3285^{++}	83-187	73-245	84-264	81-158
WBC[5]	4.0-15.0	5.5-24.8	14.5	22.5-28.0^{++}	13.5-27.0^{++}	57.3^{++}	17.5-41.5^{++}	8.0-19.5	11.0-22.4	8.0-17.5
SEGS[5]	1.6-7.5	1.4-10.4	7.3	10.8-18.2^{++}	4.9-14.9^{++}	38.4^{++}	11.5-30.7^{++}	2.4-14.8^{++}	4.1-17.5^{++}	2.0-7.2
BANDS[5]	---	0-1.4	2.8^{++}	6.4-7.6^{++}	4.1-10.0^{++}	11.5^{++}	0.2-7.5^{++}	0.1-0.8	0-0.7	0-1.4
TSP (g/dL)	6.4-7.0	5.0-8.2	8.9^{++}	5.7-6.3	5.6-6.1	6.4	6.5-7.5	5.5-7.5	6.0-7.3	6.0-7.4

*Reference values as gathered from different published literature sources
[1] Control values were obtained by pooling all pre-treatment values from all post-treatment values from untreated controls on a study-wide basis (355 measurements for each parameter)
[2] Goat sampled at death 3.8 h after receiving 10000 mg/kg bw
[3] Goats sampled 11 h after receiving 10000 mg/kg bw
[4] Goats sampled 24 h after receiving 4620 or 3090 mg/kg bw
[5] Goat sampled at death 72 h after receiving 3090 mg/kg bw
[6] Goats given 3090 mg/kg bw
[7] Goats given 1980 mg/kg bw
[++] One or more values outside of reference range

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:
Four groups consisting of five female (Spanish) goats obtained from farms were administered single oral gavage doses of glyphosate (via stomach tube) at doses of 1980, 3090, 4620, and 10000 mg/kg bw. Water was used as a vehicle in four negative control groups of five goats each. Mortality, clinical signs, body weight, haematology, clinical biochemistry, gross necropsy, histopathology were assessed during

a post-treatment observation period of 14 days.

At least three goats included in this study were discovered to be pregnant and, thus, females of different hormonal and physiological status were used. Weight and age of the test animals were widely distributed. Although randomization was applied with regard to weight, the test population was quite heterogeneous. Due to the small number of animals investigated, the relevance of the biochemical and haematological findings is questionable.

The acute oral LD_{50} was calculated to be 3530 mg/kg bw.

The study is considered acceptable in spite of the heterogeneity of the animals used. It must be taken into account that studies of this type are usually not required in Europe for active ingredients in plant protection products and that no guideline exists. The quality of the study is suitable to provide additional information about acute oral toxicity in a ruminant species. The maximum dose exceeded those used in acute toxicity studies in laboratory rodents and more parameter were assessed in this study than would be included in an acute oral toxicity study in rodents. However, pathology was confined to four of a total of 20 control animals. In addition, sometimes data obtained in the four control groups were pooled and sometimes reported separately. The acute oral toxicity of glyphosate in goats was low.

Assessment and conclusion by RMS:

CA 5.8.2/009

1. Information on the study

Data point:	CA 5.8.2
Report author	[redacted]
Report year	1987
Report title	The acute oral toxicity of the isopropylamine salt of glyphosate (MON-0139) in female goats
Report No	80007[redacted]
Document No	[redacted]-80-451
Guidelines followed in study	None stated
Deviations from current test guideline	Not applicable, non-guideline study
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes.
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

MON-0139, an aqueous formulation containing glyphosate isopropylamine salt (62.5 %) was administered as a single dose via stomach tube to five groups (N=5) of female Spanish goats at doses of 10000, 6700, 5360, 4290, or 1400 mg/kg bw. Mortality, clinical signs, body weight, haematology, clinical biochemistry, gross necropsy, and histopathology were observed for a post-treatment period of 14 days. Clinical signs, haematology, clinical biochemistry, and pathology were investigated in an effort to identify changes that are characteristic of MON-0139 intoxication and could be useful for diagnostic purposes.

The median acute oral LD_{50} was found to be 5700 mg/kg bw and the minimum lethal dosage (MLD) was 4290 mg/kg bw. The minimum dosage producing definite signs of illness (reduced feed consumption, diarrhoea) was 4290 mg/kg bw. Signs of toxicity in goats that died during the observation period included decreased feed consumption, abdominal distress, ataxia and, shortly prior to death, recumbency. One goat that died and one surviving goat each displayed an unusual "collapsing syndrome" of apparent neurological origin approximately 2 days after receiving MON-0139, while other goats displayed various other neurological signs. Sign of toxicity in goats that were sacrificed according to schedule included decreased feed consumption, diarrhoea, and loss of bodyweight. One goat developed extensive ulceration of the tongue and oral mucosa. These lesions healed completely by the end of the 14-day observation period.

No lesions considered to be treatment-related were noted at gross necropsy. No pathognomonic lesions were seen microscopically in treated goats. Mild to severe tubular nephrosis was the only consistent histopathological lesion observed in treatmentgoats died; however, this lesion was not observed in treatment goats that lived until the end of the experiment (days 14-15). This lesion is considered to be diagnostically significant for goats that die a few days after an appropriate level of exposure. Ischemic hippocampal neurons were observed in the brain of one goat that had displayed the so called "collapsing syndrome".

Changes in clinical biochemical parameters associated with toxicity included moderate elevation of serum urea nitrogen (BUN) and mild to moderate elevation of serum creatinine (SC). Slight elevations in serum glutamic oxaloacetic transaminase (SGOT) and lactic dehydrogenase (LDH) activity were also observed in terminal animals immediately prior to death. Temporary reductions in K and LDH were the only apparent treatment-related responses in goats dosed at 1400 mg/kg bw. No other diagnostically or toxicologically significant changes were observed. None of the biochemical parameters measured appeared to be involved in, or indicate the cause of, the unusual neurological manifestations seen in some goats receiving MON-0139.

Changes in circulating leukocytes associated with treatment with MON-0139 involved an increase in absolute numbers of mature (SEGS) and immature (BANDS) neutrophils with a decline in numbers of basophils and eosinophils. No consistent treatment-related changes in erythrocyte parameters were observed in this study.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:	MON-0139
Identification:	N-(phosphonomethyl)glycine isopropylamine salt
Description:	Amber liquid
Lot/Batch #:	LURT 08020
Purity:	62.5 % (46.2 % for glyphosate)
Stability of test compound:	Stable for >1 year at 29.5 ºC. Store in a cool, dry area at 18.3-29.5 ºC

Vehicle:	Water was used to dilute the test article (1:1) and to wash any residue from the container holding the dose and from the stomach tube. The total volume of water was approximately 500 mL/goat.
Test animals:	
Species:	Goat
Strain:	Spanish[1]
Source:	[redacted]
Age:	8 months to 4 years
Number/Sex:	45 females
Weight at dosing:	14.7- 47.0 kg
Acclimation period:	At least 30 days
Diet/Feed:	During acclimatisation: bermuda grass hay and a commercial goat meal containing not less than 16.0 % crude protein (Purina Goat Chow Coarse). After dosing: bermuda grass hay
Water:	Fresh water and mineral salt blocks available at all times
Housing:	During acclimatisation: Outdoor covered pens After dosing: Indoor pens
Environmental conditions:	Not reported

[1]The term Spanish is used to distinguish range meat goats from Angoras and dairy breeds. Most are of the same origin as the Mexican Criollo but they may show traces of Nubian and Toggenburg blood.

B. STUDY DESIGN AND METHODS

Animal assignment and treatment:
Groups of five of female Spanish goats received the test item, MON-0139, at dose levels of 10000, 6700, 5360, 4290, and 1400 mg/kg bw by oral gavage (rumen intubation) in a sequential manner. Initially, the test item was given at 10000 mg/kg/bw and subsequent doses were selected based on the observed responses. Four groups of negative control consisted of five animals each. They were concurrently treated with the vehicle (tap water). All goats were penned by treatment group and observed daily during pre-treatment and at least twice daily following treatment. Surviving animals were observed for 14–15 days after treatment. Blood samples were collected on three separate days during pre-treatment and 1, 3, 7, and 14 days after dosing (designated as day 0). Additional samples were then taken at unscheduled times when dictated by clinical signs or evidence of impending death.
During the initial holding period, all goats were vaccinated for enterotoxaemia, treated for internal parasites and observed for signs of disease. Only goats without visible evidence of active disease and weighing more than 13 kg,

Randomization
All goats available for experimental use were ranked according to increasing bodyweight - The resulting ranking was divided into 5 nearly equal groups. Treatment and control groups of 5 goats each were formed by randomly selecting 1 goat from each of the 5 divisions of the ranking in order that the resulting groups would be representative with respect to bodyweight of the pool from which they were selected.

Body weight
Body weights were determined prior to dosing and for surviving animals on post-dosing days 7 and 14.

Clinical Biochemistry
An atomic absorption spectrophotometer (Perkin Elmer Model 403, Perkin-Elmer Corp.) was used to

determine serum levels of calcium, magnesium, potassium, and sodium, as outlined in the manufacturer's manual. Serum levels of glucose, urea nitrogen, total protein (TP), alkaline phosphatase, lactic dehydrogenase, and glutamic oxaloacetic transaminase were determined on automated equipment (Gilford model 3500 computer directed analyser, Gilford Instruments, Inc.) according to manufacturer's procedures. Four other serum components were determined with the same automated equipment; however, different procedures and reagents were used for. these determinations, i.e., inorganic phosphorus (Inorganic Phosphorous Reagent, Worthington Diagnostics), cholinesterase (Reagent Set Kit, Biodynamics/BMC Div), gamma-glutamyl transferase (Reagent Set Kit, Biodynamics/BMC Div.) and creatine phosphokinase (CK-NAC Reagent Set Kit, Biodynamics/BMC Div.). Serum creatinine was determined on automated equipment according to manufacturer's procedures (Technicon Auto Analyser, Technicon Corporation).

Blood was collected from the external jugular vein into evacuated glass tubes (Vacutainer tubes, Becton) and allowed to clot at ambient temperature; the serum was separated by centrifugation. Serum LDH activity, CPK activity, and GLU concentration was determined as soon as possible after sample collection.

Haematology
Blood was collected from the external jugular vein into evacuated glass tubes (Vacutainer tubes, Becton) containing disodium ethylenediamine-tetraacetate. Blood smears for differential leukocyte counts were made immediately from blood containing no anticoagulant and stained with Wright's stain (Hema-Tek Slide Stainer, Ames Company). One hundred leukocytes were classified. Haemoglobin concentration was measured as cyanmethaemoglobin with a haemoglobinometer (Coulter Hemoglobinometer, Coulter Electronics). Erythrocyte count, mean corpuscular volume, haematocrit and total leukocyte count were determined with an electronic particle counter (Coulter Model ZBI with MCV/Hemat Computer and Channelyzer). Mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration values were calculated.

Pathology
All goats given MON-0139 and one randomly selected goat from each control group were subjected to post-mortem examination at the end of the post-treatment observation period (days 14-15). All surviving goats to be necropsied were killed by the intravenous administration of a commercial euthanasia solution (T-61 Solution, National Laboratories Corp., USA).
Specimens of heart, liver, kidney, spleen and other tissue with grossly visible lesions were collected only from animals dosed at 6700 and 5360 mg/kg bw, and from each of the four untreated control goats that were sacrificed. Additional specimens were collected from one goat dosed at 4290 mg/kg bw and one goat dosed at 1400 mg/kg bw. Histopathologic examination on animals of the highest dose group were not performed. Tissue sections were stained with haematoxylin and eosin and examined microscopically.

Statistics
The LD_{50} was calculated by the method of Litchfield and Wilcoxon. Group median, minimum and maximum values were calculated for each post-treatment sampling time for all clinical biochemical and haematological parameters. Control values for each treatment groups were obtained by pooling all pre-treatment values (principals with concurrent untreated controls) plus all post-treatment values from the concurrent untreated control goats and calculating for this pool the following statistics: median, minimum value, maximum value, 5^{th} quantile, and 95^{th} quantile. Medians for post-treatment determinations that fell above or below the 95^{th} or 5^{th} quantiles respectively were considered significantly greater or less than the control median ($P<0.05$). Single values for individual animals were considered to have changed significantly if they fell outside the absolute range of control values. Study-wide minimum and maximum control values were obtained by pooling control data from all 5 dose levels. The Mann-Whitney test was used for comparison of median percent change in bodyweight between goats that received MON-0139 and their concurrent controls.

II. RESULTS AND DISCUSSION

A. MORTALITY, SURVIVAL TIME, AND LD_{50}

Treatment with 10000, 6700, 5360, 4290, and 1400 mg/kg bw MON-0139 resulted in 5/5, 3/5, 2/5, 2/5, and 0/5 deaths, respectively. In the four control groups, there were no deaths. The acute oral LD_{50} for the dose-mortality data of this study was 5700 mg/kg bw MON-0139 with confidence limits of 3730 and 8710 mg/kg bw. The mortality and survival times are given in the table below.

Table 5.8.2-38: The acute oral toxicity of the isopropylamine salt of glyphosate (MON-0139) in female goats ([redacted] 1987): Mortality, survival time and median acute oral LD_{50} in female goats given MON-0139

Number of animals	MON-0139 Dosage[1] (mg/kg bw)	Mortality rate (%)	Survival time (hours)		
			Mean	Min.	Max.
5	10000	100	30.6	15.6	42.0
5	6700	60	52.5	37.0	64.0
5	5360	40	48.2	47.5	49.0
5	4290	40	69.2	53.2	85.1
5	1400	0	-	-	-
Lethal dose levels (mg/kg bw)					
LD_{50}= 5700 (confidence limits: 3730-8710) mg/kg bw					
LD_{16} = 2800 mg/kg bw					
LD_{84} = 11000 mg/kg bw					

[1] MON-0139 given as a single oral dose.

B. CLINICAL OBSERVATIONS

Clinical signs of goats that died included decreased feed consumption, abdominal distress, ataxia and, shortly prior to death, recumbency. One goat that died and one surviving goat dosed at 6700 mg/kg bw each displayed an unusual "collapsing syndrome" of apparent neurological origin approximately 2 days after receiving MON-0139 while other goats displayed various other neurological signs. One surviving goat developed extensive ulceration of the tongue and oral mucosa. These lesions healed completely by the end of the 14-day observation period.

Table 5.8.2-39: The acute oral toxicity of the isopropylamine salt of glyphosate (MON-0139) in female goats ([redacted], 1987): Prominent clinical observations in female goats given MON-0139

Number of animals	Dosage (mg/kg bw)	Observations
5	10000	DFC (5)[1], apparent colic (5); ataxia, shaking and jerking movements (2); depression (2); head bobbing (2); nystagmus (1); recumbency (4); diarrhoea (1); paddling (2); mild convulsions (2); death (5)
5	6700	Non-survivors (3): DFC (3); apparent colic (2); diarrhoea (1); bloating (3); ataxia (3); "saw-horse" stance (2); tremor (1); recumbency (3); nystagmus (2); jerky movements (2); blinking (1); terminal clonic-tonic activity (3); death (3) Survivors (2): DFC (2); bloating (1); abdominal distention (1); tremor (2); "collapsing syndrome" (1); diarrhoea (2)[2]
5	5360	Non-survivors (2): DFC (2); diarrhoea (1); ataxia (2); "saw-horse" stance (1); salivation (1); recumbency (1); tremor (l); "star-gazing" trance (l); death (2) Survivors (3): DFC (3); diarrhoea (3)

5	4290	Non-survivors (2): DFC (2); ataxia (1); "collapsing syndrome" (1); "chewing convulsions" (1); recumbency (2); salivation (1); terminal opisthotonus-like convulsions (l); death (2) Survivors (3): DFC (3); diarrhoea (3); apparent colic, salivation, lethargy, ulceration of oral mucosa (1)
5	1400	Minimal or no ill effects seen; reduced urination (?), abdominal distention (2); abortion (l)
20	0	Abortion (1)

DFC = decreased feed consumption
[1] Number of goats affected

C. BODY WEIGHT CHANGES

No statistically significant differences in body weight gain were observed between the groups treated with MON-0139 and their respective control groups. Feed consumption, although not precisely measured, was greatly reduced following treatment with MON-0139 at doses of 4290 mg/kg bw and above. For most groups, feeding activity gradually increased, reaching normal levels by the end of the study.

Table 5.8.2-40: The acute oral toxicity of the isopropylamine salt of glyphosate (MON-0139) in female goats ([redacted] 1987): Body weight changes in goats given MON-0139

Dose level (mg/kg bw)	Post treatment day weighed	Number of goats weighed	Percent change from initial weight		
Goats weighed immediately prior to death					
10000	0	1	-	-2.1	-2.1
5360-10000	1	6	-0.5	-4.4	2.4
4290-9700	2	4	-7.2	-10.1	-3.8
4290	3	1	-	-8.2	-8.2
Surviving goats weighed at scheduled times					
6700	7	2	-7.0[1]	-10.4	-3.5
Control	7	4[2]	10.3	3.8	19.3
6700	14	2	-8.1	-4.4	-11.7
Control	14	4[2]	4.5	-6.6	-14.8
5360	7	3	-0.4	-9.0	3.2
Control	7	4[2]	0	-7.2	3.7
5360	14	3	0	-3.3	2.0
Control	14	4[2]	-1.8	-4.1	3.7
4290	7	3	-13.3	-17.4	-2.4
Control	7	5	-2.8	-13.5	1.6
4290	14	3	-8.1	-23.0	1.2
Control	14	5	-4.2	-7.2	0.8
1400	7	5	-0.7[1]	-3.4	10.5
Control	7	4[2]	10.3	3.8	19.3
1400	14	4[2]	-4.8	-6.2	7.6
Control	14	4[2]	4.5	-6.6	14.8

[1] Approaches being significantly different from control median ($0.05 < P < 0.10$)
[2] Data for one goat omitted because of changes in weight brought about by parturition
Control= Concurrent control goats for proceeding group

D. POST-MORTEM OBSERVATIONS

No lesions considered to be treatment-related were noted at gross necropsy.

Table 5.8.2-41: The acute oral toxicity of the isopropylamine salt of glyphosate (MON-0139) in female goats ([redacted] 1987): Significant gross pathologic diagnoses in female goats given MON-0139 and sacrificed untreated control goats

Dose level (mg/kg bw)	D/S[1]	Body as a whole	Respiratory	Cardio-vascular	Hemic and lymphatic	Gastro-intestinal	Urogenital
10000	5/0	NGPD[2]	pulmonary oedema (2/5)[3]	NGPD	NGPD	NGPD	NGPD
6700	3/2	NGPD	pulmonary oedema (1/5)	NGPD	NGPD	bloated (2/5)	pale kidneys (1/5) renal hypertrophy (1/5)
5360	2/3	minimal fat stores (1/5)	pulmonary oedema (3/5)	NGPD	NGPD	fatty liver (4/5)	pale kidneys (2/5)
4290	2/3	NGPD	pulmonary oedema (3/5)	NGPD	NGPD	fatty liver (1/5) gallbladder oedema (1/5)	pale kidneys (2/5) renal hypertrophy (1/5)
1400	0/5	minimal fat stores (1/5)	pneumonia (1/5) pulmonary oedema (2/5)	pericarditis (1/5)	NGPD	fatty liver (l/5)	metritis (1/5) pregnancy (1/5)
Controls	0/4	minimal fat stores (1/4)	pulmonary oedema (2/4)	NGPD	NGPD	NGPD	pale kidneys (2/4) mild cystitis (1/4) metritis (1/4) endometritis (1/4)

[1] Died/sacrificed
[2] No gross pathologic diagnosis
[3] Animals affected/total animals in group

E. HISTOPATHOLOGICAL OBSERVATIONS IN SELECTED MON-0139 TREATED AND UNTREATED GOAT

Histologic examination was performed only on heart, liver, kidney, spleen and other tissues with grossly visible lesions from animals given MON-0139 at 6700 and 5360 mg/kg bw, and from each of the four untreated control goats that were sacrificed. In addition, histologic examination was performed on tissues from one goat given MON-0139 at 4290 mg/kg and on tissues from one goat given MON-0139 at 1400 mg/kg. The most consistent finding in fatally poisoned animals was mild to severe tubular nephrosis.

Table 5.8.2-42: The acute oral toxicity of the isopropylamine salt of glyphosate (MON-0139) in female goats ([redacted], 1987): Major histopathologic diagnosis in selected female goats given MON-0139 and sacrificed untreated control goats

Dose level (mg/kg bw)	Goat Number	Organ or organ system				
		Heart	Spleen	Liver and Gallbladder	Urogenital	Other
1400	550	Scar	NMD	NMD	Severe bacterial metritis[1]	N
4290	472[2]	NMD	NMD	NMD	Mild tubular nephrosis	Ischemic hippocampal neurons with focal oedema
5360	522[2]	NMD	NMD	Mild to moderate fatty change	Moderate to severe tubular nephrosis	N
	512[2]	NMD	NMD	Moderate to severe fatty	Mild to moderate tubular nephrosis	N

				change		
	520	NMD	NMD	Mild fatty change	NMD	N
	313	NMD	NMD	NMD	NMD	N
	549	Mild scaring		NMD	NMD	N
6700	407	NMD	NMD	NMD	NMD	N
	481[2]	NMD	NMD	Mild to moderate fatty change	Moderate to severe tubular nephrosis	N
	497	NMD	NMD	NMD	NMD	N
	316[2]	Focal myocarditis sarcocystosis	NMD	NMD	Moderate tubular nephrosis	N
	538[2]	NMD	NMD	NMD	Mild to moderate tubular nephrosis	N
0	528	NMD	NMD	Mild fatty change	NMD	N
	511	Multiple scars	NMD	NMD	Endometritis, retained foetal membranes	N
	494	NMD	NMD	NMD	NMD	MMCB
	461	NMD	NMD	NMD	NMD	N

NMD = No Morphologic Diagnosis (no significant pathologic change observed)
N = Organ or system not examined histologically
MMCH = Mild multifocal chronic bronchiolitis

[1] Microscopic examination of foetal tissues revealed no specific lesions
[2] Fatally poisoned
[3] Foetal tissues not examined

F. CLINICAL BIOCHEMISTRY AND HAEMATOLOGY

Slight to moderate elevations in blood urea nitrogen (BUN) and serum creatine concentrations (SC) were observed in all animals that died during the study. These findings may be related to the histopathologic kidney lesions observed in these animals. Slight elevations in serum glutamic oxaloacetic transaminase (GOT) and lactic dehydrogenase (LDH) activity were also observed in terminal animals immediately prior to death.

No other diagnostically or toxicologically significant changes were observed. None of the biochemical parameters measured appeared to be involved in, or indicate the cause of, the unusual neurological manifestations seen in some goats receiving MON-0139. The results are summarized in the following table.

Table 5.8.2-43: The acute oral toxicity of the isopropylamine salt of glyphosate (MON-0139) in female goats ([redacted] 1987): Clinical biochemical and haematological measurements (min-max values) for goats given MON-0139

			Goats that died				Goats that survived			
			Surviving <49 h		Surviving >50 h, <90 h		Definite signs of intoxication observed (8 goats)		Minimal or no signs of intoxication observed (5 goats)	
Parameter (unit)	Ref.	Control	Sampled 12-14 h before death (2 goats)	Sampled <4 h before death (5 goats)	Sampled at 24 h post-dosing (4 goats)	Sampled 6-16 h before death (3 goats)	Days 1 & 3	Days 7 & 14	Days 1 & 3	Days 7 & 14
BUN (mg/dL)	10.0-20.0	2.3-21.3	26.7-44.1	31.5-62.4	13.8-22.0	44.2-111.4	7.6-28.7	3.7-135.6	3.9-13.6	6.0-19.1
SC (mg/dL)	1.0-1.8	0.6-1.7	4.5-6.1	3.3-8.5	1.4-2.9	5.1-6.7	1.0-1.9	0.8-9.7	0.8-1.4	0.9-1.4
GLU	50.0-	37-80	120-164	17-65	74-173	57-196	60-139	50-90	40-59	29-58

Table 5.8.2-43: The acute oral toxicity of the isopropylamine salt of glyphosate (MON-0139) in female goats ([redacted] 1987): Clinical biochemical and haematological measurements (min-max values) for goats given MON-0139

			Goats that died				Goats that survived			
			Surviving <49 h		Surviving >50 h, <90 h		Definite signs of intoxication observed (8 goats)		Minimal or no signs of intoxication observed (5 goats)	
Parameter (unit)	Ref.	Control	Sampled 12-14 h before death (2 goats)	Sampled <4 h before death (5 goats)	Sampled at 24 h post-dosing (4 goats)	Sampled 6-16 h before death (3 goats)	Days 1 & 3	Days 7 & 14	Days 1 & 3	Days 7 & 14
(mg/dL)	75.0									
Na (mg/dL)	327-356	311-349	347-376	327-396	331-343	327-345	309-337	319-337	323-337	317-337
K (mg/dL)	13.6-26.1	13.4-21.2	16.2-16.5	10.9-23.2	10.3-16.9	9.0-11.0	12.2-17.6	8.0-19.5	13.9-19.5	15.0-19.4
Ca (mg/dL)	8.9-11.7	8.0-13.4	8.7-11.5	8.5-9.8	8.6-11.3	7.2-8.6	8.9-11.1	8.7-10.7	9.3-10.1	9.2-10.0
P (mg/dL)	2.9-7.3	4.1-11.9	2.9-16.0	3.5-10.0	3.9-6.4	2.9-5.9	3.5-10.2	3.8-9.7	4.5-9.3	4.9-8.8
Mg (mg/dL)	2.8-3.6	1.3-3.4	3.0-3.9	2.3-4.1	2.1-3.5	3.0-4.0	1.8-2.4	1.3-2.4	1.9-2.3	1.9-2.1
GOT (U/L)	167-513	31-151	99-100	128-459	58-107	105-145	34-77	3.7-169	53-81	50-83
LDH (U/L)	123-392	145-385	303-310	394-880	220-298	266-394	118-232	141-317	170-242	151-238
ACH (U/L)	-	240-496	405-461	388-536	344-459	409-471	293-528	209-450	273-386	255-407
ALKP (U/L)	93-387	31-902	69-283	76-282	97-422	173-476	58-594	52-542	31-323	30-307
CPK (U/L)	20-42	44-878	144-185	181-2631	110-149	183-1145	58-124	44-180	54-154	44-152
GGT (U/L)	-	12-60	19-26	23-38	30-35	30-38	23-43	21-42	15-40	11-30
WBC	4.0-15.0	5.5-17.5	12.0-14.0	3.7-21.5	9.0-23.0	10.0-18.0	5.7-19.5	8.0-35.5	7.5-12.5	7.5-20.0
SEGS	1.6-7.5	1.4-10.4	3.1-7.0	1.3-4.8	4.1-16.6	1.8-6.9	2.0-14.0	2.0-9.7	2.9-7.2	3.4-16.4
BANDS	-	0-1.4	3.6-5.9	1.3-12.1	0-4.8	1.4-9.7	0-1.0	0-1.8	0-0.3	0-0.6
LYMPH	1.8-9.0	2.4-9.2	2.6-2.8	1.0-6.5	1.4-4.9	1.7-4.4	2.0-6.7	3.1-8.4	2.9-6.4	2.0-6.3
EOS	0.1-1.5	0-1.4	0-0	0-0	0-0.1	0-0	0-0.1	0-0.7	0-0.7	0-0.4
MONO	0.1-0.9	0-0.9	0.4-0.6	0-0.9	0.1-0.4	0.1-0.5	0-0.8	0-0.7	0-0.2	0.1-3.9
BASO	0-0.2	0-0.8	0-0	0-0	0-0.1	0-0	0-0.3	0-0.6	0-0.5	0-0.4
PCV (%)	19-40	23-47	26-49	33-45	34-45	31-42	29-39	27-38	24-32	24-34
HGB (mg/dL)	8-16	7.8-16.7	9.6-15.6	10.6-15.6	10.8-14.4	10.6-14.8	10.4-12.9	9.4-12.4	11.3-12.2	9.8-11.3
RBC	7-21	12.8-24.2	15.6-23.8	16.0-26.9	19.0-24.5	16.7-22.9	15.6-23.2	15.7-21.9	18.0-19.0	15.7-17.9
MCV	15-39	15-21	17.0-18.0	15.0-19.0	15.0-18.0	15.0-18.0	15.0-9.0	13.0-9.0	14.0-21.0	15.0-1.0
MCH	5.3-8.4	4.6-7.8	6.2-6.6	4.8-7.3	5.5-6.5	5.4-6.5	5.1-7.5	5.3-7.0	5.0-6.9	5.2-7.2
MCHC (mg/dL)	32-40	28-41	31.8-36.9	31-35	32-37	32-35	32-38	31-37	33-40	34-41

Table 5.8.2-43: The acute oral toxicity of the isopropylamine salt of glyphosate (MON-0139) in female goats ([redacted], 1987): Clinical biochemical and haematological measurements (min-max values) for goats given MON-0139

			Goats that died				Goats that survived			
			Surviving <49 h		Surviving >50 h, <90 h		Definite signs of intoxication observed (8 goats)		Minimal or no signs of intoxication observed (5 goats)	
Parameter (unit)	Ref.	Control	Sampled 12-14 h before death (2 goats)	Sampled <4 h before death (5 goats)	Sampled at 24 h post-dosing (4 goats)	Sampled 6-16 h before death (3 goats)	Days 1 & 3	Days 7 & 14	Days 1 & 3	Days 7 & 14
TP (mg/dL)	6.4-7.0	5.0-8.2	6.7-8.6	5.8-7.3	7.2-7.7	6.2-7.7	5.9-8.7	5.6-7.7	6.1-7.2	5.9-7.1

Control values were obtained by pooling all pre-treatment values (principles and untreated controls) plus all post-treatment values from untreated controls on a study-wide basis (355 measurements for each parameter from 60 goats).
BUN = Urea nitrogen, SC = Creatinine, GLU = Glucose, Na = Sodium, K = Potassium, Ca = Calcium, P = Phosphorus, Mg = Magnesium, GOT = Glutamic oxaloacetic transaminase, LDH = Lactic dehydrogenase, ACH = Cholinesterase, ALKP = Alkaline phosphatase, CPK = Creatinine phosphokinase, GGT = Gamma-glutamyltransferase, WBC = Total leukocyte count, SEGS = Segmented neutrophil count, BANDS = Band neutrophil count, LYMPH = Lymphocyte count, EOS = Eosinophil count, MONO = Monocyte count, BASO = Basophil count, PCV = Microhematocrit, HGB = Haemoglobin, RBC = Erythrocyte count, MCV = Mean corpuscular volume, MCH = Mean corpuscular haemoglobin, MCHC = Mean corpuscular hemoglobin concentration, TP = Total protein

Study conclusion

The median acute oral LD_{50} was found to be 5700 mg/kg bw. The minimum lethal dose was 4290 mg/kg bw. The minimum dose producing definite signs of illness (reduced feed consumption, diarrhoea) was 4290 mg/kg bw. The lowest dose of MON-0139 given in this study (1400 mg/kg bw) produced minimal or no signs of toxicity. Signs of CNS dysfunction including behavioural abnormalities and convulsion were seen at MON-0139 doses of 4290 mg/kg bw and above.

One day of exposure to correctly treated forage should present no hazard to domestic ruminants with respect to any direct action of MON-0139 on animals consuming it. While MON-0139 did produce diarrhoea and apparent abdominal discomfort, vomiting and abnormally frequent regurgitation were not observed.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:

Five groups consisting of five female (Spanish) goats obtained from farms were administered single oral gavage (via rumen intubation) doses of the isopropylamine salt of glyphosate (MON-0139) at doses of 1400, 4290, 5360, 6700, or 10000 mg/kg bw. Water was used as a vehicle in four negative control groups of five goats each. Mortality, clinical signs, body weight, haematology, clinical biochemistry, gross necropsy, histopathology during a post-treatment observation period of 14 days.

At least three goats included to this study gave birth to kids and two aborted during this study and, thus, females of different hormonal and physiological status were used. Weight and age of the test animals were widely distributed. Although randomization was applied with regard to weight, the test population was quite heterogeneous. Due to the small number of animals investigated, the relevance of the biochemical and haematological findings is questionable.

The study is considered acceptable in spite of the heterogeneity of the animals used. It must be taken into account that studies of this type are usually not required in Europe for active ingredients in plant protection products and that no guideline exists. The quality of the study is good and it is suitable to provide additional information about acute oral toxicity in a ruminant species. The maximum dose exceeded those used in acute toxicity studies in laboratory rodents and more parameters were assessed in this study than would be included in an acute oral toxicity study in rodents. However, pathology was confined to four of a total of 20 control animals. In addition, sometimes data obtained in the four control groups were pooled and sometimes reported separately. The acute oral LD_{50} was found to be 5700 mg/kg bw. The oral acute toxicity of the isopropylamine salt of glyphosate in goats was comparable to the one of glyphosate technical acid when corrected for purity (see also CA 5.8.2/008). The acute oral toxicity for both forms of glyphosate was low in goats.

Assessment and conclusion by RMS:

CA 5.8.2/010

1. Information on the study

Data point:	CA 5.8.2
Report author	[redacted]
Report year	1987
Report title	The subacute toxicity of the isopropylamine salt of glyphosate (MON-0139) in female cattle
Report No	80002
Document No	[redacted]-82-003
Guidelines followed in study	None stated
Deviations from current test guideline	Not applicable, non-guideline study
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes.
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

MON-0139, an aqueous formulation containing glyphosate isopropylamine salt (62.4 %) was administered in seven consecutive daily doses by stomach tube to Brahman cross heifers. In a preliminary trial, MON-0139 was administered at a dose of 1000 mg/kg bw/day to two heifers. Based upon the observed response at that dose, subsequent doses of 540, 830, 1290 and 2000 mg/kg bw/day were administered to groups of three heifers each. Concurrent negative control animals (n=2 per treatment group) were treated with the

vehicle only. Mortality, clinical signs, body weight, haematology, clinical biochemistry, gross necropsy, and histopathology were observed for a post-treatment period of 14 days.

All three top dose heifers died within 1.5 days after receiving the sixth or seventh dose. In these animals, nervous system effects like head tremors, convulsions, ataxia and possible visual impairment and shortly prior to death, sternal recumbency occurred. In the group receiving 1290 mg/kg bw/day, one heifer also died after the final dose but without exhibiting signs of nervous dysfunction. Clinical signs of general toxicity included a decrease in feed intake, weight loss, diarrhoea and behavioural depression, and were noted at the two upper dose levels. Diarrhoea and decreased feed consumption were also seen in the mid dose group receiving 830 mg/kg bw/day.

The minimum lethal dose (MLD), minimum toxic dose (MTD) and no observable effect level (NOEL) were estimated to be 1290, 830, and 540 mg MON-0139/kg bw/day, respectively.

Gross pathology revealed signs of dehydration and of gastrointestinal irritation at the upper dosages (mucosal congestion of the rumen, abomasum and duodenum and erosions of the mucosa of the abomasum). High kidney weight to brain weight ratios were seen in all heifers given MON-0139 at 2000 mg/kg bw/day and two of three heifers given MON-0139 at 1290 mg/kg bw/day. High liver weight to brain weight ratios were observed in all heifers given MON-0139 at 2000 mg/kg bw/day.

Histopathologic examination was performed only on liver, kidney, and other organs with gross lesions from heifers given MON-0139 at 1290 and 830 mg/kg bw/day. Histopathological findings were confined to the kidneys and consisted of mild to marked tubular vacuolization (primarily proximal convoluted tubules) and nuclear pyknosis in many tubular epithelial cells in the heifers receiving 1290 mg/kg bw/day. No lesion was seen microscopically in liver from these heifers. Renal tubular vacuolization was not observed in heifers given MON-0139 at 830 mg/kg bw/day.

Clinical laboratory findings associated with MON-0139 doses of 1290 and 2000 mg/kg bw/day consisted of haemoconcentration (elevated PCV, HGB, RBC, and STP); increased serum levels of urea nitrogen, creatinine, GOT, LDH, CK and reduced number of circulating lymphocytes and eosinophils. In addition, heifers that died during the study period exhibited increased numbers of circulating immature (BANDSA) and mature (SEGSA) neutrophils. The surviving heifers (1290 or 830 mg MON-0139/kg bw/day) exhibited mild reductions in serum levels of sodium, inorganic phosphorus, and serum cholinesterase activity. Significant elevation of median serum magnesium concentration was observed at MON-0139 doses above 540 mg/kg bw/day. Significant reduction of median serum potassium (K) concentration was seen at all levels of MON-0139 exposure; however, the decline in K for heifers given MON-0139 at the lowest dose level (540 mg/kg bw/day) was small in magnitude and values for K remained within the limits of study-wide control values for all individuals of this group.

I. MATERIALS AND METHODS

A. MATERIALS

Non-labelled test material:	MON-0139
Identification:	N-(phosphonomethyl)glycine isopropylamine salt
Physical state:	Aqueous solution
Description:	Amber liquid
Lot/Batch #:	LBRT 08023
Source:	Monsanto Company, St. Louis, MO
Purity:	62.4 % (46.2 % for glyphosate N-(phosphonomethyl)glycine)

Stability of test compound:	Stable for >1 year at 29.5 ºC. Store in a cool, dry area at 18.3-29.5 ºC
Vehicle:	Water (approximately 500 mL/heifer)
Test animals:	
Species:	Cattle
Strain:	Brahman cross
Source:	[redacted]
Age:	Not reported (heifers)
Number/Sex:	20 females
Weight at dosing:	169 to 248 kg (mean 215.6 ± 23.1 kg)
Acclimation period:	At least 30 days
Diet/Feed:	During acclimatisation: bermudagrass hay and a commercial feed concentrate containing not less than 13.0 % crude protein (Purina Commercial Creep Chow) After acclimatisation: bermudagrass hay
Water:	Tap water, *ad libitum*
Housing:	During acclimatisation: Outdoor pens for a period of 15 to 19 days then indoor pens for a period of 11 to 15 days. After dosing: Individual indoor pens.
Environmental conditions:	Not reported
Concomitant treatment:	During the initial holding period, all heifers were vaccinated for Clostridial and Leptospiral diseases and treated for internal parasites.

B. STUDY DESIGN AND METHODS

Study dates: 02-Feb-1982 to 05-May-1982

Randomization
All heifers available for experimental use were ranked according to increasing bodyweight. The resulting ranking was divided into 3 nearly equal groups. Treatment groups of three heifers each and control groups of two heifers each were formed by randomly selecting one heifer from each of the three divisions of the ranking in order that the resulting groups would have body weights representative of the pool from which they were selected.

Animal assignment and treatment
Brahman-cross heifers were treated in a sequential manner with MON-0139 by rumen intubation as seven consecutive daily doses of 540, 830, 1000 (preliminary trial, n = 2), 1290, and 2000 mg/kg bw/day (n = 3/group). In the preliminary trial two heifers treated at 1000 mg/kg bw/day over seven consecutive days, body weighing and visual assessment of clinical condition were the only assessments performed.
The test article was given as grams of total formulation per kg body weight. Water was used to dilute the test article (1:1) and to wash any residue from the container holding the dose and from the stomach tube. The total volume of water used for these procedures was approximately 500 mL/heifer. Initially, the test item was given at 2000 mg/kg/bw/day and subsequent doses were selected based on the observed responses. Negative control animals were sham treated with tap water. All animals were penned by treatment group and observed daily during pre-treatment and at least twice daily following treatment. Surviving animals were observed for 14–15 days after treatment until end of study (day 21). Hay consumption was evaluated subjectively by observing feeding activity and visual inspection of the quantity of hay uneaten each day.

Body weight
Body weights were determined prior to dosing (day 0) and on days 6, 14, and 21. Carcass weights were determined for heifers that died during the study period.

Collection of Blood Samples
Samples of blood for clinical biochemical and haematological examination were collected on three separate days during the pre-treatment period. After the first dose of MON-0139 (day 0) samples were collected on days 2, 6, 8, 14 and 21. Samples were taken at unscheduled times when dictated by clinical signs or impending death.

Clinical Biochemistry
An atomic absorption spectrophotometer (Perkin Elmer Model 403, Perkin-Elmer Corp.) was used to determine serum levels of calcium, magnesium, potassium, and sodium, as outlined in the manufacturer's manual. Serum levels of glucose (GLU), urea nitrogen, total protein (TP), alkaline phosphatase, lactic dehydrogenase (LDH), and glutamic oxaloacetic transaminase were determined on automated equipment (Gilford model 3500 computer directed analyser, Gilford Instruments, Inc.) according to manufacturer's procedures. Four other serum components were determined with the same automated equipment; however, different procedures and reagents were used for these determinations, i.e., inorganic phosphorus (Inorganic Phosphorous Reagent, Worthington Diagnostics), cholinesterase and gamma-glutamyl transferase (Reagent Set Kit, Biodynamics/BMC Div.), and creatine phosphokinase (CK-NAC Reagent Set Kit, Biodynamics/BMC Div). Serum creatinine was determined on automated equipment according to manufacturer's procedures (Technicon Auto Analyser, Technicon Corporation).

Blood was collected from the external jugular vein into evacuated glass tubes (Vacutainer tubes, Becton) and allowed to clot at ambient temperature; the serum was separated by centrifugation. Serum LDH activity, CPK activity, and GLU concentration was determined as soon as possible after sample collection.

Haematology
Blood was collected from the external jugular vein into evacuated glass tubes (Vacutainer tubes, Becton) containing disodium ethylenediamine-tetraacetate. Blood smears for differential leukocyte counts were made immediately from blood containing no anticoagulant and stained with Wright's stain (Hema-Tek Slide Stainer, Ames Company). One hundred leukocytes were classified. Haemoglobin concentration was measured as cyanmethemoglobin with a hemoglobinometer (Coulter Hemoglobinometer, Coulter Electronics). Erythrocyte count, mean corpuscular volume, haematocrit and total leukocyte count were determined with an electronic particle counter (Coulter Model ZBI with MCV/Hemat Computer and Channelyzer). Mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration values were calculated.

Pathology
Except for the two heifers of the preliminary trial, all animals given MON-0139 and both heifers from each control group were subjected to post-mortem examination at the end of the post-treatment observation period. Surviving MON-0139 treated and control heifers to be necropsied were killed by the intravenous administration of a commercial euthanasia solution (T-61 Solution, National Laboratories Corp., USA). Organ weight was measured for kidney, liver, and brain. Specimens of heart, liver, kidney, spleen, and other tissues with gross lesions were collected and fixed in neutral, buffered 10 % formalin; however, slides for histologic study were prepared and examined only for liver, kidney and other tissues with gross lesions from animals given MON-0139 at 1290 and 830 mg/kg bw/day, and from six of the eight untreated control heifers. Tissues to be examined microscopically were embedded in paraffin, sectioned, and stained with haematoxylin and eosin.

Statistics
The MTD (minimum toxic dosage) and MLD (minimum lethal dosage) were estimated directly from the morbidity-mortality data. Group median, minimum and maximum values were calculated for each post-

treatment sampling time for all clinical biochemical and haematological parameters. Control values for each treatment group were obtained by pooling all pre-treatment values (principals with concurrent untreated controls) plus all post-treatment values from the concurrent untreated control heifers and calculating for this pool the following statistics: median, minimum value, maximum value, 5th quantile, and 95th quantile. Medians for post-treatment determinations that fell above or below the 95th or 5th quantiles respectively were considered significantly greater or less than the control median ($p \leq 0.05$). Study-wide minimum and maximum control values were obtained by pooling control data from all four dose levels. Single values for individual animals were considered to have changed significantly if they fell outside the absolute range of control values. The Mann-Whitney test was used for comparison of mean percentage change in bodyweight, mean kidney to brain weight ratios (KBR) and mean liver to brain weight ratios (LBR) between heifers that received MON-0139 and their concurrent controls.

II. RESULTS AND DISCUSSION

A. MORTALITY, SURVIVAL TIME AND MTD

All 3 animals treated with 2000 mg/kg bw/day MON-0139 and 1/3 treated with 1290 mg/kg bw/day died.

Table 5.8.2-44: The subacute toxicity of the isopropylamine salt of glyphosate (MON-0139) in female cattle ([redacted], 1987): Survival times, morbidity and mortality

Dose (mg/kg bw/day)	Animal number	Number of doses given	Toxic signs	Death	Survival time[1] (days)	Morbidity (%)	Mortality (%)
2000	41	6	Yes	Yes	6.2	100	100
	45	7			6.5		
	31	7			7.5		
1290	44	7	Yes	Yes	7.5	100	33.3
	40			No	-		
	35						
1000 (preliminary trial)	13	7	Yes	No	-	100	0
	17						
830	37	7	Yes	No	-	100	0
	38						
	39						
540	32	7	No	No	-	0	0
	33						
	42						

No mortality occurred in the eight control animals

B. CLINICAL OBSERVATIONS

In the preliminary study, treatment with 1000 mg/kg bw/day MON-0139 decreased feed intake and induced diarrhoea by the second day of treatment which continued throughout the seven day treatment period. The signs had ceased by the end of the study on day 14.

In the main study, treatment with 2000, 1290, and 830 mg/kg bw/day induced diarrhoea. Animals exposed to 2000 mg/kg bw/day MON-0139 additionally showed nervous system effects including head tremors, convulsions, ataxia, and possible visual impairment and sternal recumbency.

All eight negative control heifers in this study appeared healthy throughout the pre-treatment, treatment and post-treatment periods; no sign of illness was observed.

Table 5.8.2-45: The subacute toxicity of the isopropylamine salt of glyphosate (MON-0139) in female cattle ([redacted], 1987): Prominent clinical observations in heifers given seven daily doses of MON-0139

Number of animals	Dose (mg/kg bw/day)	Observations
3	2000	diarrhoea (3)[1], decreased feed intake (3), nasal discharge (3), foamy salivation (1), head tremors (3), belligerency (1), whole-body tremors (3), ataxia (3), head pressing (1), kicking at imaginary objects (1), apparent visual impairment (1), convulsions (1), falling (1), depression (1), recumbency (3), increased respiratory effort (2), death (3)
3	1290	diarrhoea (3) , decreased feed intake (3), depression (3), weakness (2), death (1)
2 Preliminary trial	1000	diarrhoea (2), decreased feed intake (2)
3	830	diarrhoea (3), decreased feed intake (3)
3	540	no signs of toxicity
8	0	no signs of toxicity

[1] Number of animals affected

C. BODY WEIGHT CHANGES AND FEED CONSUMPTION

Treated animals showed decreased body weight and feed intake (the feed intake was not accurately measured). In the preliminary study, treatment with 1000 mg/kg bw/day MON-0139 decreased feed intake. Treatment with 2000 and 1290 mg/kg bw/day decreased feed intake. Treatment with 1290 mg/kg bw/day induced severe weight loss and depression for the first two weeks. While feed consumption and faecal consistency was similar to that of the controls during the third week after treatment, the animals remained thin and weakened. For the control animals, feed intake and character of the faeces remained normal throughout the study.

Table 5.8.2-46: The subacute toxicity of the isopropylamine salt of glyphosate (MON-0139) in female cattle ([redacted] 1987): Mean percent body weight changes in heifers given MON-0139

Dose (mg/kg bw/day)	Day of treatment	Mean Percentage change from initial body weight (N)			
		MON-0139 treated heifers		Control heifers	
2000	6	-1.2	(3)	-1.29	(2)
1290	6	-6.5[1]	(3)	-2.1	(2)
	14	-12.0[2]	(2)	-1.6	(2)
	21	-9.6	(2)	-3.4	(2)
830	6	-5.2[1]	(3)	-1.5	(2)
	14	-4.6	(3)	-0.4	(2)
	21	-2.4[1]	(3)	5.1	(2)
540	6	0.3	(3)	-0.7	(2)
	14	1.0	(3)	2.7	(2)
	21	0.4	(3)	1.5	(2)

[1] Approaches being significantly different from concurrent control mean ($p<0.083$)
[2] Approaches being significantly different from concurrent control mean ($p<0.121$)
N= number of heifers weighed

D. POST-MORTEM OBSERVATIONS

Few significant gross lesions were noted in heifers that died during the study period; however, at the time of necropsy each was judged to be dehydrated or to have suffered loss of body weight. Nonspecific (agonal)

lesions included aspiration of rumen contents, petechial haemorrhage associated with the epicardium and spleen and pulmonary oedema. Post-mortem evidence of diarrhoea was observed in three of these heifers and no faeces were present in the rectum of a fourth heifer. Other lesions observed in these animals potentially attributable to treatment included erosions of the abomasal mucosa and congestion of the mucosa of the rumen, abomasum, and duodenum. One heifer administered 2000 mg/kg bw/day exhibited hyphaema (blood in the anterior chamber) in both eyes. No treatment-related lesions were observed at necropsy in the other eight MON-0139 treated heifers that were sacrificed at the end of the observation period (day 21 or 22). High kidney weight to brain weight ratios were seen in all heifers given MON-0139 at 2000 mg/kg bw/day and two of three heifers given MON-0139 at 1290 mg/kg bw/day. High liver weight to brain weight ratios were observed in all heifers given MON-0139 at 2000 mg/kg bw/day. These observations became rare or absent at lower MON-0139 dose. Mean KBR was greater than but not significantly different from mean KBR of concurrent controls for MON-0139 doses of 2000 and 1290 mg/kg bw/day. Mean KBR was less than but not significantly different from concurrent controls for heifers given MON-0139 at 830 or 540 mg/kg bw/day. Findings for LBR were similar. A mycotic dermititis was observed in many animals of this study, including untreated control heifers. Major gross pathologic findings noted in treated and control heifers are presented in the following table and unless indicated otherwise were judged to be unrelated to treatment and due to common agents such as parasites or the method of killing.

Table 5.8.2-47: The subacute toxicity of the isopropylamine salt of glyphosate (MON-0139) in female cattle ([redacted], 1987): Mean kidney and liver weight divided by brain weight for heifers given MON-0139 and concurrent controls

Dose (mg/kg bw/day)	Number of heifers	Kidney/Brain	Liver/Brain
2000	3	1.86[2]	7.57[2]
0[1]	2	1.25	6.45
1290	3	1.49[2]	5.96
0[1]	2	0.96	4.81
830	3	1.14[2]	5.75
0[1]	2	1.27	6.08
540	3	1.18	6.14
0[1]	2	1.19	5.92

[1] Concurrent controls for preceding MON-0139 treated group
[2] Approaches being significantly different from concurrent control mean (P <0.083)

E. HISTOPATHOLOGICAL OBSERVATIONS

Microscopic examination of kidney from the heifer given MON-0139 at 1290 mg/kg bw/day that died revealed marked renal tubular vacuolization. The proximal portion of the convoluted tubules appeared to be most severely involved. In addition, many tubular epithelial cells contained pyknotic nuclei. Histologic examination of the abomasum revealed multifocal superficial mucosal erosions, which appeared to be of recent development based on the minimal extent of the associated cellular reaction. Segmental congestion of the ileum (noted grossly) was characterized microscopically by focal necrosis and inflammation of the mucosa overlying Peyer's patches that extended superficially into the lymphoid tissue. Bacterial colonization of these areas in the ileum was marked. Blood in the anterior chamber of both eyes of one heifer was confirmed microscopically and was judged to have been the result of trauma.

The two surviving heifers given MON-0139 at 1290 mg/kg bw/day each had mild renal tubular vacuolisation. Focal vasculitis and perivasculitis was noted in the livers of one animal and one control animal and was considered to be the result of parasite migrations.

There were no significant treatment-related microscopic lesions observed in the tissues studied from heifers given MON-0139 at 830 mg/kg bw/day or tissues studied from control heifers. Dermatomycosis consistent with lesions caused by *Trichophyton spp.* was observed in both treated and control animals.

Table 5.8.2-48: The subacute toxicity of the isopropylamine salt of glyphosate (MON-0139) in female cattle ([redacted], 1987): Major gross pathologic findings in heifers given MON-0139 and in untreated control heifers

Animal number	Body as a whole	Skin	Respiratory	Cardio-vascular	Haemic & Lymphatic	Digestive	Urogenital
2000 mg MON-0139/kg bw/day							
31-12[1]	Dehydration; weight loss	Dermatitis	Aspiration (agonal)	Petechiae, epicardial	Petechiae, capsule of spleen	Erosions, abomasal mucosa[2]; congestion abomasum rumen ; diarrhoea[2]; HLBR	NGL; HKBR[2]
45-12[1]	Dehydration (moderate)	Dermatitis	Nodules subpleural; Oedema pulmonary (mild)	Petechiae, epicardial	NGL	Congestion, abomasal and duodenal mucosa[2]; Distension, gall bladder, diarrhoea, HLBR[2]	NGL; HKBR[2]
41-12[1]	Weight loss	Dermatitis	Oedema pulmonary	Petechiae, epicardial	NGL	Congestion, ileal mucosa; diarrhoea[2]; congestion, liver; HLBR[2]	NGL; HKBR[2]
30-13[3]	NGL	Dermatitis	NGL	NGL	NGL	NGL	NGL
4-13[3]	Weight loss	Dermatitis	NGL	NGL	NGL	NGL	Infarct, rt. kidney
1290 mg MON-0139/kg bw/day							
40-22	Weight loss; serious atrophy of fat	Dermatitis	Petechiae, pleura	Petechiae, epicardial	NGL	Scars, capsule of liver	NGL
35-22	NGL	Dermatitis	NGL	NGL	NGL	NGL	NGL; HKBR[2]
44-22[1]	Dehydration	NGL	NGL	Hemocyst, heart valve	NGL	Erosions, abomasal mucosa; Congestion mucosa of ileum	NGL; HKBR[2]
34-23[3]	NGL	Dermatitis	Petechiae trachea mucosa and pleura	NGL	NGL	Petechiae, duodenum; congestion; Peyer's patches	Petechiae urinary bladder
9-23[3]	NGL	Dermatitis	NGL	NGL	NGL	Erosion oesophagus	NGL
830 mg MON-0139/kg bw/day							
38-32	NGL	NGL	NGL	NGL	NGL	Congestion colon, round ligament	NGL
37-32	NGL	NGL	NGL	NGL	NGL	Scars (2), liver capsule	NGL
39-32	NGL	Dermatitis	NGL	NGL	NGL	NGL	NGL
28-33[3]	NGL	NGL	NGL	NGL	Congestion, spleen	Petechiae, abomasal mucosa; Congestion ileal mucosa	Congestion vulvar mucosa
11-33[3]	NGL	Dermatitis	NGL	NGL	NGL	Congestion, ileum and cecum	NGL
540 mg MON-0139/kg bw/day							
42-42	NGL	NGL	Focus, subpleural	NGL	NGL	Erosions, oesophagus,	HKBR

Table 5.8.2-48: The subacute toxicity of the isopropylamine salt of glyphosate (MON-0139) in female cattle ([redacted] 1987): Major gross pathologic findings in heifers given MON-0139 and in untreated control heifers

Animal number	Body as a whole	Skin	Respiratory	Cardio-vascular	Haemic & Lymphatic	Digestive	Urogenital
						HLBR	
32-42	NGL	NGL	Red foci, subpleural	NGL	NGL	Congestion, ileum and Peyer's patches; mottling, liver	NGL
33-42	NGL	NGL	NGL	NGL	NGL	NGL	NGL
29-43[3]	NGL	NGL	NGL	NGL	NGL	Lipoma	NGL
20-43[3]	NGL	Dermatitis	NGL	NGL	Lymphoid hyperplasia pharyngeal nodes (mild)	NGL; parasitism	NGL

[1] Fatally poisoned, all other animals -were sacrificed at the end of the observation period
[2] Observations (non-agonal) potentially attributable to treatment
[3] Untreated control heifers for preceding group
NGL = No Gross Lesions
HLBR = High liver weight to brain weight ratio relative to controls
HKBR = High kidney weight to brain weight ratio relative to controls

F. CLINICAL BIOCHEMISTRY AND HAEMATOLOGY

Treatment with 1290 and 2000 mg/kg bw/day MON-0139 increased haematocrit, haemoglobin, red blood cells and increased serum levels of total protein, urea nitrogen (BUN), creatinine and serum glutamic oxaloacetic transaminase (GOT), lactic dehydrogenase (LDH), and creatine phosphokinase (CPK) activities.
The haematologic alterations were considered to be due to haemoconcentration secondary to fluid shifts resulting from diarrhoea.
Elevations in CPK, GOT, and LDH activities were attributed to muscle damage resulting from convulsions and/or prolonged sternal recumbency.
Slight elevations of BUN and creatinine may have been due to decreased renal perfusion produced by dehydration secondary to diarrhoea. However, the presence of histopathological kidney lesions at 1290 mg/kg bw/day and changes in serum electrolyte levels at several doses suggest that these changes may have been partly due to some renal impairment.
An increase in the number of neutrophils and a decrease in the number of lymphocytes observed at 1290 and 2000 mg/kg bw/day probably represented a response to stress-induced release of corticosteroids.

Table 5.8.2-49: The subacute toxicity of the isopropylamine salt of glyphosate (MON-0139) in female cattle ([redacted] 1987): Days after treatment on which median values for clinical laboratory measurements performed on heifers given MON-0139 for 7 days were significantly different from concurrent median control values

	Dose (mg/kg bw/day)			
Parameter	**2000**	**1290[1]**	**830**	**540**
BUN	6	2, 6, 7, 8, 14	-	8[2]
CREAT	6	7, 8	-	8[2]
GLU	-	7	-	-
Na	6	6[2], 14[2]	-	-
K	6[2]	6[2], 8[2], 14[2]	6[2]	14[2]

Ca	6[2]	-	8[2]	-
P	6	7, 21[2]	2	-
Mg	6	7, 8	2	-
GOT	6[3]	2, 6, 7, 8, 14, 21	-	-
LDH	6[3]	6, 7	-	-
SACH	-	14[2]	21[2]	-
ALKP	-	7	-	-
CKN	6	2, 6, 7, 8	-	-
GGT	-	-	-	8, 14, 21
WBC	-	-	-	-
SEGSA	6	-	-	-
BANDSA	6	-	-	-
LYMPHSA	6[2]	6[2]	-	-
EOSA	2[4], 6[4]	6[4], 8[4]	-	14[2], 21[2]
MONOSA	-	-	-	-
BASOA	-	-	-	-
PCV	6	6[3], 7, 8	-	-
HGB	6	6, 7, 8	-	-
RBC	6[3]	6, 7, 8	-	-
MCV	-	-	-	-
MCH	-	-	-	-
MCHC	-	6, 21	-	-
STP	6	6, 7, 8	-	-

[1] Represents data from three animals on days 2 and 6 and two animals on days 8, 14, and 21. Entries for day 7 represent one animal, and the value is outside the range of study-wide control values

[2] Medians significantly lower than the concurrent median control value; entries without a symbol represent medians significantly greater than the concurrent median control values.

[3] Noteworthy non-significant increase

[4] Noteworthy non-significant decrease

BUN = Urea nitrogen, CREAT = Creatinine, GLU = Glucose, Na = Sodium, K = Potassium, Ca = Calcium, P = Inorganic phosphorus, Mg = Magnesium, GOT = Glutamic oxaloacetic transaminase, LDH = Lactic dehydrogenase, SACH = Cholinesterase, ALKP = Alkaline phosphatase, CKN = Creatine phosphokinase, GGT = Gamma-glutamyltransferase, WBC = Total leukocyte count, SEGSA = Segmented neutrophil count, BANDSA = Band neutrophil count, LYMPHSA = Lymphocyte count, EOSA = Eosinophil count, MONOSA = Monocyte count, BASOA = Basophil count, PCV = Microhematocrit, HGB = Haemoglobin concentration, RBC = Erythrocyte count, MCV = Mean corpuscular volume, MCH = Mean corpuscular haemoglobin, MCHC = Mean corpuscular haemoglobin concentration, STP = Total protein

Table 5.8.2-50: The subacute toxicity of the isopropylamine salt of glyphosate (MON-0139) in female cattle ([redacted] 1987): Clinical biochemical and haematological measurements (min-max values) for heifers given MON-0139

Parameter (unit)	Ref.[1,2]	Control[3]	Death on day 6 or 7		Surviving heifers			
			Sampled 6-14 h before death (3 heifers)	Sampled 34-38 h before death (2 heifers)	Signs of intoxication observed (5 heifers)		No signs of intoxication observed (3 heifers)	
					Days 2, 6, 8	Days 14 & 21	Days 2, 6, 8	Days 14 & 21
BUN (mg/dL)	6.0-27.0	4.3-24.0	55.5-100.0	26.5-65.6	7.3-85.2	6.5-47.3	5.0-13.8	8.2-21.5
CREAT (mg/dL)	0.20-2.60	1.30-2.55	8.62-11.30	4.7-11.1	1.55-9.55	1.50-2.55	1.55-2.35	1.40-2.20
GLU (mg/dL)	45-130	46-180	104-229	137-257	68-151	65-110	65-89	71-87
Na (mg/dL)	304-350	317-358	348-392	319-339	309-339	312-345	329-337	317-331
K	15.2-26.5	11.8-21.0	11.7-19.1	11.6-12.4	8.9-15.4	7.1-17.8	15.4-	14.2-

Table 5.8.2-50: The subacute toxicity of the isopropylamine salt of glyphosate (MON-0139) in female cattle ([redacted] 1987): Clinical biochemical and haematological measurements (min-max values) for heifers given MON-0139

Parameter (unit)	Ref.[1,2]	Control[3]	Death on day 6 or 7		Surviving heifers			
			Sampled 6-14 h before death (3 heifers)	Sampled 34-38 h before death (2 heifers)	Signs of intoxication observed (5 heifers)		No signs of intoxication observed (3 heifers)	
					Days 2, 6, 8	Days 14 & 21	Days 2, 6, 8	Days 14 & 21
(mg/dL)							17.0	15.8
Ca (mg/dL)	9.4-12.2	8.6-13.4	8.2-11.6	8.5-9.3	9.2-10.9	9.5-11.4	9.9-12.0	9.9-11.7
P (mg/mL)	4.5-9.3	5.4-9.9	6.9-20.7	9.9-111.3	5.7-10.5	3.9-9.0	6.0-7.6	6.9-8.0
Mg (mg/mL)	1.20-3.50	1.58-2.48	3.27-4.46	2.23-3.51	1.88-3.47	2.03-2.48	1.83-2.13	2.03-2.28
GOT (U/L)	8.5-93	27-70	56-132	62-133	33-189	41-87	37-72	32-64
LDH (U/L)	8-302	475-737	694-965	666-759	398-1186	465-705	540-731	494-729
SACH (U/L)	-	197-412	315-421	288-334	200-326	220-333	230-314	211-323
ALKP (U/L)	94.-170	28-169	62-251	73-126	27-149	28-126	53-137	58-130
CKN (U/L)	-	45-714	274-6988	502-2079	113-9234	60-177	61-698	51-94
GGT (U/L)	-	8-22	16-22	14-16	10-19	11-22	10-18	11-20
WBC	4.0-10.0	4.1-13.4	8.3-12.1	6.9-12.7	4.9-9.2	4.8-9.7	4.5-9.2	4.8-8.0
SEGSA	0.6-4.5	0.6-5.8	1.4-5.1	3.9-6.5	0.9-2.8	1.1-3.1	0.9-2.3	0.6-1.4
BANDSA	0-1.0	0-0.2	0-0.8	0.2-0.9	0-0.1	0-0.1	0-0.1	0
LYMPHSA	1.8-7.5	2.7-8.3	2.0-8.1	2.6-4.7	2.1-6.8	2.9-6.9	3.5-7.2	4.1-7.0
EOSA	0-2.0	0-1.8	0	0	0-0.7	0-1.4	0-0.5	0-0.2
MONOSA	0.1-0.7	0-0.9	0-0.7	0.1-0.6	0-0.5	0.1-0.5	0-0.3	0-0.2
BASOA	Rare	0-0.1	0	0-0.1	0-0.2	0-0.2	0-0.1	0
PCV (%)	24.0-40.0	33.8-54.1	50.7-71.3	53.5-57.4	39.3-56.5	39.3-47.2	41.4-46.8	42.4-43.7
HGB (g/L)	8.0-14.0	10.7-19.5	19.1-25.5	21.3-23.0	13.4-20.2	15.2-18.5	15.1-16.8	15.5-16.5
RBC	5.0-9.0	7.9-14.1	11.8-17.9	13.3-15.8	9.6-12.9	10.0-11.7	10.4-11.8	10.8-11.7
MCV (fL)	40.0-60.0	36.0-49.5	40.5-48.0	37.0-40.5	39.0-44.5	37.5-43.5	38.0-40.5	36.0-39.5
MCH (pg)	11.0-17.8	10.6-17.9	14.2-16.2	14.6-16.0	13.5-16.7	14.7-16.8	13.1-14.9	13.2-15.3
MCHC (g/dL)	30.0-36.0	27.9-40.0	35.8-37.7	39.8-40.1	31.5-39.6	34.0-41.7	34.8-37.7	35.6-38.7
STP (g/dL)	5.9-8.6	5.7-9.5	7.4-9.6	7.9-7.9	6.4-9.0	6.1-7.8	6.2-7.6	5.9-7.3

[1] Mitruka, BM and Rawnsley, HM: Clinical Biochemical and Haematological Reference Values in Normal Experimental Animals. Masson Publishing USA, Inc., New York, 1977.
[2] Archer, RK and Jeffcott, LB (eds.): Comparative Clinical Haematology, Blackwell Scientific Publications, London, 1977.
[3] Control values were obtained by pooling all pre-treatment values (principal and untreated controls) plus all post-treatment values from untreated controls on a study wide basis (99 measurements for each parameter from 20 heifers).
[4] Cells x $10^3/\mu L$
[5] Cells x $10^6/\mu L$

Table 5.8.2-50: The subacute toxicity of the isopropylamine salt of glyphosate (MON-0139) in female cattle ([redacted] 1987): Clinical biochemical and haematological measurements (min-max values) for heifers given MON-0139

Parameter (unit)	Ref.[1,2]	Control[3]	Death on day 6 or 7		Surviving heifers			
			Sampled 6-14 h before death (3 heifers)	Sampled 34-38 h before death (2 heifers)	Signs of intoxication observed (5 heifers)		No signs of intoxication observed (3 heifers)	
					Days 2, 6, 8	Days 14 & 21	Days 2, 6, 8	Days 14 & 21

BUN = Urea nitrogen, CREAT = Creatinine, GLU = Glucose, Na = Sodium, K = Potassium, Ca = Calcium, P = Inorganic phosphorus, Mg = Magnesium, GOT = Glutamic oxaloacetic transaminase, LDH = Lactic dehydrogenase, SACH = Cholinesterase, ALKP = Alkaline phosphatase, CKN = Creatine phosphokinase, GGT = Gamma-glutamyltransferase, WBC = Total leukocyte count, SEGSA = Segmented neutrophil count, BANDSA = Band neutrophil count, LYMPHSA = Lymphocyte count, EOSA = Eosinophil count, MONOSA = Monocyte count, BASOA = Basophil count, PCV = Microhematocrit, HGB = Haemoglobin concentration, RBC = Erythrocyte count, MCV = Mean corpuscular volume, MCH = Mean corpuscular haemoglobin, MCHC = Mean corpuscular hemoglobin concentration, STP = Total protein

Study conclusion

Exposure of Brahman-cross heifers for seven consecutive days to MON-0139 resulted in clinical signs of toxicity at doses of 830 mg/kg bw/day and above which included loss of appetite and diarrhoea. Mortality was observed at 1290 and 2000 mg/kg bw/day only. In addition, nervous system effects consistent with overt acute toxicity and impending death (head tremors, convulsions, ataxia, etc.) were observed at 2000 mg/kg bw/day. Changes in several haematological parameters observed at 1290 mg/kg bw/day and above may have been secondary to fluid and blood volume alterations resulting from the diarrhoea. Serum chemistry changes and histopathologic lesions indicative of renal damage were observed at 1290 mg/kg bw/day. The no-observable-effect level (NOEL) under the conditions of this study was considered to be 540 mg/kg bw/day.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:

An aqueous solution of the isopropylamine salt of glyphosate (MON-0139) was administered on seven consecutive days by ruminal intubation to four groups of three female cattle (heifers) at 0, 540, 830, 1290 and 2000 mg/kg bw/day. Water was used as a vehicle to give a constant dosing volume of 500 mL. Four concurrent negative controls groups of two heifers each were sham treated with water. Mortality, clinical signs, body weight, haematology, clinical biochemistry, gross necropsy, histopathology were assessed during a post-treatment observation period of 14 days.

Clinical signs of toxicity occurred at doses of 830 mg/kg bw/day and above, including loss of appetite and diarrhoea. Mortality occurred at doses of 1290 and 2000 mg/kg bw/day. Nervous system effects (head tremors, convulsions, ataxia, etc.) were observed at 2000 mg/kg bw/day prior to death.

At the two upper dose levels, haematological changes (haemoconcentration) probably resulted from diarrhoea. Serum chemistry changes and histopathologic lesions indicative of renal damage were observed at 1290 mg/kg bw/day. An increase in ASAT, creatine phosphokinase and lactate dehydrogenase activity suggests rather muscle than liver damage since there were no histological liver findings. Muscle damage could result from convulsions or prolonged sternal recumbency. Some further biochemical findings (blood urea nitrogen, creatinine) could be well due to dehydration but, in particular in the presence of histological kidney lesions, might also provide evidence of renal function impairment.

The statistical calculations in this study were based on very small group sizes of three treated and two negative control animals. Histopathology was confined to slides prepared for liver, kidney and tissues with gross lesions obtained from animals receiving doses of 1290 and 830 mg/kg bw/day and from 6 of the 8 control heifers. Limited data on organ weights was provided as kidney or liver to brain ratios only.

The study is considered acceptable despite the low number of animals per dose group. The quality of the study is suitable to provide additional information about subacute oral toxicity in a ruminant species. The lowest dose of 540 mg/kg bw/day can be considered a NOAEL for toxic effects in this study. Subacute oral toxicity of the isopropylamine salt of glyphosate in young cattle (heifers) was low. Forage application rate for grazing cattle to receive a dose equivalent to the NOAEL is estimated to be 77 pounds per acre (86 kg/ha), an unrealistically high use rate.

Assessment and conclusion by RMS:

CA 5.8.2/011

1. Information on the study

Data point:	CA 5.8.2
Report author	[redacted]
Report year	1987
Report title	Irritating effect of Glyphosate, Surfactant and Roundup on Stomach and small Intestine in Dogs
Report No	2309496
Document No	Not reported
Guidelines followed in study	None stated
Deviations from current test guideline	Not applicable
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities.
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

This study evaluated the irritating effect of Roundup® (herbicide consisting of isopropylamine salt of glyphosate at 41 %, surfactant MON-0818 at 15 %, and water) and its ingredients on mucosa of fasted dogs. The irritating effects of Roundup, glyphosate and surfactant on gastric and small intestinal mucosa were tested *in vivo* using Beagle dogs. Histopathological findings were compared with effects caused by 0.25 N hydrochloric acid as positive control agent and with unchanged tissue samples. All test substances were applied directly to mucosae of anaesthetized dogs.

Direct application of Roundup, glyphosate acid, and surfactant caused only mild damage to the stomach and intestine. The severity of the damage was equivalent to that caused by 0.25 N hydrochloric acid. The test materials tended to affect the small intestine more than the stomach. Roundup and its surfactant caused decomposition of the tip of villi in the small intestine. Roundup, glyphosate and surfactant caused oedema and congestion of top mucosal layers and degeneration of epithelium in the stomach.

The oedema and ablation of epithelium were considered to have resulted from interstitial exudation due to congestion-caused microcirculatory disorder. As for the small intestine, in particular, congestion of villi produced oedema in the space between absorptive epithelium and basal membrane, which subsequently pushed up the epithelium to the extent that it might be ablated and detached.

These effects were more severe with the Roundup formulation than with either the IPA salt or the surfactant. The intestine appeared to be more affected than the stomach. The severity of the damage was equivalent to that caused be 0.25 N hydrochloric acid. Clinical, autopsy and experimental evidence indicate a potential for gastrointestinal damage from glyphosate components of glyphosate formulations, but the frequency of severe injury appears to be low. Irritation was more severe with the Roundup formulation than with either the IPA salt or the surfactant alone. The intestine appeared to be more affected than the stomach. The severity of the damage was equivalent to that caused be 0.25 N hydrochloric acid.

The IPA salt of glyphosate, Roundup herbicide (41 % IPA) and the surfactant MON-0818 (15 % of which is contained in Roundup) and 0.25 N hydrochloric acid solution (control) were directly administered on the gastric and small intestinal mucosa of fasted male beagle dogs. The specimens were examined microscopically and evaluated for mucosal damage in comparison with normal gastric and intestinal tissues. Direct application of Roundup® herbicide, and the surfactant caused mild mucosal damage in the stomach and intestine. These effects were more severe with the Roundup formulation than. with either the IPA salt or the surfactant. The intestine appeared to be more affected than the stomach. The severity of the damage was equivalent to that caused be 0.25 N hydrochloric acid. The results suggest that these substances could significantly contribute to the toxicity of glyphosate products.

I. MATERIALS AND METHODS

A. MATERIALS

Test material 1:	Undilute Roundup Formulation
Identification:	41 % isopropylamine (IPA) salt of glyphosate
Lot #:	Oda B6E20 88.10
Purity:	Not reported
Stability of test compound:	Not reported
Test material 2:	Isopropylamine salt of glyphosate
Identification:	Isopropylamine salt of glyphosate (MON139)
Lot#:	LIRT09023
Purity:	63.2 % assay
Stability of test compound:	Not reported
Test material 3:	
Identification:	Undilute Surfactant (MON-0818)
Lot/Batch #:	Not reported
Purity:	Not reported

Stability of test compound:	Not reported
Positive control:	
Identification:	0.25 N hydrochloric acid
Source:	Kokusan Kagaku Co. Ltd.
Purity:	Not reported
Stability of test compound:	Not reported
Vehicle and/ or positive control:	Distilled water
Test animals:	
Species:	Dog
Strain:	Beagle
Source:	[redacted]
Age:	Approximately 15 months
Sex/Number:	Male, one test group consisting of 4to 8 animals
Body weight:	12-15 kg
Acclimation period:	Not reported
Diet/Feed:	DS (Oriental Yeast Co. Ltd.) , *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Stainless steel two-stage cages (Watering System Co.)
Environmental conditions:	Temperature: 23 ± 2 °C Humidity: 55 ± 10 % Air changes: not reported Photocycle: 12 hours light/dark cycle

B. STUDY DESIGN AND METHODS

Administration of test solutions
Roundup was applied undiluted. IPA salt of glyphosate and surfactant were diluted with distilled water to 41 % and 15 % respectively for application. The positive control 6N hydrochloric acid was diluted to 0.25 N solution with distilled water.

Beagle dogs, fasted for a day, were anesthetized with Ketamine and Skisamesonium, and the abdominal incision was performed. The stomach was cut open along the greater curvature with a cautery knife. The anterior wall of the stomach was everted to expose the mucosal tissue. In order to prevent the reflex of bile, etc., cheese cloth was inserted into the oesophagus and the pylorus. Using a silk suture, the gastric mucosa was pulled up to stuff cheese cloth into the serosa side of the posterior wall , thus making two 3cm diameter depressions on the surface of the body of stomach. Into these concavities, the test materials were placed, and left for 30 minutes. Whenever the test material was spilled or reduced in volume for other reasons, a sufficient amount of the test material was added. The small intestine was ligatured at two positions which were 10 cm apart. When doing this, with silk sutures care was taken not to stop blood circulation in the area concerned. A Teflon-coated catheter was inserted into the intestinal duct for administration of test solutions. Each test article was left in the test portion of the intestinal duct for 30 minutes as in the case of the stomach. After the 30-minute application, the stomach was immediately washed with physiological saline and the treatment portion of the stomach was incised for collection. The small intestine was cut open to evert the inner mucosal lining and washed with physiological saline.
The test material-applied portions of stomach and small intestine were fixed with 10 % formalin buffer and their specimens were prepared by a routine method of haematoxylin and eosin stain. These specimens were examined microscopically and evaluated for mucosal damage in comparison with normal gastric and

intestinal tissues.

II. RESULTS AND DISCUSSION

A. HISTOPATHOLOGY OF GASTRIC MUCOSA

The stomach had slight oedema on the mucosa, congestion of the mucosa and very slight degeneration of the epithelium, after Roundup was treated. Application of glyphosate and surfactant also caused similar changes. Glyphosate affected the stomach approximately the same way and to the same degree as Roundup. As for surfactant, congestion of the mucosa was slightly milder than that for Roundup, but other observations were similar. A 0.25 N hydrochloric acid brought the same changes as Roundup, though the oedema and congestion of mucosa were slightly milder than those for Roundup were, while the degeneration of epithelium was slightly more severe.

B. HISTOPATHOLOGY OF SMALL INTESTINE MUCOSA

Roundup caused congestion of villi, oedema of the furthest portion of villi, degeneration and ablation of absorptive epithelium and dilatation of crypts. Glyphosate and surfactant showed the similar changes, though surfactant had slightly milder effect in oedema and glyphosate and surfactant had slightly milder effect in congestion of villi than Roundup. Surfactant did not cause dilatation of crypts, which the other two test materials did. A 0.25 N hydrochloric acid was similar in effect to Roundup, though the severity of the changes brought by the hydrochloric acid was a little higher or similar to that by Roundup.

There was no remarkable change in the mucosa of a normal stomach. The Roundup- treated stomach had slight oedema and congestion of mucosal layers and degeneration of epithelium. There were similar changes in similar severity observed in the stomach treated with glyphosate. Surfactant had slightly milder effect in congestion of mucosa than Roundup , but there were no other differences between surfactant and Roundup as to the changes and their severity. The 0.25 N hydrochloric acid applied stomach displayed damages on mucosa similar to those for Roundup.

There was no remarkable change in a normal intestine. Roundup treatment caused congestion of villi, oedema of villi, degeneration and ablation of epithelium and dilatation and crypts. The glyphosate treated intestine also showed similar changes to the same degree , though its congestion of villi was less severe than that for Roundup. As for surfactant, the same observations except dilatation of crypts were made, but the congestion and oedema of villi were slightly milder than those for Roundup. A 0.25 N hydrochloric acid showed the same changes as Roundup, but the severity of those changes was slightly higher than , or at least the same as that for Roundup. In some cases, decomposition of the tip of villi was observed in the intestines treated with Roundup or surfactant.

Table 5.8.2-51: Irritating effect of Glyphosate, Surfactant and Roundup on Stomach and small Intestine in Dogs ([redacted], 1987): Histopathological findings

	Findings of stomach after treatment with				Findings of intestine after treatment with			
	Roundup n=5	Glyphosate n=5	Surfactant n=4	HCl n=4	Roundup n=8	Glyphosate n=8	Surfactant n=8	HCl n=5
	Congestion of mucosa				**Congestion of villi**			
None	-	-	2	1	-	-	1	-
Very slight	-	1	1	2	-	1	2	-
Slight	4	3	1	1	2	5	3	-
Moderate	1	1	-	-	5	2	2	4
Severe	-	-	-	-	1	-	-	1
	Oedema of mucosa				**Oedema of villi**			
None	-	-	-	-	-	-	-	-
Very slight	-	1	1	2	2	2	5	-
Slight	5	4	3	2	6	6	3	5
Moderate	-	-	-	-	-	-	-	-
Severe	-	-	-	-	-	-	-	-
	Degeneration of epithelium				**Degeneration and ablation of epithelium**			
None	-	-	-	-	-	-	-	-
Very slight	5	5	4	2	2	2	3	-
Slight	-	-	-	2	5	4	4	5
Moderate	-	-	-	-	1	2	1	-
Severe	-	-	-	-	-	-	-	-
					Dilatation of crypts			
None	-	-	-	-	3	1	8	4
Very slight	-	-	-	-	2	3	-	1
Slight	-	-	-	-	3	4	-	-
Moderate	-	-	-	-	-	-	-	-
Severe	-	-	-	-	-	-	-	-

n = number of animals

Study conclusion

Roundup, glyphosate and surfactant caused oedema and congestion of top mucosal layers and degeneration of epithelium in the stomach. Treatment of 0.25 N hydrochloric acid also caused the same changes, but the severity was similar to or slightly lower than that by Roundup. As for the intestinal treatment, the three test articles caused congestion of villi, oedema of tips of villi and degeneration and ablation of epithelium. In addition to these, glyphosate and Roundup treated tissues showed dilatation of crypts. A 0.25 N hydrochloric acid also showed the same changes, but the severity of those changes was slightly higher than, or at least the same as that for Roundup. The mucosal damage in the stomach and small intestine caused by treatment of Roundup, glyphosate of surfactant was only mild, equivalent to that caused by 0.25 N hydrochloric acid. The intestine was more severely damaged than the stomach in every case.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:

The 63.2 % concentrate IPA salt of glyphosate, undiluted Roundup herbicide (41 % IPA, 15 % MON-0818 surfactant), the undiluted surfactant MON-0818, and 0.25 N hydrochloric acid solution (control) were directly administered on the gastric and small intestinal mucosa of fasted male beagle dogs. The specimens were examined microscopically and evaluated for mucosal damage in comparison with normal gastric and intestinal tissues. Direct application of Roundup® herbicide, and the surfactant caused mild mucosal damage in the stomach and intestine. These effects were more severe with the

Roundup formulation than with either the IPA salt or the surfactant. The intestine appeared to be more affected than the stomach. The severity of the damage was equivalent to that caused be 0.25 N hydrochloric acid.
The data are considered as supplemental information indicating that highly concentrated glyphosate IPA salt has irritating properties on the stomach and intestine of dogs, nevertheless has a lower impact than the undiluted Roundup herbicide.

Assessment and conclusion by RMS:

CA 5.8.2/012

1. Information on the study

Data point:	CA 5.8.2
Report author	[redacted]
Report year	1980
Report title	Acute intraperitoneal toxicity of glyphosate in female rat
Report No	Not reported
Document No	Not reported.
Guidelines followed in study	None stated.
Deviations from current test guideline	Not applicable.
Previous evaluation	Not accepted in RAR (2015).
GLP/Officially recognised testing facilities[1,2]	Not specified.
Acceptability/Reliability:	Supportive Route of exposure (intraperitoneal injection); deficiencies in reporting (only limited information on test material, test animals and results available).
Category study in AIR 5 dossier (L docs)	Category 4a

2. Full summary

The test substance, N-phosphono-methyl-glycine, was evaluated for its acute intraperitoneal toxicity potential, by exposing groups of ten Sprague-Dawley strain female rats to doses of 620, 760, 940, 1040 and 1260 mg/kg bw. The test substance was diluted in propylene-glycol in water.
Mortality occurred in the 4 highest dose groups. In the highest dose group (1260 mg/kg bw), 10/10 animals died. In the next lower dose group (1040 mg/kg bw), 8/10 animals died followed by 4/10 and 1/10 deceased animals at 940 mg/kg bw and 760 mg/kg bw, respectively. Transient dyspnoe due to string irritation of viscera was observed. In the deceased animals, superficial liver necrosis, punctiform bleedings of the peritoneum and exudatum between the pleura surfaces were seen. The extent of fibrophosis extending to the viscera was proportional to the time of survival. The late deaths were considered to be caused by

peritonitis instead of test substance-induced toxicity.
The acute intraperitoneal LD_{50} was calculated to be 919 ± 42 mg/kg bw.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Identification:	N-phosphono-methyl-glycine
Description:	Not reported
Lot/Batch #:	Not reported
Purity:	Not reported
Stability of test compound:	Not reported
Vehicle and/ or positive control:	Propylene glycol in water/ None

Test animals:

Species:	Rat
Strain:	Wistar
Source:	Not reported
Age:	Not reported
Sex:	Females
Weight at dosing:	190 - 210 g
Acclimation period:	Not reported
Diet/Food:	Normal laboratory rodent food (LATI), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Not reported
Environmental conditions:	Temperature: 20 ± 2 °C Humidity: 55 - 75 % Air changes: 8/hour Hours light/dark cycle: Not reported

B. STUDY DESIGN AND METHODS

In life dates: Not reported

Animal assignment and treatment:
The dosage of the test substance was 50 % concentration of the test substance in 10 % propylene-glycol in water.
The dose levels are shown in the following table.

Table 5.8.2-52: Acute intraperitoneal toxicity of glyphosate in female rat ([redacted], 1980): Dose levels

Sex	No. of rats	Dose level (mg/kg bw)
Female	10	620
	10	760

	10	940
	10	1040
	10	1260

No details on observations for mortality and clinical signs of toxicity is available.

II. RESULTS AND DISCUSSION

A. MORTALITY
The occurrence of deaths is shown in the following table.

Table 5.8.2-53: Acute intraperitoneal toxicity of glyphosate in female rat ([redacted], 1980): Mortality

Sex	No. of rats	Dose level (mg/kg bw)	Survival time of dead animals (days)	Mortality Dead animals (%)
Female	10	620	all survived	0/10 (0)
	10	760	9	1/10 (10)
	10	940	7-13	4/10 (40)
	10	1040	6-11	8/10 (80)
	10	1260	5-10	10/10 (100)

B. CLINICAL OBSERVATIONS
No test substance related symptoms were observed.
According to the autopsy findings the observed transient limb anemia and the dispnoe were probably due to the strong irritation of viscera caused by the i.p. injection.

C. BODY WEIGHT
No data on body weight available.

D. NECROPSY
In the dead animals, superficial liver necrosis, punctiform bleedings of the peritoneum, exudatum between the pleura surfaces were seen. The extent of fibrophosis extending to the viscera was proportional to the time of survival. The late deaths were considered to be caused by peritonitis instead of test substance induced toxicity.

Study conclusion
The acute intraperitoneal LD_{50} for the test substance N-phosphono-methyl-glycine was calculated to be 919 ± 42 mg/kg.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:
Acute intraperitoneal toxicity of glyphosate was tested in rats. There is no OECD guideline available for this endpoint. As to be expected, toxicity was higher by this route as compared to oral, dermal or inhalation exposure. Due to the route of exposure and deficiencies in reporting, this study provides only supplementary information. Based on the study results, the acute intraperitoneal LD_{50} is 919 mg/kg bw in female rats.

Assessment and conclusion by RMS:

CA 5.8.2/013

1. Information on the study

Data point:	CA 5.8.2
Report author	[redacted]
Report year	1978
Report title	Acute toxicity study (intraperitoneal injection) of CP67573 on the rats
Report No	[redacted]-79-109
Document No	Not reported
Guidelines followed in study	None stated
Deviations from current test guideline	Not applicable
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities[1,2]	No
Acceptability/Reliability:	Supportive Route of exposure (intraperitoneal injection); body weights not reported.
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary

The test substance CP67573 was evaluated for its acute intraperitoneal toxicity potential by a single injection to Wistar rats (10 animals/group/sex). The test substance was applied at a dose of 182, 255, 357, 422 and 500 mg/kg bw to males and 255, 357, 500, 700, 980 and 1372 mg/kg bw to females. The animals were observed for a period of 14 days. The behavioural and pathological findings suggest that the test substance acts on the central nervous system and digestive organs. The acute intraperitoneal LD_{50} in male and female rats was calculated to be 281 and 467 mg/kg bw, respectively.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Identification:	CP67573
Description:	White powder
Lot/Batch #:	Not specified

Purity:	98.4 % glyphosate
Stability of test compound:	Not specified
Vehicle and/ or positive control:	0.5 % tragant solution

Test animals:

Species:	Rat
Strain:	Wistar-Imamichi
Source:	[redacted] (not further specified)
Age:	5 weeks
Sex:	Males and females
Weight at dosing:	Male: 95 - 102 g; female: 94 - 100 g
Acclimation period:	Not specified
Diet/Food:	Fumabashi Mojyo Co., Ltd. chow, *ad libitum*
Water:	Drinking water, *ad libitum*
Housing:	Individual
Environmental conditions:	Temperature: 24 ± 1 °C Humidity: 50 - 60 % Air changes: 12/hours Photocycle: 14 hours light/10 hours dark cycle

B. STUDY DESIGN AND METHODS

In life dates: not specified

Animal assignment and treatment
The test substance suspended in 0.5 % tragant solution was prepared just prior to use in varying concentrations to maintain a dose volume of 0.5 mL/100 g bw.
The dose levels are shown in the following table below.

Table 5.8.2-54: Acute toxicity study (intraperitoneal injection) of CP67573 on the rats ([redacted] 1978): Dose levels

Sex	No. of rats	Dose level (mg/kg bw)
Male	10	182
	10	255
	10	357
	10	422
	10	500
Female	10	255
	10	357
	10	500
	10	700
	10	980
	10	1372

Observations for mortality and clinical signs of toxicity were made during the 14-day post-exposure period. Body weights of individual rats were measured on Day 0, 7 and 14. All rats were subjected to a grosspathological examination.

The LD_{50} and 95 % confidence limits were calculated by Lithchfield-Wilcoxon method used mortality of Day 7.

II. RESULTS AND DISCUSSION

A. MORTALITY

The occurrence of deaths is shown in the following table.

Table 5.8.2-55: Acute toxicity study (intraperitoneal injection) of CP67573 on the rats ([redacted], 1978): Mortality

Sex	No. of rats	Dose level (mg/kg bw)	Day									Mortality (%)	
			1			2	3	4	5	6	7	8-14	Days 0-7*	
			1 h	6 h	24 h									
Male	10	182	0	0	0	0	0	0	0	0	0	0	0/10	(0)
	10	255	0	0	0	0	2	1	0	1	0	0	4/10	(40)
	10	357	0	0	0	1	1	2	2	0	1	2	7/10	(70)
	10	422	0	0	0	3	4	1	0	0	0	2	8/10	(80)
	10	500	0	0	0	2	6	2	0	0	0	0	10/10	(100)
Female	10	255	0	0	0	0	0	0	0	0	0	0	0/10	(0)
	10	357	0	0	0	0	0	1	1	0	0	4	2/10	(20)
	10	500	0	0	0	1	4	0	1	0	0	1	6/10	(60)
	10	700	0	0	0	1	2	3	1	2	0	1	9/10	(90)
	10	980	0	5	0	3	0	0	0	1	0	1	9/10	(90)
	10	1372	0	10	0	0	0	0	0	0	0	0	10/10	(100)

* The LD_{50} was calculated after 7 days, due to further deaths in the second week the 14-day LD_{50} would have been lower in both cases.

B. CLINICAL OBSERVATIONS

Thirteen minutes after administration of CP67573, ventral posture, decrease of spontaneous activity, piloerection, salivation and decrease in skin temperature were observed in all animals of both sex. These signs were not observed on the followingmorning in the lowest dose groups of males and females (182 mg/kg bw and 255 mg/kg bw, respectively). In other dose groups, these symtoms were observed for 36 to 48 hours.

In dead animals disappearance of righting reflex and coma was observed. Animals died in ventral posture.

C. BODY WEIGHT

Body weights were not reported.

D. NECROPSY

Gross pathological findings present in deceased animals were increase in circumference of the abdomen; ascites, hyperplasia in intestine wall; adhesion, transformation and slight hypertrophy of liver lobes (with hypertrophy of peritoneum); haemorrhage of mucosa in fundamental stomach; spot haemorrhage in thymus and peyr patches; hyperemia in adrenals.

The signs observed at autopsy of the survivors were as follows: adhesion, slight hypertrophy and transformation of liver lobes and adhesion of stomach and pancreas, hyperplasia of spleen capsule in all animals. The animals that increased in circumference of the abdomen revealed a decrease in body weights and hypertrophy of cecum.

Since the changes of the liver were due to hyperplasia of peritoneum induced by mechanophysical stimulation of injected suspension, the observed findings in the liver were not attributed to a test substance specific induced toxicity.

Study conclusion
The acute intraperitoneal LD_{50} for the test substance CP67573 in males and females was calculated to be 281 mg/kg bw and 467 mg/kg bw, respectively. The 95 % confidence limits were 210 - 340 mg/kg bw and 383 - 592 mg/kg bw for males and females, respectively.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:
Acute intraperitoneal toxicity of glyphosate was tested in rats. There is no OECD guideline available for this endpoint. As to be expected, toxicity was higher by this route as compared to oral, dermal or inhalation exposure. Due to the route of exposure, this study provides only supplementary information. Based on the study results, the acute intraperitoneal LD_{50} is 281 mg/kg bw and 467 mg/kg bw in male and female rats, respectively.

Assessment and conclusion by RMS:

CA 5.8.2/014

Information on the study

Data point	CA 5.3.3
Report author	[redacted]
Report year	2012
Report title	Glyphosate acid - *In Vitro* Absorption through Abraded Rabbit Skin using [^{14}C]-glyphosate
Report No	JV2182-REG
Document No	Not reported
Guidelines followed in study	OECD 428 (2004)
Deviations from current test guideline (OECD 428, 2004)	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (E docs)	Category 2a

2. Full summary
The purpose of this study was to determine the *in vitro* percutaneous absorption of glyphosate acid through abraded rabbit skin following a 6-hour exposure period and subsequent 18 hour monitoring period. This study was designed to assess the potential dermal penetration of test material through rabbit skin and will be of use in estimating the systemic dose achieved in a previous *in vivo* rabbit dermal toxicity study (see CA 5.3.7/01, [redacted], 1982). Therefore, the application rate and exposure conditions used in this study

were calculated to be equivalent to 5000 mg/kg bw/day as applied to rabbits in the *in vivo* dermal study (CA 5.3.7/01).

^{14}C-glyphosate was incorporated into a wet cake preparation prior to application. The preparations were applied as a paste to abraded rabbit skin membranes at a rate of 79.8 mg/cm^2 (corresponding to 48.3 mg glyphosate acid/cm^2) and left un-occluded for an exposure period of 6 hours, after which the skin surface was washed. The absorption process was followed by taking samples of the receptor fluid (physiological saline) at recorded intervals throughout a total time-period of 24 hours. The distribution of glyphosate within the test system and a 24-hour absorption profile were determined. All samples were analysed by liquid scintillation counting (LSC).

The results of this *in vitro* study indicate the dermal absorption of glyphosate through abraded rabbit skin is slow. The vast majority of glyphosate will be washed off during normal washing procedures. The mean total amount of glyphosate absorbed after 24 hours was 2.42 % of the applied dose. The reported total potentially absorbable amount, represented by the mean absorbed dose together with the mean amount in the remaining dermis was 2.66 %.

Materials and methods

A. Materials

1. Test materials:

a) Non radio-labelled test substance:

Identification:	MON 77973 (glyphosate acid)
Description:	White wet cake
Lot/Batch #:	GLP-1103-21149-T
Chemical purity:	85.14 % as glyphosate acid (purity: 95.93 %)
Stability of test compound:	Expiry date: 2012-03-09

b) Analytical reference standard:

Identification:	Glyphosate acid
Lot/Batch #:	GLP-0810-1915-A
Chemical purity:	Not reported

c) Radio-labelled test substance

Identification:	^{14}C-glyphosate (as glyphosate acid) [phosphonomethylene-^{14}C]-Glyphosate
Lot/Batch #:	4675JJN002-1
Radiochemical purity:	96.7 % (confirmed by analysis)
Specific activity:	48 mCi/mmol; 1776 MBq/mmol; 2523µCi/mL; 9.35 MBq/mL

2. Test skin source:

Species:	Rabbit
Strain:	New Zealand White Albino
Source:	Harlan
Age:	At least 12 weeks
Type:	Complete pelt

B: Study design and methods

Preparation of skin samples:
Skin pelts from New Zealand White albino rabbits at least 12 weeks old were obtained from Harlan. The skin samples were transported on cold blocks and were stored on arrival at -20 °C, the day after sacrifice. The skin samples arrived clipped and excised and were examined for scars and blemishes. Any extraneous subcutaneous tissue was removed after defrosting and the pelts clipped further if necessary. The pelts were given an identifying number and individually stored frozen, at approximately -20 °C, on aluminium foil until required for use.

Test substance preparation
The doses were prepared, to mimic as closely as possible a 5000 mg/kg bw dose from a previous rabbit *in vivo* study (CA 5.3.7/01, [redacted], 1982). The dose equivalency was calculated on a dose per unit area of skin basis using an average *in vivo* rabbit weight of 2.78 kg. The doses were prepared as close to the time of application as was practicable.

Radioactive stock solution of ^{14}C-glyphosate
The radiolabelled ^{14}C-glyphosate was supplied as a solution in water.

Trial preparation of the radiolabelled glyphosate acid
Glyphosate acid trial preparation was prepared using the method described below, with the exception that a different volume or smaller amounts of radioactivity or unlabelled material were used, where applicable. Three individual vials were prepared as part of the trial preparation, to assess dosing methodology. The paste like composition of the dose preparation was investigated to ensure that it visually provided good skin contact during application to the membranes.

Preparation of radiolabelled glyphosate acid
Firstly 8008 mg of non-labelled glyphosate wet cake was added to a vial, followed by 4162 µL of radiolabelled glyphosate stock solution, providing a nominal 3.85 mg of glyphosate (40 MBq) radioactivity. 5 mL of water was then added and the preparation mixed thoroughly. The preparation was then freeze dried to remove the water added and the water present in the wet cake. When dry, the glyphosate wet cake preparation was then weighed to confirm the removal of all the water. Approximately 521 mg of the dried wet cake preparation was then added to 8 individual vials together with approximately 300 µL of saline to each vial to create a paste. A final weight of each vial was recorded and the preparation was thoroughly mixed with a spatula into a paste before dosing.

Preparation of non-labelled glyphosate acid
To demonstrate that the dose preparations have a close contact during the application procedure, an additional dose preparation without radiolabel was prepared according to the procedure described above.

Analyses of dose preparations
The radioactivity content of the stock solution was determined by liquid scintillation counting (LSC) analyses of sub-samples of solvent dilutions. The radiochemical purity of the radiolabelled stock solution of the test substance was determined by thin layer chromatography (TLC) using unlabelled test substance as reference standard.
The radioactivity content and homogeneity of the dose preparations were checked by LSC analyses. The radiochemical purity and stability was measured by TLC analyses.

Preparation of diffusion cells
The skin membranes were placed in static glass diffusion cells providing an exposure area of 2.54 cm^2 of skin. The cells had a receptor volume of approximately 4.5 mL.
An integrity test was performed by measuring the electrical resistance (ER) across the skin membranes. Non-abraded membranes with a resistance of 1.5 - 5 kΩ were considered having a normal integrity and used for the skin abrasion. Rabbit skin was abraded using a blunt spatula drawn over the skin area approximately six to eight times, in the form of a grid, in order to mimic 'Draize' abrasion as conducted in

the in vivo study (CA 5.3.7/01, [redacted], 1982). After the abrasion a further integrity test was performed by measuring the electrical resistance (ER) across the skin membranes. For abraded skin samples membranes with ER values in the range of 0.7 – 1.0 kΩ were selected for the study.
Cells were selected such that the application rate was represented by eight intact skin samples from five different animals. Physiological saline was chosen as receptor fluid. The skin surface temperature was maintained at 32 ± 1 °C using a water bath.

Test substance application and sampling
Prior to dosing a pre-treatment a sample of 500 µL was taken from each diffusion cell, and replaced by an equal amount of fresh receptor fluid.
Each dose formulation was applied to the abraded skin membrane as a dried glyphosate acid wet cake paste and spread over the skin surface using a spatula. The weight of each individual preparation and spatula were recorded before and after dosing to allow the applied dose to be calculated.

Each dose was applied at the nominal rate of 79.8 mL/cm^2 exposed skin area (202.8 mg/cell), corresponding to 48.3 mg glyphosate/cm^2. The applications were left un-occluded for 24 hours.
Receptor fluid samples (500 µL) were taken by an auto-sampler at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 16, 20 and 24 hours after application. After each sampling the removed amount of receptor fluid was replaced by an equal amount of fresh receptor fluid.
After the 6-hour sampling, the skin samples were washed by gently swabbing the application site application site with at least three natural sponges pre-wetted with 3 % Teepol L® in water. Decontamination was shown to be complete following assessment of residual radioactivity levels on the skin surface with a Geiger counter. Two further sponges, pre-wetted with water, were used to further swab the surface.

Terminal procedures
After the last sampling, 24 hours after application the remaining receptor fluid was discarded. The receptor chamber was rinsed with receptor fluid that was also discarded.
The donor chamber was carefully removed and the underside wiped with a single natural sponge, pre-wetted with 3 % Teepol L® in water, which was added to the wash sponges. The donor chamber was washed with deionised water and a sample was taken for LSC analysis.
The epidermal surface of the skin was decontaminated by gently swabbing the application site with natural sponges pre-wetted with 3 % Teepol L® in water. Decontamination was shown to be complete following assessment of residual radioactivity levels on the skin surface with a Geiger counter. The skin surface was washed with further sponges pre-wetted with water. All the sponges were combined and digested in Soluene 350® and made up to a recorded volume. A sample was taken for analysis.

Due to the fragility of the abraded skin samples tape stripping could not be performed. Instead a heat separation technique was used to separate the epidermis from the dermis.

The skin was carefully removed from the receptor chamber and the flange area cut away and digested in Soluene 350® and aliquots taken for analysis by LSC.

The remaining skin disc was placed dermis side down, on cling film. A second piece of cling film was then used to cover the epidermis side. A 200 g weight was placed in a water bath at 65 ºC for an hour prior to use. The weight was placed onto the epidermal surface with moderate pressure for approximately 90 seconds. The epidermis was peeled away from the dermis using forceps. The dermis was digested in Soluene 350® and aliquots taken for analysis by LSC. The epidermis was digested in Soluene 350® and the whole sample analysed by LSC.

Analysis of samples
The radiochemical purity and stability of the ^{14}C-glyphosate preparations was determined by TLC using silica gel plates and methanol:water:acetic acid (6 : 3 : 0.5, v/v/v). Radioactivity on the TLC plates was measured using a Packard Instant Imager (SOP E003). Unlabelled material was visualised under UV light

at 254 nm.
For visualising the test material on the TLC plates a 2 % ninhydrine solution in acetone was used. In addition, for analyses of dose preparations K2 cellulose plates and a revised solvent system (methanol: water:acetic acid (8 : 1.5 : 0.5, v/v/v) was used.

Liquid samples of the receptor fluid, washing solutions, digested wash sponges, and digested dermis and epidermis were measured by LSC using a Packard 3100 TR LSC counter and Goldstar as scintillation fluid. Results of the analysis of the samples of receptor fluid collected in the study were expressed as amounts of glyphosate in the receptor solution in terms of µg/cm^2. The amounts absorbed, rates of absorption (µg/cm^2/h) and 'percentage of dose absorbed' were calculated. Membranes with absorption profiles that indicate membrane damage during the course of the experiment have been excluded from calculations. The results of the mass balance and distribution determinations were expressed in terms of amount absorbed and 'percentage of applied dose.
The absorbed dose was considered the glyphosate detected in the receptor fluid, while the potentially biologically available proportion of the dose was regarded as the sum of absorbed dose and the amount recovered from the dermis. The test material removed from the surface of the epidermis by the washing procedure, as well as the glyphosate recovered from the epidermis at the end of the exposure was considered unabsorbed.

Results

A. ANALYSES OF THE ^{14}C-GLYPHOSATE STOCK SOLUTION
TLC analysis of the ^{14}C-glyphosate stock solution confirmed a radiochemical purity of greater than 95 %. LSC analysis revealed a radioactivity content of 72.1 MBq, equivalent to a concentration of 0.924 mg/mL. The stock solution was homogeneous with a 1.31 % deviation between the replicates.

B. ANALYSES OF DOSE PREPARATIONS
LSC analyses confirmed the mean application rate to be 48.3 mg glyphosate/cm^2. The dose preparations had low variability between the replicates analysed (1.66 – 6.26 %) and, considering the physical nature of the preparation, the dose preparations were considered to have acceptable homogeneity.

C. MEMBRANE INTEGRITY CHECK
Based on the ER measurements eight cells with abraded skin samples were selected for the absorption study.

D. DERMAL ABSORPTION OF GLYPHOSATE
Absorption profiles were assessed from eight abraded skin samples. Since one skin sample showed an atypical absorption profile, this was excluded from the calculation of means and SD.

The determined distribution of radioactivity are summarised in the table **Error! Reference source not found.**below.

Table 5.8.2-56: Glyphosate acid - *In Vitro* Absorption through Abraded Rabbit Skin using [^{14}C]-glyphosate ([redacted], 2012): Summary of results for dermal absorption of ^{14}C-glyphosate

Dose preparation		
Applied dose "wet cake" [mg/cm^2]	79.8	
Applied dose glyphosate [mg/cm^2]	48.3	
Number of cells assessed	7	
	Distribution of radioactivity (mean values)	
	µg/cm^2	% of applied dose
Surface compartment		
Dermis (after heat separation)	118 ± 19.4	0.243 ± 0.040

Skin wash at 6 hours	42802 ± 3008	87.9 ± 6.30
Skin wash at 24 hours	1159 ± 1224	2.38 ± 2.51
Donor chamber	59.2 ± 56.9	0.121 ± 0.117
Receptor compartment		
Receptor fluid 0 – 24 h	1177 ± 244	2.42 ± 0.503
Total absorbed*	**1177**	**2.42**
Epidermis (after heat separation)	20.1 ± 9.97	0.041 ± 0.020
Flange area	132 ± 68.6	0.270 ± 0.141
Total potentially absorbable**	**1295**	**2.663**
Total recovery	**45468 ± 2096**	**93.3 ± 4.46**
Absorption rates 0 – 24h [µg/cm²/h]	53.1 ± 10.2	-

SD: Standard deviation; * Amount in receptor fluid; ** Total potentially absorbable = total absorbed + remaining dermis (after heat separation)

The total recovery of the individual cells was in the range of 87.3 % to 98.2 %, with an overall mean recovery of 93.3 % of applied dose.

The majority of the applied glyphosate acid (mean 87.9 %) was washed off the skin at 6 hours, with a further 2.38 % washed off at 24 hours. A small proportion (0.041 %) of the dose applied was recovered from the epidermis, with 0.243 % remaining in the dermis.

The mean rate of absorption of glyphosate acid between 0-1 hours was 47.0 µg/cm²/h, which increased to 166 µg/cm²/h between 1 – 4 hours. The mean absorption rate subsequently slowed to 72.3 µg/cm²/h between 4-10 hours and declined further to 13.3 µg/cm²/h for the remainder of the absorption period (10 – 24 hours). The overall absorption rate (0 – 24 hours) was 53.1 µg/cm²/h.

The mean amount of glyphosate acid that penetrated abraded rabbit skin into the receptor fluid over the entire 24-hour experimental period was 1177 µg/cm², corresponding to 2.42 % of the applied dose.

Considering that the amount found in the remaining dermis after 24 h was potentially available and could further penetrate through the skin, the total amount of glyphosate potentially available was 2.66 % of the applied dose.

3. Assessment and conclusion

Assessment and conclusion by applicant:

In this study, the *in vitro* percutaneous absorption of glyphosate acid through abraded rabbit skin was assessed following a 6-hour exposure period and a subsequent 18 hour monitoring period. The study was conducted according to OECD 428 (2004) and in compliance with GLP regulations. There were no deviations from OECD 428 (2004). Therefore, the study was considered valid.

The results of this *in vitro* study indicate the dermal absorption of glyphosate through abraded rabbit skin is slow. The vast majority of glyphosate will be washed off during normal washing procedures. The mean total amount absorbed after 24 hours was 2.42 % of the applied dose. The reported total potentially absorbable amount, represented by the mean absorbed dose together with the mean amount in the remaining dermis was 2.66 % of the applied dose. Applying this to the repeated dose rabbit dermal toxicity NOAEL of 5000 mg/kg bw/day in Johnson (CA 5.3.3/008) a systemic NOAEL in rabbit repeated dose studies is determined to be 133 mg/kg bw/day.

Assessment and conclusion by RMS:

RMS should indicate if they agree to the results and conclusions of the APPL.

Literature evaluation

A literature search for the active substance glyphosate was performed in accordance to the provisions of

the EFSA Guidance "Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) 1107/2009" and updated Appendix to this Guidance document. The following publications were found relevant and reliable for this section and the summaries are thus presented below and are part of the general discussion at the beginning of the section.

Table 5.8.2-57: Publications on toxicological effects of glyphosate

Annex Point	Study & Substance(s)	Study type	Reliability and restriction comments	Result
CA 5.8.2/015	Forsythe *et al.*, 2018 Glyphosate (Batch: not specified, Purity: not specified)	*in vitro:* Environmental Toxin Screening Using Human-Derived 3D Bioengineered Liver and Cardiac Organoids	Glyphosate not characterized No positive control to validate test system Effects noted at doses not physiogically feasible *in vivo*	Glyphosate was shown to reduce organoid integrity and viability at doses from 250 µM to 2.5 mM. IC_{50} values based on ATP activity of liver and cardiac organoids were found to be 10.53 and 10.85 mM, respectively. When cardiac organoids were exposed to 0.25 mM an effect was found on beating rate. Exposure to 2.5 mM for 2 days resulted in all organoids stopping beating.
CA 5.8.2/016	Mesnage *et al.*, 2018 - Glyphosate (Batch: not specified, Purity: ≥96 %) - quizalofop-p-ethyl - isoxaflutole - mesotrione	*in vitro:* Transcriptome and metabolome analysis of HepaRG human liver cells	No positive control No cytotoxicity assessment to determine appropriate study concentration range	4 active substances were tested for their effect on the transcriptome and metabolome profile of differentiated HepaRG cells at 0.06, 6, and 600 µM. Glyphosate was found to be only weakly toxic inducing little change in transcriptome profiles when compared to the other test substances. Metabolomics analysis of HepaRG cells exposed to glyphosate at 0.06 µM revealed a significant decrease in long chain fatty acid- and polyunsaturated fatty acid-levels. At glyphosate concentrations of 6 and 600 µM, lower lipid levels were

Table 5.8.2-57: Publications on toxicological effects of glyphosate

Annex Point	Study & Substance(s)	Study type	Reliability and restriction comments	Result
				observed (not statistically significant).

CA 5.8.2/015

1. Information on the study

Data point:	CA 5.8.2
Report author	Forsythe, S.D. *et al.*
Report year	2018
Report title	Environmental Toxin Screening Using Human-Derived 3D Bioengineered Liver and Cardiac Organoids
Document No	doi: 10.3389/fpubh.2018.00103 ISSN: 2296-2565
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary

In this study the toxicity of glyphosate for liver and cardiac organoids was investigated in the concentration range from 25 µM to 25 mM. The endpoints considered were cell viability, ATP activity and beating rate of the cardiomyocytes. Glyphosate was shown to reduce organoid integrity and viability at doses from 250 µM to 2.5 mM. The IC_{50} values based on ATP activity of liver and cardiac organoids were found to be 10.53 and 10.85 mM, respectively. When cardiac organoids were exposed to glyphosate at 0.25 mM a non-statistically significant effect was found on beating rate. Exposure to 2.5 mM for 2 days resulted in all organoids stopping beating.

I. MATERIALS AND METHODS

Test material
The purity and source of glyphosate were not reported.

Liver and cardiac cell sources, culture and organoid formation
For liver organoids, all cells used were commercially sourced, human primary cells. Hepatic stellate cells (HSCs) were expanded in culture for two passages before cryopreservation for use in organoid formation. During expansion, HSCs were cultured in 90 % high glucose DMEM and 10 % fetal bovine serum on a rat tail collagen I coating (10 g/cm^2) at 37 °C with 5 % CO_2. Primary human hepatocytes were thawed according to the manufacturer's instructions using hepatocyte thawing medium. Kupffer cells were also thawed following the manufacturer's instructions. After thawing, primary human hepatocytes were plated on collagen coated (10 g/cm^2) 6-well culture plates, using hepatocyte plating medium at a density of ~ 150,000 cells/cm^2. Cells were incubated at 37 °C with 5 % CO_2 for 4 hours before adding matrigel as an overlay. Following further incubation for 24 hours, fresh HCM medium was added. For cardiac organoids,

induced pluripotent stem cell-derived cardiomyocytes (iPSC CMs) were commercially sourced from Axiogenesis (cat. #COR.4U Cardiomyocytes). Human primary cardiac fibroblasts were commercially sourced from ScienCell (cat. #6330). Prior to organoid formation, iPSC CMs were cultured on tissue culture plastic for 48 hours in COR.4U medium until cells began beating spontaneously. At this point, iPSC CMs were harvested using trypsin-EDTA. The liver cells were combined in a cell seeding mixture comprised of 90 % HCM medium, 10 % heat-inactivated fetal bovine serum, and rat tail collagen I (10 ng/µL). Liver organoids were produced with a mixture of 80 % hepatocytes, 10 % hepatic stellate cells, and 10 % Kupffer cells. Approximately 1,500 cells per 40 µL medium were used to form aggregates in each well of non-adherent, round-bottom, 96-well plates to produce spherical organoids. Cardiac organoids were produced similarly. IPSC CMs were suspended in cardiomyocyte maintenance medium. Fibroblasts were added as 10 % of the total cell number, and the volume was adjusted to reach a cell density of 10,000 cells/mL. 100 µL of cell suspension was pipetted into each well of non-adherent, round-bottom, 96-well plates to produce spherical organoids resulting in approximately 1,000 cells/organoid. Well plates were incubated and observed daily until organoid formation, and then immediately used in experiments.

Preparation of test item stock solutions
Glyphosate was dissolved in DI H_2O for environmental toxins to reach 50 mM. Serial dilutions were performed in media for each cell type until all concentrations were created at 2X final concentration. Glyphosate was assessed in the concentration range from 25 µM to 25 mM.

Live/dead staining
Organoids were isolated from 96-well low adhesion round bottom plates, suspended in Hystem hydrogel in a 20 µL construct, and placed into 12-well plates to immobilize organoids in a 3D extracellular matrix environment. Each concentration of glyphosate premixed at 1X concentration in media according to organoid type was added to individual wells and organoids were allowed to incubate under their respective conditions for 2 days at 37 °C with 5 % CO_2. Studies were performed using n = 5 or higher for all conditions. Medium was then removed and organoids were assessed by LIVE/DEAD® Viability/Cytotoxicity Kit assays. Specifically, 2.0 µM calcein AM and 4.0 µM ethidium homodimer in PBS was added to each well and was allowed to incubate for 1 hour. Imaging was then performed by macro-confocal microscopy and composite images were created with ethidium bromide red fluorescence representing dead nuclei and calcein AM green fluorescence representing live cells.

ATP activity assays
Glyphosate was added in a premix 2× concentration of 100 µL solution to each well of a 96-well plate containing an organoid with 100 µL of medium and allowed to incubate for 2 days at 37 °C with 5 % CO_2 with n = 6 or higher. Medium was then removed from each well leaving 100 µL of media remaining along with the organoid. Next, 100 µL of Cell-Titer Glo Luminescent Cell Viability Assay solution was added to each well along with 100 µL added to 100 µL DMEM in a Costar Black Polystrene 96-well assay plate and allowed to incubate for 10 minutes at room temperature shielded from light. The entire contents of the wells containing organoids were then added to the Costar Black Polystrene 96-well assay plate wells and the contents were read on a Vertias Microplate Luminometer using default settings. Values were then averaged among the different groups and graphed for analysis using Graph Pad Prism© software.

Heart beat assay
Fully formed cardiac organoids in the wells of a 96-well non-adhesive round bottom plate were placed under a Leica DMIL LED microscope to allow for the recording of natural beat rates in 20 s videos (n = 3). The plate was then returned to the 37 °C, 5 % CO_2 incubator for 5 minutes to ensure that organoids did not experience significant temperature decreases, which can detrimentally impact beating rates. The process was repeated until all experimental subject organoids under the test compound concentrations described above, but at varying time points, had been recorded. Glyphosate was added as 2 × 100 µL concentration to each well of a 96-well low adhesion round bottom plate containing 100 µL of normal medium with a cardiac spheroid, and allowed to incubate for 30 minutes at 37 °C. The plate was then recorded, 3 organoids at a time, in the process listed above until all organoids were recorded and the plate was returned to the incubator. The process was then repeated 24 and 48 hours later. The 20 s videos were then analyzed by

counting the beats for each video and multiplied by 3 to scale values to beats per minute. A beat was defined as the beginning of the contractile movement of the organoid. A beat did not need reach conclusion to be counted. If multiple beating regions were observed then the beating of the largest multi-cell structure was used to calculate the beating rate.

Statistics
The data are generally presented as the means of number of replicates ± SD. All data were graphed and analyzed for significance using a Student's T-test. For ATP activity assays p-values were considered significant when <0.01. For beating kinetic assays p-values were considered significant when <0.05. Data samples were eliminated from the experimental groups if they fell outside of two SDs from the experimental group averages. Sample sizes (generally n = 5 or n = 6, depending on the experiment as described) were determined based on preliminary experiments. These sample sizes, with the typically observed SDs, allowed statistical significance at $\alpha = 0.05$ with statistical power greater than 80 %. The IC_{50} was calculated using the Graph Pad Prism© software.

II. RESULTS AND DISCUSSION

Organoid production and viability
Liver organoids were successfully formed after 4 days whereas it took 7 days for the cardiac organoids to allow for self-propagating beating to occur. Viability for both was confirmed to be greater than 95 % using live/dead imaging.

Organoid viability
Live/dead staining of liver organoids was used to visualize indications of cytotoxicity due to exposure to glyphosate. Integrity and viability of liver organoids began to show a steady reduction when exposed to glyphosate concentrations from 250 µM to 2.5 mM. In contrast, cardiac organoids maintained integrity during all testing, but cell death occurred when exposed to glyphosate concentrations from 2.5 to 25 mM.

Organoid ATP activity
After testing original three doses (n ≥6) centered on doses described in the literature, two further trials narrowed the range containing the IC_{50}. The IC_{50} values were found to be similar for cardiac and liver organoids for glyphosate, i.e. 10.85 and 10.53 mM, respectively. For liver organoids, 10 mM of glyphosate resulted in a statistically significant decrease in ATP activity (p <0.001) and for cardiac organoids, 5 mM of glyphosate resulted in statistically significant decrease in ATP activity (p <0.001).

Cardiac organoid beating rates
The time points to test physiological reactions to glyphosate exposure were chosen based on previous studies testing calcium channel impairment in 2D cardiomyocyte populations and defined as immediate (30 minutes), short term (1 day), and long term (2 days). Although not statistically significant, glyphosate demonstrated toxicity at 250 µM and higher, with beat rates at 250 µM slowing on day one among all organoids and two of the three organoids ceasing beating on day 2. At 2.5 mM, exposure for one day caused two spheroids to cease beating entirely, with one-third showing a 50 % reduction in beat rate. By day 2 all organoids ceased beating.

Study conclusion
Glyphosate live/dead imaging varied greatly, with significant cell death in liver organoids and little cell death in cardiac organoids at 2.5 mM. ATP values recorded at 10 mM showed a large difference between liver and cardiac organoids (0.568 and 0.804, respectively), displaying a more gradual decrease in cell viability for the liver organoids as compared to the cardiac organoids. The IC_{50} values of liver and cardiac organoids were found to be similar i.e. 10.53 and 10.85 mM, respectively. When cardiac organoids were exposed to glyphosate at concentrations from 0.25 mM on an effect on beating rate was observed with a complete stop of beating at 2.5 mM after 2 days of exposure.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:
In this study the toxicity of glyphosate for liver and cardiac organoids was investigated in the concentration range from 25 µM to 25 mM. The endpoints considered were cell viability, ATP activity and beating rate of the cardiomyocytes. Glyphosate was shown to reduce organoid integrity and viability at doses from 250 µM to 2.5 mM. The IC_{50} values based on ATP activity of liver and cardiac organoids were found to be 10.53 and 10.85 mM, respectively. When cardiac organoids were exposed to glyphosate at 0.25 mM a non-statistically significant effect was found on beating rate. Exposure to 2.5 mM for 2 days resulted in all organoids stopping beating.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate used was not characterized, no positive controls were used to validate the organoid test systems and the concentrations at which most of the effects have been observed are physiologically not feasible in *in vivo* experimental models.

Reliability criteria for *in vitro* toxicology studies

Publication: Forsythe *et al*., 2018	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	N	Purity and source were not reported.
Only glyphosate acid or one of its salts is the tested substance	N	Also lead, mercury and thallium were assessed.
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	Y?	Liver and cardiac organoids. Origin of hepatic cells not sufficiently documented.
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	NA	
Test concentrations in physiologically acceptable range (< 1 mM)	Partly	Test concentrations at which effects were seen were > mM.
Cytotoxicity tests reported	Y	
Positive and negative controls	N	No positive controls used.
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	

Reliability not assignable		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate used was not characterized, no positive controls were used to validate the organoid test systems and the concentrations at which most of the effects have been observed are physiologically not feasible in *in vivo* experimental models.		

Assessment and conclusion by RMS:

CA 5.8.2/016

1. Information on the study

Data point:	CA 5.8.2
Report author	Mesnage, R. *et al.*
Report year	2018
Report title	Ignoring Adjuvant Toxicity Falsifies the Safety Profile of Commercial Pesticides
Document No	doi.org/10.3389/fpubh.2017.00361 E-ISSN: 2296-2565
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary

Currently, the health risk assessment of pesticides in the European Union and in the United States focuses almost exclusively on the stated active principle. Nonetheless, adjuvants can also be toxic in their own right with numerous negative health effects having been reported in humans and on the environment. Despite the known toxicity of adjuvants, they are regulated differently from active principles, with their toxic effects being generally ignored. Adjuvants are not subject to an acceptable daily intake, and they are not included in the health risk assessment of dietary exposures to pesticide residues. Here, this gap in risk assessment by reference to glyphosate, the most used pesticide active ingredient is illustrated. The case of neonicotinoid insecticides, which are strongly suspected to be involved in bee and bumblebee colony collapse disorder is also investigated. Authors of studies sometimes use the name of the active principle (for example glyphosate) when they are testing a commercial formulation containing multiple (active principle plus adjuvant) ingredients. This results in confusion in the scientific literature and within regulatory circles and leads to a misrepresentation of the safety profile of commercial pesticides. Urgent action is needed to lift the veil on the presence of adjuvants in food and human bodily fluids, as well as in the environment (such as in air, water, and soil) and to characterize their toxicological properties. This must be accompanied by regulatory precautionary measures to protect the environment and general human population from some toxic adjuvants that are currently missing from risk assessments.

I. MATERIALS AND METHODS

Test Material
Glyphosate (purity ≥96 %) was purchased from Sigma-Aldrich, Gillingham, Dorset, UK.

HepaRG cell culture
Differentiated HepaRG™ cells (HPR 116) were purchased from Biopredic International (Rennes, France). Cells were thawed, suspended and plated in general purpose medium (Williams' E medium + GlutaMAX™) containing the ADD670 supplement. Cells were kept in general purpose medium until day 8, when the culture becomes well organized and includes well-delineated trabeculae and many canaliculi-like structures. At this time, the culture is composed of primitive biliary epithelial cells and mature hepatocytes with basal metabolic activities similar to freshly isolated primary cells. From day 8 to day 14, cells were switched to the test medium composed of Williams' E medium + GlutaMAX™ supplemented with 2 % fetal bovine serum and 1 % DMSO, as well as different concentrations of glyphosate or solvent as a control. Glyphosate was tested at 0.06 µM (concentration representative of low environmental exposure), 6 µM, and 600 µM.

Library generation and RNA-sequencing
A 100 ng aliquot of total RNA from each sample was used to prepare total RNA libraries using the KAPA Stranded RNA-Seq Kit with RiboErase, and samples were randomised before preparation. Polymerase chain reaction (PCR) was performed for 14 cycles for final library amplification. Resulting libraries were quantified using the Qubit 2.0 spectrophotometer and average fragment size assessed using the Agilent 2200 Tapestation. The transcriptome of HepaRG cells exposed to glyphosate was sequenced employing this strategy, except that the libraries were prepared as previously described. A total of 3 separate sequencing pools were created using equimolar quantities of each sample with compatible indexes: 2 with 17 samples each, and one with 16 samples. Paired-end reads of 75bp were generated for each library using the Illumina NextSeq®500 in con-junction with the NextSeq®500 v2 high-output 150-cycle kit.

Mass spectrometry-based metabolomics
Approximately 5,000,000 hepaRG cells per sample were harvested from the 6 well-plates to obtain a sufficient quantity of material to perform the metabolomics experiment. Cells were detached using 0.05 % trypsin EDTA, and centrifuged to eliminate trypsin residues. Finally, cell pellets were frozen at -80 °C pending analysis. Metabolomics analysis of the frozen cell pellets was conducted by Metabolon Inc. The sample extracts were stored overnight under nitrogen before preparation for analysis. The resulting extract was analysed on four independent instrument platforms: two different separate RP/UPLC-MS/MS with positive ion mode electrospray ionization (ESI), a RP/UPLC-MS/MS with negative ion mode ESI, as well as by hydrophilic-interaction chromatography(HILIC)/UPLC-MS/MS with negative ion mode ESI as previously described. Compounds were identified by comparison to library entries of purified standards or recurrent unknown entities. Biochemical identifications are based on 3 criteria: retention index within a narrow retention time/index (RI) window of the proposed identification, accurate mass match to the library +/- 10 ppm, and the MS/MS forward and reverse scores between the experimental data and authentic standards. The use of all three data points can be utilized to distinguish and differentiate biochemicals. Peaks were quantified using the area-under-the-curve method.

Statistics
Metabolome data analysis was performed using in-house services of Metabolon Inc. Biochemical data were normalized with respect to protein concentration as determined by the Bradford assay. The Welch's two-sample t-test was used to test whether control and treatment group means are different from two independent populations. This version of the two-sample t-test allows for unequal variances. FDR methods and estimated q-values were used to account for the highest number of false positive results caused by the high number of statistical tests. The RNA-seq data analysis was performed using the new version of the Tuxedo protocol with HISAT2, StringTie and Ballgown. A standard linear model-based comparison of transcript abundance was performed without adjusting for other covariates to identify differentially

expressed transcripts for each group. Although 3 concentrations were tested for glyphosate, no multigroup comparisons were used because dose spacing was too large to allow reliable conclusions to be drawn from these methods. Instead, pairwise comparisons were used.

II. RESULTS AND DISCUSSION

Control, untreated cell cultures presented no visible signs of aging after a 6-day exposure. Transcriptome profiles of HepaRG cells were then determined using the Illumina-based RNA sequencing platform. The highest concentration of glyphosate tested caused significant changes in transcriptome profiles. Alterations in gene expression caused by the 2 lowest concentrations (0.06 and 6 µM) failed to pass the statistical threshold that took into account the high number of tests performed. A total of 7 transcripts had their levels altered ($p < 0.05$) with glyphosate at 600 µM. The number of genes disturbed by the exposure to glyphosate was insufficient to use a functional annotation tool for pathway enrichment analysis. It is not clear if glyphosate lacks hepatic toxic effects at these concentrations or if this experimental design lacks sensitivity to detect hepatic effects of weak toxicants. To further explore changes in liver metabolism caused by glyphosate in greater detail, a global metabolome profiling of HepaRG cells exposed to three concentrations of glyphosate was explored. The Metabolon HD4 platform detected 802 named biochemicals in the HepaRG samples. Overall, glyphosate did not cause significant alterations in metabolome composition. However, exposure did cause a significant decrease in long chain fatty acids (LCFAs) and polyunsaturated fatty acids (PUFAs). HepaRG cells exposed to the lowest concentration of glyphosate tested (0.06 µM) showed the most dramatic effects in the levels of these fatty acids as either significant or trends towards significant lower levels. At the higher glyphosate concentrations of 6 µM and 600 µM, lower lipid levels were also observed but these did not reach statistical significance.

Study conclusion

An in-depth investigation was conducted of transcriptome profile alterations in HepaRG human liver cells caused by exposure to pesticide active ingredients. Glyphosate was found to be only weakly toxic inducing little change in transcriptome profiles. Interestingly, a follow-up metabolomics analysis of HepaRG cells treated with the lowest (0.06 µM) concentration of glyphosate revealed a significant decrease in the levels of LCFAs and PUFAs. Although these findings from an *in vitro* tissue culture model system cannot be readily translated to effects *in vivo*, they are nevertheless indicative of differences in toxicity potency between pesticide ingredients. The exact nature of this low dose effect of glyphosate cannot be determined from this single experiment, but it is possible that at higher concentrations, more overtly toxic mechanisms are masking the effects on lipids. Another possibility is that a saturation effect is occurring once the low dose is exceeded bearing in mind that glyphosate levels found in the HepaRG cells during the metabolomics analysis increased by 3.7- and 336.35-fold at the intermediate and highest concentrations tested compared to the negative controls. Glyphosate was the least toxic of the compounds tested in this study.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:

Together with 3 other herbicide active ingredients (quizalofop-p-ethyl, isoxaflutole and mesotrione) the effect of glyphosate on the transcriptome and metabolome profile of differentiated HepaRG cells was investigated at 0.06, 6 and 600 µM. Glyphosate was found to be only weakly toxic inducing little change in transcriptome profiles when compared with the other herbicides tested. A follow-up metabolomics analysis of HepaRG cells exposed to glyphosate at 0.06 µM revealed a significant decrease in the levels of long chain fatty acids (LCFAs) and polyunsaturated fatty acids (PUFAs). At the higher glyphosate concentrations of 6 and 600 µM, lower lipid levels were also observed but these did not reach statistical significance. It is not clear, however, how these findings from an *in vitro* tissue culture model can be

translated to effects *in vivo*.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because no positive control was used and no cytoxicity tests were performed to optimise the concentration range to be explored.

Reliability criteria for *in vitro* toxicology studies

Publication: Mesnage *et al*., 2018	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Glyphosate (purity ≥96 %). Source: Sigma-Aldrich, Gillingham, Dorset, UK.
Only glyphosate acid or one of its salts is the tested substance	N	Also three other pesticide active ingredients were tested (quizalofop-p-ethyl, isoxaflutole and mesotrione).
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	Y	
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	NA	
Test concentrations in physiologically acceptable range (< 1 mM)	Y	0.06, 6 and 600 µM.
Cytotoxicity tests reported	N	
Transcriptomics and metabolomics methods described	Y	
Positive and negative controls	N	No positive controls included.
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Reliability not assignable		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because no positive control was used and no cytoxicity tests were performed to optimise the concentration range to be explored.		

Assessment and conclusion by RMS:

CA 5.8.3 Endocrine disrupting properties

Glyphosate was investigated for potential endocrine disrupting properties in several guideline- and GLP-conform *in vitro* and *in vivo* studies. In an androgen receptor binding assay, glyphosate did not show any activity for androgen receptor binding (CA 5.8.3/001). No interaction of glyphosate with the estrogen receptor (ER) was determined in an ER binding assay (CA 5.8.3/003); and no evidence of agonistic activity on the human ERα was identified in an ER transcriptional activation assay (CA 5.8.3/002). Further, a human recombinant aromatase assay revealed no inhibitory effect of glyphosate on aromatase activity (CA 5.8.3/004); and in a H295R steroidogenesis assay glyphosate was tested negative for changes in testosterone as well as 17β-estradiol production, demonstrating that glyphosate does not impact hormone production (CA 5.8.3/009).

A literature search resulted in six ~~four~~ publications, which investigated the effects of glyphosate exposure on granulosa, adipose stromal, TM4 Sertoli, MCF-7, MDA-MB-231, T47D, JEG3, VM7Luc4E2 and T47D-KBluc cells. The results of these non-guideline *in vitro* studies suggested that glyphosate partly interfered with main functional parameters of these cells (e.g. mitochondrial enzymatic activity; estrogen response element (ERE)-mediated transcription of genes, altered gene expression of genes involved in cell cycle regulation, stimulation by steroid hormones and cell death; ERα protein expression). No estrogenic activity was observed in two cell lines or via the two human estrogen receptor (hER) subtypes, hERα and hERβ. The effects of glyphosate on cell viability and cell proliferation were heterogeneous in these publications. For most of the effects no concentration-response relationship could be established. However, some authors concluded that glyphosate could affect reproductive function. Others stated that the effects were only observed at high concentrations *in vitro* and humans exposed to glyphosate would not exhibit effects at realistic exposure levels (CA 5.8.3/012; CA 5.8.3/013, CA 5.8.3/014, CA 5.8.3/015, CA 5.8.3/018). The six ~~four~~ publications on cellular effects of glyphosate are summarized in a table presented above the first publication in the respective summary section (CA 5.8.3/012).

The effects reported in the literature could not be corroborated by any *in vivo* regulatory toxicology study. In an uterotrophic assay in ovariectomized rats, glyphosate did not demonstrate biological activity consistent with agonism of natural estrogens when administered orally at doses up to 1000 mg/kg bw/day (CA 5.8.3/005). Similarly, a pubertal development and thyroid function assay in intact juvenile/peripubertal female rats did not reveal any glyphosate-related estrogenic or anti-estrogenic effects, nor was there any evidence of direct test substance-related effects on pubertal development or thyroid function following oral administration of glyphosate at doses up to 1000 mg/kg bw/day (CA 5.8.3/008). When male rats were orally exposed to glyphosate at doses up to 1000 mg/kg bw/day, neither in a Hershberger assay in peripubertal orchidoepididymectomized rats (CA 5.8.3/006) nor in a pubertal development and thyroid function assay in intact juvenile/peripubertal male rats (CA 5.8.3/007) any androgenic or anti-androgenic effects or evidence of primary glyphosate-related effects on pubertal development or thyroid function could be observed.

Further, an assessment on the potential endocrine disrupting properties of glyphosate in accordance with Commission Regulation (EU) 2018/605 and a (Q)SAR screening under the guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009 were performed. Glyphosate has not been shown to induce EATS-mediated adversity and no EATS-related endocrine activity was observed *in silico, in vitro,* and *in vivo* for humans and mammals as well as for non-target organisms (CA 5.8.3/010, CA 5.8.3/011).

In summary, glyphosate was negative for estrogen, androgen, thyroid, and steroidogenesis (EATS)-mediated endocrine disrupting properties based on the results of the regulatory endocrine-studies performed. The effects reported in a limited number of publications *in vitro* were not consistent with the findings of the valid OCSPP and OECD guideline studies.

Table 5.8.3-1: Studies on endocrine disrupting properties with glyphosate

Annex Point	Study	Study type	Substance(s)	Reference list- related category§	Result
CA 5.8.3/001	[redacted] 2012	*in vitro:* Androgen receptor binding assay	Glyphosate acid (Batch: GLP-1103-21149-T, Purity: 95.93 % glyphosate acid, 85.14 % calculated glyphosate content)	Valid, Category 2a	Glyphosate did not show any activity for androgen receptor binding.
CA 5.8.3/002	[redacted] 2012	*in vitro:* Estrogen receptor alpha transcriptional activation assay	Glyphosate acid (Batch: GLP-1103-21149-T, Purity: 95.93 % glyphosate acid, 85.14 % calculated glyphosate content)	Valid, Category 2a	Glyphosate did not act as an agonist of the human estrogen receptor alpha (hERα), showing less than 10 % of the induction potential of 17β-estradiol at the highest concentration tested.
CA 5.8.3/003	[redacted], 2012	*in vitro:* Estrogen receptor binding assay	Glyphosate acid (Batch: GLP-1103-21149-T, Purity: 95.93 % glyphosate acid, 85.14 % calculated glyphosate content)	Valid, Category 2a	Glyphosate did not show any activity for estrogen receptor binding.
CA 5.8.3/004	[redacted] 2012	*in vitro:* Aromatase assay	Glyphosate acid (Batch: GLP-1103-21149-T, Purity: 95.93 % glyphosate acid, 85.14 % calculated glyphosate content)	Valid, Category 2a	Increasing concentrations of glyphosate (10^{-10} M to 10^{-3} M) showed no decrease in aromatase activity. The mean aromatase activity at all tested glyphosate concentrations was ≥ 99.67 % compared to control. Therefore, glyphosate did not inhibit aromatase activity.
CA 5.8.3/005	[redacted], 2012	*in vivo:* Uterotrophic assay	Glyphosate acid (Batch: GLP-1103-21149-T, Purity: 95.93 % glyphosate acid, 85.14 % calculated glyphosate content)	Valid, Category 2a	Glyphosate did not demonstrate or mimic biological activities consistent with agonism of natural estrogens at all applied doses including the limit dose of 1000 mg/kg bw/day.
CA 5.8.3/006	[redacted] 2012	*in vivo:* Hershberger assay	Glyphosate acid (Batch: GLP-1103-21149-T, Purity: 95.93 % glyphosate acid, 85.14 % calculated glyphosate content)	Valid, Category 2a	Glyphosate was negative for androgenicity and anti-androgenicity in the Hershberger assay all applied doses including

					the limit dose of 1000 mg/kg bw/day.
CA 5.8.3/007	[redacted] 2012	*in vivo:* Pubertal development and thyroid function in intact juvenile/ peripubertal male rats, Crl:CD(SD)	Glyphosate acid (Batch: GLP-1103-21149-T, Purity: 95.93 % glyphosate acid, 85.14 % calculated glyphosate content)	Valid, Category 2a	There was no evidence of any direct glyphosate -related androgenic or anti-androgenic effect, nor was there any evidence of direct glyphosate-related effects on pubertal development or thyroid function at all applied doses including the limit dose of 1000 mg/kg bw/day.
CA 5.8.3/008	[redacted], 2012	*in vivo:* Pubertal development and thyroid function in intact juvenile/ peripubertal female rats, Crl:CD(SD)	Glyphosate acid (Batch: GLP-1103-21149-T, Purity: 95.93 % glyphosate acid, 85.14 % calculated glyphosate content)	Valid, Category 2a	There was no evidence of any glyphosate-related estrogenic or anti-estrogenic effect, nor was there any evidence of glyphosat-related effects on pubertal development or thyroid function at all applied doses including the limit dose of 1000 mg/kg bw/day.
CA 5.8.3/009	Hecker *et al.*, 2011	*in vitro:* Steroidogenesis assay	Glyphosate, not further specified	Valid, Category 2a	Glyphosate did not alter estradiol and testosterone production in the H295R steroidogenesis assay.
CA 5.8.3/010	[redacted] [redacted] [redacted]., 2020	Assessment on the endocrine disrupting properties of the active substance glyphosate in accordance with Commission Regulation (EU) 2018/605	Glyphosate	Valid, Category 1	Glyphosate has not been shown to induce EATS-mediated adversity or endocrine activity in humans, mammals, and non-target organisms.
CA 5.8.3/011	[redacted], 2020	(Q)SAR screening on endocrine disrupting potential of Glyphosate under the Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009	Glyphosate	Valid, Category 1	Five QSAR tools (OECD QSAR Toolbox, Vega, Endocrine Disruptome, Danish QSAR database and OPERA ToxCast COMPARA/CERAPP consensus models) were applied for predictions of potential endocrine activity of glyphosate. Overall, there is no indication that glyphosate has endocrine disrupting activity.

§: The category describes the acceptability/reliability of the study within the AIR 5 submission. This category does not describe

hazard classification. (for details please refer to the Doc ID: 110054-B-GRG_Jun_2020)

CA 5.8.3/001

1. Information on the study

Data point:	CA 5.8.3
Report author	[redacted]
Report year	2012
Report title	Glyphosate: Androgen Receptor Binding (Rat Prostate Cytosol) Screening Assay
Report No	6500V-100334ARB
Document No	CTX-11-026
Guidelines followed in study	US OPPTS/OCSPP 890.1150 (2009)
Deviations from current test guideline (OCSPP 890.1150, 2009)	None
Previous evaluation	Yes, accepted in EFSA peer review on endocrine disrupting properties (2017)
GLP/Officially recognised testing facilities	Yes
Comment on GLP	The control and reference substances were not characterized in accordance with GLP standards, nor was the stability under storage conditions at the test site determined in accordance with GLP standards. However, the substances were purchased from commercial suppliers, and the manufacturers' certificates of analysis were used to define the purity. Verification of the concentration, stability and homogeneity of the test, control, and reference substances in the test system were not determined in accordance with GLP. The test, control, and reference substances were soluble in their vehicles, dosing solutions were prepared immediately prior to use, and the control and reference substances met their performance criteria. Therefore, these exceptions do not affect the validity of the study.
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive summary

In this androgen receptor (AR) binding assay, ventral prostate cytosol from Sprague Dawley rats was used as source of AR to conduct both a saturation binding experiment and a competitive binding experiment. The saturation binding experiment was conducted to demonstrate that the AR isolated from rat prostate cytosol was present in reasonable amount and was functioning with appropriate affinity for the radiolabelled reference androgen (R1881) prior to routinely conducting AR competitive binding experiments. The competitive binding assay was conducted to measure the binding of a single concentration of [^{3}H]-R1881 (1 nM) in the presence of increasing concentrations of glyphosate (ranging from 10^{-10} to 10^{-3} M). The assay included dexamethasone as weak positive control and unlabelled R1881 as positive control (or non-labelled receptor ligand).

Three valid runs of the assay were completed. All concentrations were tested in triplicates. In the first valid,

independent run, the mean specific binding of [^{3}H]-R1881 was >95 % at every glyphosate concentration tested, except for 10^{-9} M, classifying it as a "non-binder" for this run. The mean specific binding of [^{3}H]-R1881 for 10^{-9} M glyphosate was 66.5 %. However, this was due to a clear outlier where the hydroxyapatite pellet might have been lost. Excluding this value, a mean specific binding of 96.0 % was determined, which concurs with the other runs. In the second and third valid runs, the mean specific binding of [^{3}H]-R1881 was >93 % and >92 %, respectively, classifying glyphosate as a "non-binder" in both runs. The weak positive control dexamethasone had a $LogIC_{50}$ of -4.6 M in each of the three runs. The $LogIC_{50}$ of R1881 was -9.0 M, -9.1 M and -9.0 M in the first, second, and third run, respectively.

Under the conditions of the present study, glyphosate was classified as a "non-binder" in all three valid runs resulting in a final classification of a non-binder to the androgen receptor.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Identification:	Glyphosate acid
CAS No.:	1071-83-6
Description:	White crystalline solid
Lot/Batch No.:	GLP-1103-21149-T
Purity:	95.93 % glyphosate acid 85.14 % calculated glyphosate content
Stability of test compound:	Not reported. Expiry 2012-03-09
Vehicle and positive controls:	
Solvent/vehicle used:	Low salt TEDG buffer (10 mM Tris, 1 mM sodium molybdate, 1.5 mM EDTA, 10 % glycerol, and 1 mM DTT, pH 7.4)
Positive Control (non-labelled receptor ligand):	R1881 Source: Sigma-Aldrich Lot/Batch No.: 060M4638 Purity: 98 % CAS No.: 965-93-5 Solvent used: DMSO
Weak Positive Control:	Dexamethasone Source: Sigma-Aldrich Lot/Batch No.: 1419230 Purity: 98.9 % CAS No: 50-02-2 Solvent used: DMSO
Radioactive receptor ligand:	
Identification:	[^{3}H]-R1881
Source:	Perkin-Elmer
Lot/Batch No.:	653698
Radiochemical Purity:	>97 %
Specific activity:	85.1 Ci/mmol, certification date 2011-02-24
Solvent used:	Working Assay buffer = TEDG+PI buffer (10 mM Tris, 1 mM sodium molybdate, 1.5 mM EDTA, 10 % glycerol, 1 mM DTT, and 0.5 % Protease Inhibitor (PI) (v/v), pH 7.4)

Test system: Rat ventral prostate cytosol was prepared from male Sprague Dawley rats (90 days of age) castrated at most one day prior delivery/ preparation. The prostate tissue was inspected for healthy appearance and excess fascia were trimmed if necessary. The prostates were weighed, placed in ice-cold TEDG buffer, and homogenized. The cytosol was isolated, pooled, and kept ice-cold. The protein concentration was 8.8 mg/mL.

Test concentrations: Serial dilutions of glyphosate, the reference standard R1881, and the weak positive control dexamethasone were prepared to achieve the concentrations shown in the table below.

Table 5.8.3-2: Glyphosate: Androgen Receptor Binding (Rat Prostate Cytosol) Screening Assay ([redacted] 2012): Competitor final molar (M) concentrations in competitive binding assay[1, 2]

Positive control R1881	Weak positive control Dexamethasone	Test substance Glyphosate
1×10^{-6}	1×10^{-3}	1×10^{-3}
1×10^{-7}	1×10^{-4}	1×10^{-4}
1×10^{-8}	1×10^{-5}	1×10^{-5}
1×10^{-9}	1×10^{-6}	1×10^{-6}
1×10^{-10}	1×10^{-7}	1×10^{-7}
1×10^{-11}	1×10^{-8}	1×10^{-8}
-	1×10^{-9}	1×10^{-9}
-	1×10^{-10}	1×10^{-10}

[1] Each sample contained 10 µL of the solvent control, positive control (non-radiolabelled R1881), weak positive control, or test substance (with the exception of tubes used to evaluate non-specific binding, which contained 30 µL (100X) of non-radiolabelled R1881), 50 µL of triamcinolone acetonide, 30 µL [^{3}H]-R1881, and 300 µL of prostate cytosol.
[2] Each concentration of each chemical was run in triplicate.

B. STUDY DESIGN AND TEST PERFORMANCE

Experimental dates: 2011-10-25 to 2011-11-01

Saturation (radioligand) binding experiment
A saturation binding experiment measuring total and non-specific binding of [^{3}H]-R1881 was performed to demonstrate that the AR was present in reasonable amount and had the appropriate affinity for the R1881 ligand. The methods and results from the saturation binding experiment were not included in the final study report. However, a statement of the laboratory verifying that the saturation binding curve data were acceptable according to the US EPA OPPTS/OCSPP 890.1150 (2009) was included in the appendix of the study report.

Competitive binding experiment
The competitive binding experiment was performed to measure the binding of a single concentration of [^{3}H]-R1881 (final concentration 1 nM), to the AR in the presence of increasing concentrations of glyphosate. A summary of the experimental conditions for the competitive binding experiment is presented in the table below. The specific activity of [^{3}H]-R1881 was adjusted for decay over time prior to each run. The adjusted specific activity was 82.0, 82.0, and 81.9 Ci/mmol for the first, second, and third run of the assay, respectively. Each assay consisted of three independent runs conducted on three different days, and each run contained triplicates at each concentration. In addition to glyphosate, concentrations of R1881

(positive control) and dexamethasone (weak positive control) were also evaluated in the competitive binding experiment to ensure that the test system was behaving as expected.

Table 5.8.3-3: Glyphosate: Androgen Receptor Binding (Rat Prostate Cytosol) Screening Assay ([redacted] 2012): Summary of experimental conditions for competitive binding experiment

Source of receptor		Rat prostate cytosol
Concentration of radioligand		1 nM
Concentration of receptor		6.8 mg/mL, sufficient to bind 10 - 15 % of
Concentration of glyphosate (as serial dilutions)		100 pM to 1 mM
Temperature		4 ± 2 °C
Incubation time		16 - 20 h
Composition of assay buffer (TEDG+PI)	Tris	10 mM (pH 7.4)
	EDTA	1.5 mM
	Glycerol	10 % (v/v)
	Protease Inhibitor	0.5 % (v/v)
	DTT	1 mM
	Sodium molybdate	1 mM

C. DATA ANALYSIS

Classification of test material

The classification of glyphosate as a binder or non-binder was made on the basis of the average results of three non-concurrent runs, each of which met the performance criteria. Each run was individually classified as follows:

Binder: The lowest point on the fitted response curve within the range of the data was <50 %.

Equivocal: The lowest point on the fitted response curve within the range of the data was >50 % but <75 % (i.e. radioligand displacement is ≥25 % but <50 %).

Non-Binder: The lowest point on the fitted response curve within the range of the data is >75 % (i.e. <25 % displacement of radioligand), or the data do not fit the model.

Descriptors for receptor binding

In circumstances when AR receptor binding was measured, the following parameters were determined.

- B_{max}: Maximal binding capacity
- K_d: Dissociation constant (nM), measures the affinity of the receptor for its natural ligand.
- IC_{50}: Concentration of the test substance at which 50 % of the radioligand is displaced from the receptor.

II. RESULTS AND DISCUSSION

A. SATURATION BINDING EXPERIMENT

Results from the saturation binding experiment were not provided in the final study report. However, the laboratory provided a statement in the appendix of the study report indicating that the saturation binding curve data were acceptable according to the US EPA OPPTS/OCSPP 890.1150 (2009). Reference control results included in the competitive binding experiment confirm that functional AR was present in sufficient amounts and functioning with appropriate affinity.

Summarised saturation binding data of the performing laboratory were submitted following a request by the US EPA (MRID 48843501). The dissociation constant (K_d) for [^{3}H]-R1881 was 0.613 ± 0.041 nM and the estimated B_{max} was 0.817 ± 0.049 fmol/100 µg protein for the single batch of prostate cytosol that was prepared. The mean and individual K_d values were below the range reported in the US EPA validation program (0.685 to 1.57 nM). However, confidence in these values is high according to the goodness of fit (R^2 = 0.957 - 0.984) and the small variation among runs.

B. COMPETITIVE BINDING EXPERIMENT

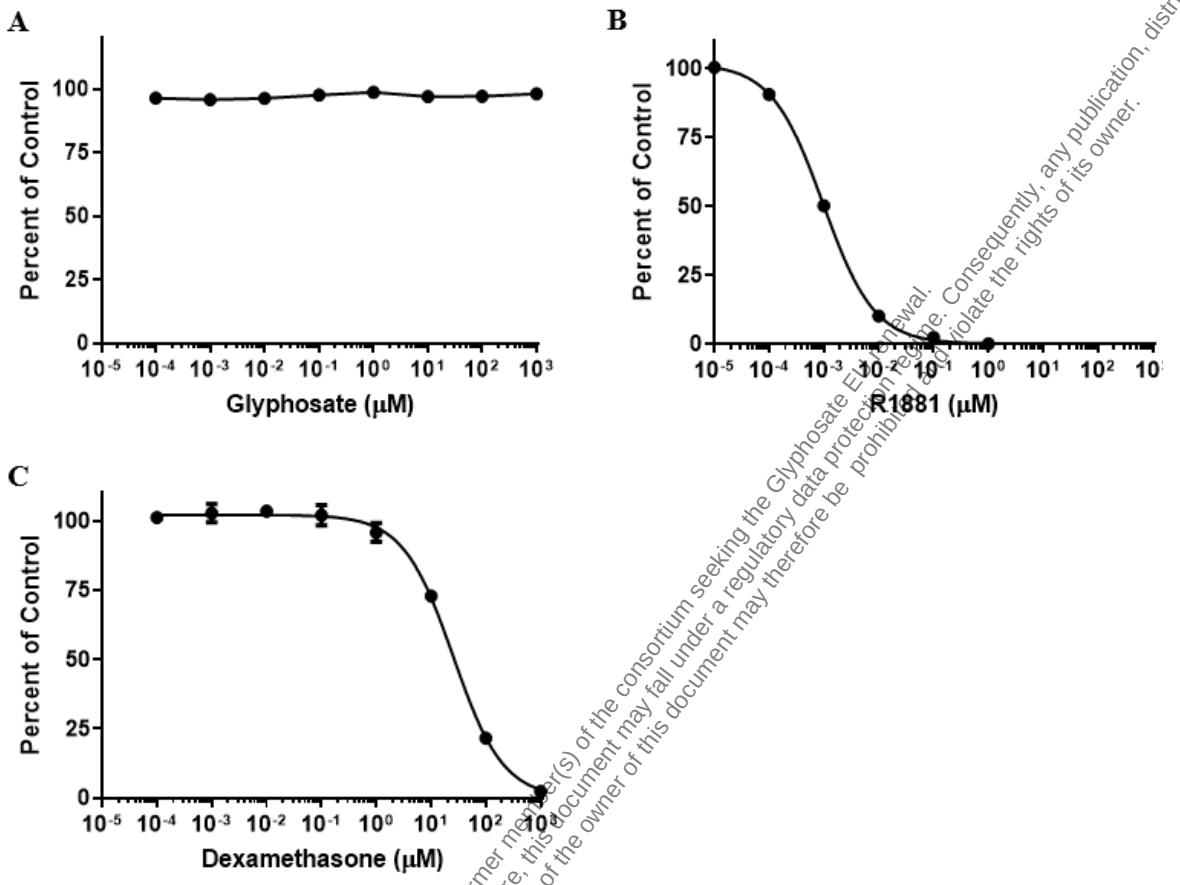
Results from three valid runs of the competitive binding experiment are presented in the table and the figure below. In the first valid, independent run, the mean specific binding of [^{3}H]-R1881 was >95 % at every soluble concentration tested for glyphosate, except for 10^{-9} M, classifying it as a non-binder for this run. The mean specific binding of [^{3}H]-R1881 for 10^{-9} M glyphosate was 66.5 %; however, this was due to a clear outlier where it appeared the hydroxyapatite (HAP) pellet might have been lost. Excluding this value yielded a mean specific binding of 96.0 %, which concurs with the other runs. In the second and third valid, independent runs, the mean specific binding of [^{3}H]-R1881 was >93 and >92 %, respectively, classifying glyphosate as a non-binder in both of these runs. The weak positive control dexamethasone had a $LogIC_{50}$ of -4.6 M in each of the three runs. The $LogIC_{50}$ of R1881 was -9.0 M, -9.1 M and -9.0 M in the first, second, and third run, respectively, demonstrating low variability.

Table 5.8.3-4: Glyphosate: Androgen Receptor Binding (Rat Prostate Cytosol) Screening Assay ([redacted] 2012): Competitive binding assay of glyphosate with androgen receptor from rat prostate cytosol

Parameter	Substance	Run 1[1]	Run 2[1]	Run 3[1]	Mean ± SEM
r^2 (unweighted)	R1881	NR	NR	NR	NA
	Dexamethasone	NR	NR	NR	NA
	Glyphosate	NR	NR	NR	NA
Log IC_{50} (M)	R1881	-9.0	-9.1	-9.0	9.03 + 0.019
	Dexamethasone	-4.6	-4.6	-4.6	-4.6
	Glyphosate	NA	NA	NA	NA
IC_{50} (nM)	R1881	1.0	0.7947	1.0	0.9316 + 0.039
	Dexamethasone	25100	25100	25100	25100
	Glyphosate	NA	NA	NA	NA

[1] The mean is reported for the concurrent replicates within each run.
r^2 = Goodness of fit (r^2 is more appropriately expressed as a range, as opposed to a mean). For the test substance, these parameters could not be calculated.
NR = Not reported.
NA = Not applicable since binding was not evident.

Figure 5.8.3-1: Glyphosate: Androgen Receptor Binding (Rat Prostate Cytosol) Screening Assay ([redacted] 2012): Percent [^{3}H]-R1881 bound to the androgen receptor in the presence of glyphosate (A), unlabelled R1881 (B, strong positive control), or dexamethasone (C, weak positive control) (mean of three valid runs ± SEM)



III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study is performed in accordance with the current US OPPTS/OCSPP 890.1150 (2009). The study is therefore considered valid. In the conducted binding studies, glyphosate did not show any activity for androgen receptor binding in all three valid and independent runs of the assay. Thus, glyphosate was classified as a non-binder to the androgen receptor.

Assessment and conclusion by RMS:

CA 5.8.3/002

1. Information on the study

Data point:	CA 5.8.3
Report author	[redacted]
Report year	2012

Report title	Estrogen Receptor Transcriptional Activation (Human Cell Line (HeLa-9903)) Screening Assay with Glyphosate
Report No	6500V-100334ERTA
Document No	CTX-11-028
Guidelines followed in study	US OPPTS/OCSPP 890.1300 (2009), OECD 455 (2009)
Deviations from current test guideline (OCSPP 890.1300, 2009) (OECD 455, 2016)	No major deviations to US OPPTS/OCSPP 890.1300 (2009). Hill slope value for 17α-estradiol, $LogPC_{50}$ and $LogPC_{30}$ value for 17α-methyltestosterone were outside the required range. However, these deviations from the ranges were minor and were not considered to impact the interpretation of results. Following deviations were noted to the current OECD 455 (2016): no ER antagonist assay was performed; demonstration of proficiency was stated but no data were included in study report; vehicle control (DMSO) for positive control was not tested; and some values (see above) were outside the required range. However, these deviations were not considered to affect the final outcome of the ER agonist assay.
Previous evaluation	Yes, accepted in EFSA peer review on endocrine disrupting properties (2017).
GLP/Officially recognised testing facilities	Yes
Comment on GLP	The control and reference substances were not characterised in accordance with Good Laboratory Practice Standards, nor was the stability under storage conditions at the test site determined in accordance with Good Laboratory Practice Standards. However, the substances were purchased from commercial suppliers, and the manufacturers' certificates of analysis were used to define the purity. Verification of the test concentrations, stability and homogeneity of the test, control, and reference substances in the cell culture media were not determined in accordance with Good Laboratory Practice. The test, control, and reference substances were soluble in their vehicles, dosing solutions were prepared immediately prior to use and were shown to be solutions by visual inspection and the control and reference substances met their performance criteria. Therefore, these exceptions do not affect the validity of the study.
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive summary

The ability of glyphosate to act as an agonist of human estrogen receptor alpha (hERα) was evaluated in an estrogen receptor (ER) transcriptional activation assay using the hERα-HeLa-9903 cell line. In two valid runs, stably-transfected hERα-HeLa-9903 cells were exposed to glyphosate at concentrations of 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, and 10^{-3} M in cell culture medium. Preliminary assessments showed that glyphosate concentrations up to 10^{-3} M did not result in any precipitation or cytotoxicity (≥20 % reduction in cell viability). The cells were exposed to the test substance for 24 ± 2 hours and luciferase activity was measured. Each assay run included 17β-estradiol as a positive control and strong estrogen reference substance, 17α-estradiol as a weak estrogen reference substance, 17α-methyltestosterone as a very weak agonist reference substance, and corticosterone as a negative control. The positive control and reference substances induced the appropriate responses, with minor exceptions (Hill slope value for 17α-estradiol,

$LogPC_{50}$ and $LogPC_{10}$ value for 17α-methyltestosterone were slightly outside the required range), and confirmed the sensitivity and performance of the assay.

Glyphosate demonstrated no evidence of interaction with the hERα over the concentration range tested in the HeLa-9903 model system, showing less than 10 % of the induction potential of 17β-estradiol at the highest concentration tested.

Thus, glyphosate was not identified as an agonist of human estrogen receptor alpha (hERα) under the conditions of the present study.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Identification:	Glyphosate acid
CAS No.:	1071-83-6
Description:	White crystalline solid
Lot/Batch No.:	GLP-1103-21149-T
Purity:	95.93 % glyphosate acid 85.14 % calculated glyphosate content
Stability of test compound:	Not reported. Expiry: 2012-03-09
Vehicle, positive controls, and negative control:	
Solvent/vehicle used:	HeLa-9903 cell culture medium
Strong Positive Control (PC) estrogen:	17β-estradiol Source: Sigma-Aldrich Lot/Batch No.: 110M0138V Purity: 100 % CAS No.: 50-28-2 Solvent used: DMSO
Weak PC estrogen:	17α-estradiol Source: Sigma-Aldrich Lot/Batch No.: 041M4065V Purity: 99.72 % CAS No.: 57-91-0 Solvent used: DMSO
Negative Control (NC):	Corticosterone Source: Sigma-Aldrich Lot/Batch No.: BCBC6322V Purity: 99.2 % CAS No.: 50-22-6 Solvent used: DMSO
Very Weak Agonist:	17α-methyltestosterone Source: Sigma-Aldrich Lot/Batch No.: 060M1543V Purity: 99 % CAS No.: 58-18-4 Solvent used: DMSO

Test system: Stably-transfected hERα-HeLa-9903 cells were obtained from the Japanese Collection of Research Bioresources (JCRB) Cell Bank, Osaka 567-0085, Japan. Cells were free of mycoplasma infection and were maintained in Eagle's Minimum Essential Medium (EMEM) without phenol red, supplemented with 60 mg/L of kanamycin (antibiotic) and 10 % dextran coated-charcoal-stripped foetal bovine serum (DCC-FBS), in a CO_2 incubator (5 % CO_2) at 37 ± 1 °C.
The cells used in the preliminary study, first valid run, and second valid run of the assay were passages 26, 27, and 16 (a new vial from cryopreservation), respectively.

Test concentrations: Six concentrations each of glyphosate, 17β-estradiol, 17α-estradiol, corticosterone, and 17α-methyltestosterone were included in each valid run of the assay as shown in the table below.

Table 5.8.3-5: Estrogen Receptor Transcriptional Activation (Human Cell Line (HeLa-9903)) Screening Assay with Glyphosate ([redacted], 2012): Concentration range of glyphosate and reference substances in the ER transactivation assay

Reference substance	CAS No.	Concentration range (M)	Class
Glyphosate	1071-83-6	10^{-10} - 10^{-3}	Test substance
17β-estradiol	50-28-2	10^{-14} - 10^{-8}	Strong estrogen
17α-estradiol	57-91-0	10^{-12} - 10^{-6}	Weak estrogen
Corticosterone	50-22-6	10^{-10} - 10^{-4}	Negative control
17α-methyltestosterone	58-18-4	10^{-11} - 10^{-5}	Very weak estrogen

B. STUDY DESIGN AND TEST PERFORMANCE

Experimental dates: 2011-09-20 to 2011-10-21

Preliminary cytotoxicity/ precipitation assays

In order to identify a suitable top concentration for use in the ER transcriptional activation assays, preliminary precipitation and cytotoxicity assays were conducted with glyphosate. Cytotoxicity (i.e. ≥20 % reduction in cell viability) was assessed using a two-read propidium iodide uptake assay at the following glyphosate concentrations: $10^{-6\ 5}$, 10^{-6}, $10^{-5\ 5}$, 10^{-5}, $10^{-4\ 5}$, 10^{-4}, $10^{-3\ 5}$, and 10^{-3} M; 125 µM digitonin was used as a positive control for cell death. No precipitation or cytotoxicity was observed by visual inspection at the highest glyphosate test concentration of 10^{-3} M.

ER transcription activation assay

The hERα-HeLa-9903 cells were plated in a 96-well culture plate at a density of ~1 x 10^4 cells/100 µL/well. After a 3-hour (minimum) post-seeding incubation period, the medium was replaced with 150 µL/well of culture medium containing vehicle control (VC), glyphosate, or the reference chemicals at the concentrations identified in the table above. The final concentration of DMSO in the culture medium for the reference substances was held constant at 0.1 % (v/v). Glyphosate dosing solutions were prepared in tissue culture medium. The tissue culture plates were incubated in an incubator under an atmosphere of 5 % CO_2 and at 37 ± 1 °C for 24 ± 2 hours prior to measuring luciferase activity.

C. DATA ANALYSIS

Relative transcriptional activity

Relative transcriptional activity of glyphosate was expressed as percent of the maximally inducing

concentration of the positive control, 1 nM 17β-estradiol.

Data interpretation

The data were interpreted according to the following steps (according to the test guideline):

1. Where appropriate, $LogPC_{50}$, $LogPC_{10}$, $LogEC_{50}$ and Hill slope values were calculated using XLfit from IDBS version 5.2.0.0 (Surrey, UK).
2. For the test substance, the maximum response relative to the positive control (RPC_{max}) was determined. In each individual run of the transcriptional activation assay, if RPC_{max} was <10 %, the test substance was considered to give a negative response for hERα agonism.
RPC_{max} = Maximum level of response induced by a test chemical, expressed as a percentage of the response induced by the positive control (17β-estradiol at 1 nM) on the same plate.
3. For each individual run of the transcriptional activation assay, the acceptability of the data was evaluated using the following criteria:
    a. The mean normalized luciferase signal of the PC (1 nM 17β-estradiol) should be at least 4-fold that of the mean VC on each plate.
    b. The fold induction corresponding to the PC_{10} value of the concurrent PC is greater than 1 + 2 SD of the fold induction value (=1) of the VC. [PC_{10} = Concentration of a test chemical at which the response is 10 % of the response induced by the positive control (17β-estradiol at 1 nM) in each plate.]
    c. The results of the four reference substances should be within acceptable ranges.
4. If the acceptability criteria outlined above were met, that run of the transcriptional activation assay was considered to be valid.
5. The test substance was considered negative if RPC_{max} <10 % in at least two valid runs of the transcriptional activation assay. The test substance was considered positive if RPC_{max} ≥10 % in at least two valid runs of the transcriptional activation assay.

II. RESULTS AND DISCUSSION

A. PRELIMINARY CYTOTOXICITY/ PRECIPITATION ASSAYS

Cytotoxicity for glyphosate did not exceed the defined threshold for cytotoxicity of 20 % reduced cell viability and precipitation of glyphosate was not observed at the highest test concentration of 10^{-3} M. Therefore, the final concentrations of glyphosate tested in the two valid runs of the transcriptional activation assay were 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, and 10^{-10} M.

B. ER TRANSCRIPTION ACTIVATION ASSAY

In the main assays, cytotoxicity (≥20 % reduction in cell viability) and precipitation were not observed at any of the tested concentrations of glyphosate. In the two valid, independent runs of the assay, the highest mean induction values for glyphosate (RPC_{max}) were 2.4 ± 1.4 % and 0.8 ± 0.3 % over the concentration range tested in the first and second run (see table and figure below), respectively. These mean induction values were clearly below the criterion for a positive response (i.e. maximum response relative to the positive control, RPC_{max} of glyphosate was <10 % of the induction produced by 1 nM 17β-estradiol) over the concentration range tested. The mean response for 1 nM 17β-estradiol showed a concentration-dependent response with mean induction of approximately 120-fold.

In both valid runs of the assay, the positive control and reference substances induced the appropriate responses meeting the performance standards with some exceptions as follows:

- In the second valid run of the assay, the Hill Slope value for 17α-estradiol was slightly higher than the guideline suggested criteria (2.3 compared to the range of 0.9 to 2.0).
- In both valid runs of the assay, the Log PC_{50} value for 17α-methyltestosterone was not reached. However, induction was able to reach 40.8 - 42.6 % of the positive control.
- In both valid runs of the assay, the Log PC_{10} value for 17α-methyltestosterone was slightly higher than the guideline suggested criteria (-5.9 for both valid runs, compared to the

range of -6.2 to -8.0).

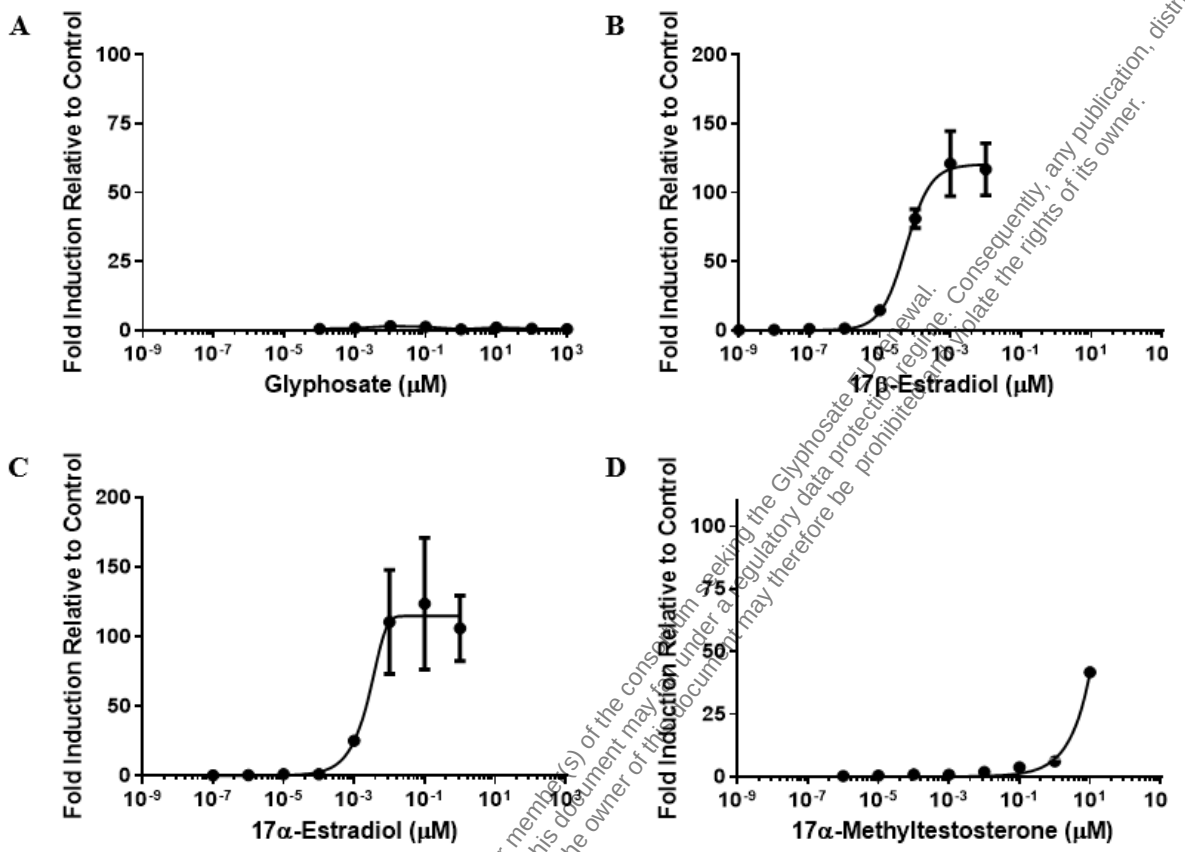
These exceptions were considered minor and not to have affected interpretation and final outcome of the assay results, as the responses of 17β-estradiol, 17α-estradiol, 17α-methyltestosterone, and corticosterone were characteristic of a strong estrogen, a weak setrogen, a weak agonist, and a negative compound, respectively. The US EPA convened a Scientific Advisory Panel (SAP) in 2013 to comment on the significance of minor deviations for performance criteria for the reference substances. The SAP concluded that minor deviations from the performance criteria do not affect the validity of these studies.

Table 5.8.3-6: Estrogen Receptor Transcriptional Activation (Human Cell Line (HeLa-9903)) Screening Assay with Glyphosate ([redacted] 2012): Relative transcriptional activation (RTA) induced by glyphosate

Parameter	RTA (mean ± SD); % of Positive Control (PC)			
	Run 1		Run 2	
Glyphosate concentration (M)	Mean	± SD	Mean	± SD
10^{-3}	0.5	0.8	0.5	0.3
10^{-4}	1.3	1.1	0.3	0.2
10^{-5}	2.4	1.4	0.7	0.3
10^{-6}	1.8	1.1	0.8	0.3
10^{-7}	0.8	1.0	0.0	0.3
10^{-8}	1.8	1.5	0.3	0.3
10^{-9}	1.2	1.6	0.0	0.3
10^{-10}	1.1	1.0	-0.1	0.3
RPC_{max}	2.4 ± 1.4 %		0.8 ± 0.3 %	
PC_{10}	NA		NA	

NA = not applicable and could not be calculated because all responses were below a PC_{10} indicating that glyphosate did not induce estrogen receptor-mediated transactivation.

Figure 5.8.3-2: Estrogen Receptor Transcriptional Activation (Human Cell Line (HeLa-9903)) Screening Assay with Glyphosate ([redacted] 2012): Percent induction following 24 ± 2 h of exposure to glyphosate (A), 17β-estradiol (B, strong positive control), 17 α-estradiol (C, moderately strong positive control), or 17 α-methyltestosterone (D, weak positive control) (mean of two valid assays ± SEM)



III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study is performed in accordance with the current US OPPTS/OCSPP 890.1150 (2009) with no major deviations. The study is also performed in accordance with the current OECD 455 (2016), however the following deviations were noted: no ER antagonist assay was performed; demonstration of proficiency was stated but no data were included in study report and vehicle control (DMSO) for positive control was not tested. These deviations were not considered to affect the final outcome of the ER agonist assay. The study is therefore considered valid. Based on the results of the two valid transcriptional activation assays performed in the HeLa-9903 model system, glyphosate did not act as agonist of the human estrogen receptor alpha (hERα) over the concentration range tested (maximum concentration 10^{-3} M). The highest mean induction value of glyphosate was 2.4 ± 1.4 % (RPC_{max}), which is well below 10 % of the response of the positive control (1 nM 17β-estradiol) and represents a negative response for hERα agonism.

Thus, glyphosate was not identified as an agonist of human estrogen receptor alpha (hERα) under the conditions of the present study.

Assessment and conclusion by RMS:

CA 5.8.3/003

1. Information on the study

Data point:	CA 5.8.3
Report author	[redacted]
Report year	2012
Report title	Glyphosate: Estrogen Receptor Binding (Rat Uterine Cytosol) Screening Assay
Report No	6500V-100334ERB
Document No	CTX-11-029
Guidelines followed in study	US OPPTS/OCSPP 890.1250 (2009)
Deviations from current test guideline (OCSPP 890.1250, 2009) (OECD 493, 2015)	None to US EPA OPPTS/OCSPP Test Guideline 890.1250 (2009). Current OECD 493 (2015) is not applicable (present study obtained the estrogen receptor from rat uterine cytosol preparations; OECD 493 (2015) applies to two fully validated human recombinant receptor ERα (hrERα) *in vitro* test methods).
Previous evaluation	Yes, accepted in EFSA peer review on endocrine disrupting properties (2017).
GLP/Officially recognised testing facilities	Yes
Comment on GLP	The control and reference substances were not characterised in accordance with GLP standards, nor was the stability under storage conditions at the test site determined in accordance with GLP standards. However, the substances were purchased from commercial suppliers, and the manufacturers' certificates of analysis were used to define the purity. Verification of the test concentrations, stability and homogeneity of the test, control, and reference substances in the test system were not determined in accordance with GLP. The test, control, and reference substances were soluble in their vehicles, dosing solutions were prepared immediately prior to use, and the control and reference substances met their performance criteria. Therefore, these exceptions do not affect the validity of the study.
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive summary

In this estrogen receptor binding assay, the ability of glyphosate to interact with the estrogen receptor (ER) was evaluated. Cytosol isolated from Sprague Dawley rat uteri was used as source of ER to conduct a saturation binding experiment and a competitive binding experiment according to US EPA OPTTS test guideline 890.1250 (2009). The saturation binding experiment was conducted to demonstrate that the ER contained in the rat uterine cytosol was present in reasonable amount and was functioning with appropriate affinity for the radiolabelled reference estrogen, [^{3}H]-17β-estradiol, prior to routinely conducting the ER competitive binding experiment. The competitive binding experiment was conducted to measure the binding of a single concentration of [^{3}H]-17β-estradiol (1 nM) to the ER, in the presence of increasing

concentrations of glyphosate (ranging from 10^{-10} - 10^{-3} M). All concentrations were tested in replicates of three. The duration of incubation was 16 - 20 hours at 4 °C.

Four runs were conducted, however, the first run was considered invalid because the results for 17β-estradiol (positive control) did neither fit the guideline-established acceptance criteria nor did they fall within the range of historical data. Mean specific binding was >81 %, >95 %, and >111 % at every concentration of glyphosate tested in the first, second, and third valid runs of the assay, respectively, and averaged >100 % across the concentration range. Glyphosate showed no evidence of competing with estradiol for the ER and was classified as "non-interacting" for all three valid runs.

The weak positive control, 19-norethindrone met the validity criteria for each of the runs, and each assay had a Log IC_{50} value of -5.5 M. The Log IC_{50} values for 17β-estradiol also met the validity criteria and were -9.1 M, -9.0 M and -8.9 M in the first, second, and third valid runs, respectively. The negative control octyltriethoxysilane was correctly concluded not to interact with the ER.

Based on the results of the present Estrogen Receptor Binding Assay, glyphosate is not a ligand for the ER and is classified as not-interacting with the ER.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Identification:	Glyphosate acid
CAS No.:	1071-83-6
Description:	White crystalline solid
Lot/Batch No.:	GLP-1103-21149-T
Purity:	95.93 % glyphosate acid 85.14 % calculated glyphosate content
Stability of test compound:	Not reported. Expiry: 2012-03-09
Vehicle, positive controls, and negative control:	
Solvent/vehicle:	Assay buffer, i.e. TEDG+PI buffer (composition: 10 mM Tris, 1.5 mM EDTA, 1 mM DTT, 0.5 % Protease Inhibitor (PMSF) (v/v), 10 % glycerol, pH ~7.4)
Positive Control (non-labelled receptor ligand):	17β-estradiol Source: Sigma-Aldrich Lot/Batch No.: 110M0138V Purity: 100 % CAS No.: 50-28-2 Solvent used: DMSO
Weak Positive Control:	19-norethindrone Source: Sigma-Aldrich Lot/Batch No.: 030M1359V Purity: 99 % CAS No.: 68-22-4 Solvent used: DMSO

Negative Control: Octyltriethoxysilane
Source: Sigma-Aldrich
Lot/Batch No.: 24996KK
Purity: 99.34 %
CAS No.: 2943-75-1
Solvent used: DMSO

Radioactive receptor ligand:

Identification: $[^3H]$-17β-estradiol

Source: Perkin-Elmer

Lot/Batch No.: 650702

Radiochemical purity: >97 %

Specific activity: 130.2 Ci/mmol, certification date: 2011-05-06

Solvent used: DMSO

Test system: Rat uterine cytosol prepared from 12 - 13-week old female Sprague Dawley rats ovariectomized seven days prior to being euthanized. Uterine tissue was inspected for signs of residual ovarian tissue after ovariectomy. The uteri were weighed, placed in ice-cold TEDG+PI buffer, and homogenized. The cytosol was isolated, pooled and kept ice-cold. The protein concentration of the cytosol was 1.1 mg/mL.

Test concentrations: Dilutions of glyphosate, 19-norethindrone, octyltriethoxylsilane, 17β-estradiol are shown in the table below.

Table 5.8.3-7: Glyphosate: Estrogen Receptor Binding (Rat Uterine Cytosol) Screening Assay ([redacted] 2012): Competitor final molar (M) concentrations in competitive binding assay[1, 2]

Positive Control 17β-Estradiol	Weak Positive Control 19-Norethindrone	Negative Control Octyltriethoxysilane	Test Substance Glyphosate
1.0×10^{-7}	1.0×10^{-4}	1.0×10^{-3}	1.0×10^{-3}
1.0×10^{-8}	3.16×10^{-5}	1.0×10^{-4}	1.0×10^{-4}
3.16×10^{-9}	3.16×10^{-6}	1.0×10^{-5}	1.0×10^{-5}
1.0×10^{-9}	1.0×10^{-6}	1.0×10^{-6}	1.0×10^{-6}
3.16×10^{-10}	3.16×10^{-7}	1.0×10^{-7}	1.0×10^{-7}
1.0×10^{-10}	1.0×10^{-7}	1.0×10^{-8}	1.0×10^{-8}
1.0×10^{-11}	3.16×10^{-8}	1.0×10^{-9}	1.0×10^{-9}
-	3.16×10^{-9}	1.0×10^{-10}	1.0×10^{-10}

[1] Each sample contains: 10 µL of either the test substance, weak positive control, negative control, solvent control, or positive control; 390 µL of TEDG+PI buffer with $[^3H]$-17β-estradiol; and 100 µL of uterine cytosol (with ER), for a total of 500 µL.
[2] Each concentration of each chemical was run in triplicate.

B. STUDY DESIGN AND TEST PERFORMANCE

Experimental dates: 2011-11-03 to 2011-12-03

Saturation (radioligand) binding experiment

A saturation binding experiment to measure total and non-specific binding of $[^3H]$-17β-estradiol was performed to demonstrate that the ER was present in reasonable concentrations and had appropriate affinity for the native ligand. The methods and results from the saturation binding experiment were not included in

the final study report. However, a statement of the laboratory verifying that the saturation binding curve data were acceptable according to the US OPPTS/OCSPP 890.1250 (2009) was included in the appendix of the study report.

Competitive binding experiment

The competitive binding experiment was performed to measure the binding of a single concentration of [^{3}H]-17β-estradiol (1 nM) to the ER in the presence of increasing concentrations of glyphosate. A summary of the experiment conditions for the competitive binding experiment is included in the table below. The specific activity of [^{3}H]-17β-estradiol was adjusted for decay over time prior to each run. The adjusted specific activity was 126.6, 126.5, and 126.1 Ci/mmol for the first, second, and third valid runs of the assay, respectively.

Each assay consisted of three independent valid runs, which were conducted on three different days, and each run of the assay consisted of triplicates at each test concentration. In addition to glyphosate, concentrations of 17β-estradiol, 19-norethindrone, and octyltriethoxysilane were also evaluated in the competitive binding experiment to ensure that the test system was behaving as expected.

Table 5.8.3-8: Glyphosate: Estrogen Receptor Binding (Rat Uterine Cytosol) Screening Assay ([redacted], 2012): Summary of conditions for competitive binding experiment

Source of receptor		Rat uterine cytosol
Concentration of radioligand		1 nM
Concentration of receptor		Sufficient to bind 10 - 15 % of radioligand
Concentration of glyphosate (as serial dilutions)		100 pM to 1 mM
Temperature		4 ± 2 °C
Incubation time		16 - 20 hours
Composition of assay buffer (TEDG+PI buffer)	Tris	10 mM (pH 7.4)
	EDTA	1.5 mM
	Glycerol	10 % (v/v)
	Protease Inhibitor (Phenylmethylsulfonyl fluoride (PMSF))	0.5 % (v/v)
	DTT	1 mM

C. DATA ANALYSIS

Classification of test material

The test material was classified based on the average of three valid runs of the assay. Each run was individually classified as follows:

Interactive = Lowest point on the fitted curve within the range of the data is <50 % (i.e. >50 % of the radiolabelled estradiol has been displaced from the ER).

Not interactive = There are usable data points at or above 10^{-6} M and either the lowest point on the fitted response curve within the range of the data is >75 % (i.e. <25 % of the radiolabelled estradiol has been displaced from the ER), or a binding curve cannot be fitted and the lowest average percent binding among concentration groups in the data is >75 %.

Equivocal up to the limit of concentrations tested = If there are no data points at or above a test chemical concentration of 10^{-6} M and either a binding curve can be fit but ≤50 % of the radiolabelled estradiol has been displaced from the ER, or a binding curve cannot be fit and the lowest average percent binding among concentration groups in the data is >50 %.

Equivocal = A run is classified as equivocal if it does not fall into any of the categories above.

Descriptors for receptor binding

In circumstances when ER receptor binding was measured, the following parameters were determined:

- B_{max}: Maximum specific binding number (fmol ER/100 µg cytosol protein) measures the concentration of active receptor sites.
- K_d: Dissociation constant (nM), measures the affinity of the receptor for its natural ligand.
- IC_{50}: Concentration of the test substance at which 50 % of the radioligand is displaced from the receptor.

II. RESULTS AND DISCUSSION

A. SATURATION BINDING EXPERIMENT

Results from the saturation binding experiment were not provided in the study report, although the laboratory provided a statement in the Appendix of the study report indicating that the saturation binding curve data were acceptable according to the respective US OPPTS/OCSPP 890.1250 (2009). Reference control results included in the competitive binding experiment confirmed that functional ER was present in sufficient amounts and functioning with appropriate affinity.

Summarised saturation binding data from the performing laboratory were submitted following a request by the Agency (MRID 48843501). The protein concentrations used in the saturation binding runs varied between each run, and were approximately 3- to 6-fold greater than recommended in the test guideline (160 to 320 µg versus 50 ± 10 µg, respectively). The K_d for [^{3}H]-17β-estradiol was 0.331 ± 0.061 nM, and the estimated B_{max} was 74.55 ± 3.03 fmol/100 µg protein for the prepared rat uterine cytosol. The K_d for each run was within the expected test guideline range of 0.03 - 1.5 nM.

B. COMPETITIVE BINDING EXPERIMENT

Four runs of the assay were conducted; however, the first run was considered invalid because the positive control 17β-estradiol did not meet the guideline-established acceptance criteria or was outside the range of historical control data. The remaining three runs met all acceptance criteria and are considered valid.

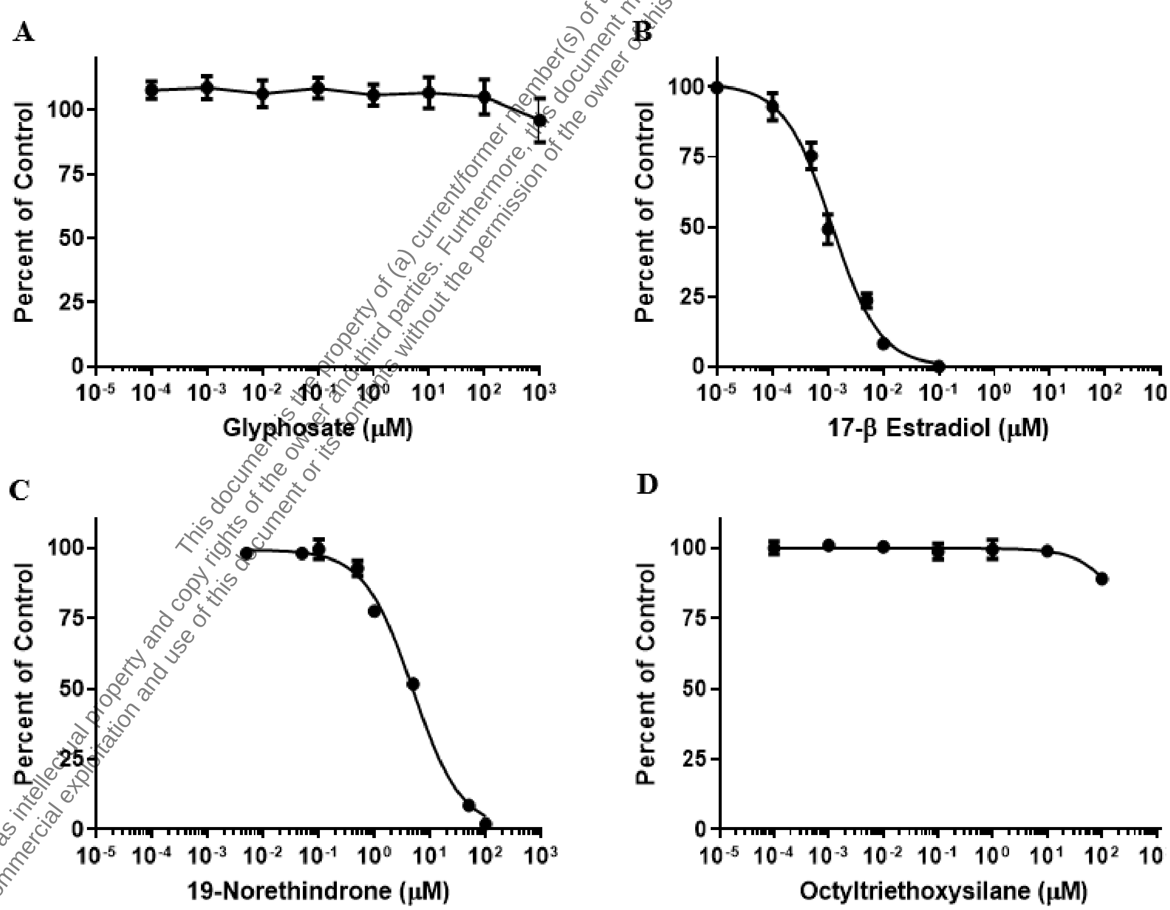
Results from the valid runs of the competitive binding experiment are presented in the table below. Specific binding was >81 %, >95 %, and >111 % at every concentration of glyphosate tested in the first, second, and third valid runs of the assay, respectively, and averaged >100 % across the concentration range (see figure below). Mean specific binding of the positive control, 17β-estradiol, and the weak positive control, 19-norethinodrone, met the guideline-established performance criteria for the assay in the three valid runs of the assay. The curve for the reference material showed that increasing concentrations of unlabelled 17β-estradiol displaced [^{3}H]-17β-estradiol in a manner consistent with one-site binding (see figure below). The Log IC_{50} of 17β-estradiol was -9.1 M, -9.0 M and -8.9 M in the first, second, and third valid run, respectively. 19-Norethindrone, had a Log IC_{50} of -5.5 M for each of the three valid runs. Confidence in these numbers is high due to the small variation. Mean specific binding of octyltriethoxysilane, the negative control, was >88 % at all concentrations except 10^{-3} M in the first and second valid assays, where the mean specific binding was 46.0 % and 47.4 %, respectively at 10^{-3} M. This phenomenon of lower mean specific binding observed in the first and second valid assays has previously been observed at the highest test concentration of octyltriethoxysilane and is associated with compound precipitation, rather than true competitive inhibition.

Table 5.8.3-9: Glyphosate: Estrogen Receptor Binding (Rat Uterine Cytosol) Screening Assay ([redacted] 2012): Results of competitive binding assay of glyphosate with estrogen receptor from rat uterine cytosol

Parameter	Compound	Run 1[1]	Run 2[1]	Run 3[1]	Mean ± SEM
r^2 (unweighted)	17β-estradiol	NR	NR	NR	NA
	19-Norethinodrone	NR	NR	NR	NA
	Glyphosate	NR	NR	NR	NA
Log IC_{50} (M)	17β-estradiol	-9.1	-9.0	-8.9	-9.0 ± 0.03
	19-Norethinodrone	-5.5	-5.5	-5.5	-5.5
	Glyphosate	NA	NA	NA	NA
IC_{50} (nM)	17β-estradiol	0.7947	1	1.26	1.02 ± 0.074
	19-Norethinodrone	3160	3160	3160	3160
	Glyphosate	NA	NA	NA	NA

[1] The mean is reported for the concurrent replicates within each run.
r^2 = Goodness of fit (r^2 is more appropriately expressed as a range, as opposed to a mean). For the test substance, these parameters could not be calculated.
NR = Not reported.
NA = Not applicable since binding was not evident.

Figure 5.8.3-3: Glyphosate: Estrogen Receptor Binding (Rat Uterine Cytosol) Screening Assay ([redacted] 2012): Relative amount of [³H]-17β-estradiol bound to the estrogen receptor in the presence of glyphosate (A), unlabelled 17β-estradiol (B, positive control), 19-norethindrone (C, weak positive control), or octyltriethoxysilane (D, negative control) (mean of three valid assays, ± SEM)



III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study is performed in accordance with the current US OPPTS/OCSPP 890.1250 (2009). Deviations from the current OECD 493 (2015) is not applicable as the present study obtained the estrogen receptor from rat uterine cytosol preparations and the OECD 493 (2015) applies to two fully validated human recombinant receptor ERα (hrERα) *in vitro* test methods. The study is therefore considered valid. No interaction of glyphosate with the estrogen receptor was determined in the performed saturation and competitive binding experiment. Based on these results, glyphosate was classified as "non-interacting" in all three valid independent runs of the Estrogen Receptor Binding Assay. Thus, under the conditions reported, glyphosate was assigned a final classification of "non-interacting" for binding to the estrogen receptor.

Assessment and conclusion by RMS:

CA 5.8.3/004

1. Information on the study

Data point:	CA 5.8.3
Report author	[redacted]
Report year	2012
Report title	Glyphosate: Human Recombinant Aromatase Assay
Report No	6500V-100334AROM
Document No	CTX-11-027
Guidelines followed in study	US OPPTS/OCSPP 890.1200 (2009)
Deviations from current test guideline (OCSPP 890.1200, 2009)	None
Previous evaluation	Yes, accepted in EFSA peer review on endocrine disrupting properties (2017).
GLP/Officially recognised testing facilities	Yes
Comment on GLP	The positive control substance was not characterised in accordance with GLP standards, nor was the stability under storage conditions at the test site determined in accordance with GLP standards. However, the substance was purchased from commercial supplier, and the manufacturers' certificate of analysis was used to define the purity. Concentrations of test substance and control substances were not verified using analytical methods. In view of the short-term nature of studies of this type, no analyses of stability, homogeneity or achieved concentration(s) were conducted on preparations of the test substance or positive control chemicals, either before or after the treatment phase.

	This exception is not considered to have affected the integrity of the study and is not required in the US OPPTS 890.1200 guideline. For the reference control compounds, stability was demonstrated by an appropriate response in the assay system. Proficiency testing done to certify analysts was not performed under GLP and the results of proficiency testing are not included in the final report. However, the raw data was provided to US EPA by the test facility and support the expected designations of inhibitor or non-inhibitor for each of the proficiency chemicals (econazole, fenarimol, nitrofen, and atrazine) as well as the positive control (4-OH ASDN).
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive summary

Glyphosate was evaluated for the potential to inhibit aromatase catalytic activity. In an *in vitro* aromatase (CYP19) assay, glyphosate was incubated with human recombinant aromatase (human CYP19 [Aromatase] and P450 reductase Supersomes^{TM}) and tritiated androstenedione ([^{3}H]ASDN) at concentrations of 10^{-10} M to 10^{-3} M for 15 minutes to assess the effect of glyphosate on aromatase activity. The solvent vehicle was 0.1 M sodium phosphate buffer for glyphosate, ethanol for ASDN, and dimethylsulfoxide (DMSO) for the positive control inhibitor 4-hydroxyandrostenedione (4-OH ASDN).

Aromatase activity was determined by measuring the amount of tritiated water produced at the end of a 15-minute incubation for each concentration of the used substances. Four independent runs of the assay were conducted; however, the first run was unacceptable because of incorrect standard preparation. Each run included a full activity control, a background activity control, a positive control series (10^{-10} M to 10^{-5} M) using the known inhibitor 4-OH ASDN in duplicate per concentration, and the test substance series (10^{-10} M to 10^{-3} M) in triplicate per concentration.

Aromatase activity in the full activity controls was 0.593 nmol/mg protein/min, which was greater than the minimum recommended aromatase activity of 0.1 nmol/mg protein/min. The response of the full activity controls and background controls was acceptable for each run.

For the positive control substance (4-OH ASDN), aromatase activity results were within the recommended ranges for the performance criteria. The estimated Log IC_{50} for 4-OH ASDN averaged -7.29 M and the Hill slope was -0.96.

For glyphosate, mean aromatase activity was 99.67 ± 1.86 % of vehicle control at the lowest tested concentration of 10^{-10} M and 109.3 ± 5.53 % of the vehicle control at the highest tested concentration of 10^{-3} M. The average aromatase activity was ≥99.67 % of the control at all tested glyphosate concentrations for all runs. Hill slope estimates were not determined for glyphosate because it never achieved >25 % inhibition. Thus, in three independent runs of the assay, increasing concentrations of glyphosate showed no decrease in aromatase activity.

Based on the data of the present study, glyphosate did not inhibit aromatase activity under the reported experimental conditions, and was therefore classified as a non-inhibitor of aromatase activity.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Identification:	Glyphosate acid
CAS No.:	1071-83-6
Description:	White crystalline solid
Lot/Batch No.:	GLP-1103-21149-T
Purity:	95.93 % glyphosate acid 85.14 % calculated glyphosate content
Stability of test compound:	Not reported. Expiry: 2012-03-09
Vehicle, non-labelled substrate, and positive control:	
Solvent/vehicle used:	0.1 M sodium phosphate buffer (pH 7.4)
Non-radio labelled aromatase substrate:	4-Androstene-3,17-dione (ASDN) Source: Steraloids, Inc. Lot/Batch No.: L1712 Purity: 99.8 % CAS No.: 63-05-8 Solvent used: Ethanol
Positive control (aromatase inhibitor):	4-hydroxyandrostendione (4-OH ASDN) Source: Sigma-Aldrich Lot/Batch No.: 081K2133 Purity: 99.6 % CAS No.: 566-48-3 Solvent used: DMSO

Radiolabelled substrate:

Identification:	[1β-^{3}H]-Androstenedione ([^{3}H]ASDN)
Source:	Perkin-Elmer
Lot/Batch No.:	619344
Radiochemical purity:	>97 %
Specific activity:	26.3 Ci/mmol, certification date 2010-08-06
Concentration of stock:	15 - 30 Ci/mmol
Solvent used:	Ethanol

Test system:

Identification:	Human CYP19 (Aromatase) and P450 reductase Supersomes™ (BD Gentest™, BD Biosciences)
Lot/Batch No.:	19701
Protein concentration:	3.7 mg/mL
Cytochrome C reductase activity:	540 nmol cytochrome C reduced/mg protein/min
Aromatase activity:	5.7 pmol product/pmol P450/min
Test concentrations:	Dilutions of glyphosate and the positive control 4-OH ASDN were tested as shown in the table below. A full activity control (containing all test components plus solvent control) and a background activity control (containing all test components except NADPH plus solvent control) were also performed.

Table 5.8.3-10: Glyphosate: Human Recombinant Aromatase Assay ([redacted] 2012): Competitor Final Molar (M) Concentrations in Aromatase Assay[1]

Positive control 4-OH ASDN	Test substance Glyphosate
1×10^{-5}	1×10^{-3}
1×10^{-6}	1×10^{-4}
$1 \times 10^{-6\,5}$	1×10^{-5}
1×10^{-7}	1×10^{-6}
$1 \times 10^{-7\,5}$	1×10^{-7}
1×10^{-8}	1×10^{-8}
1×10^{-9}	1×10^{-9}
1×10^{-10}	1×10^{-10}

[1] In each run of the assay, the full activity control and background activity control were run in replicates of four; the positive control was run in duplicate, and glyphosate was run in triplicate.

B. STUDY DESIGN AND TEST PERFORMANCE

Experimental dates: 2011-10-17 to 2011-10-21

Proficiency Testing
Proficiency testing of the CYP19 aromatase assay was conducted in three independent assay runs using the same methods as described below for the aromatase assay. Each run included evaluation of the positive control (4-OH ASDN) and the four recommended proficiency chemicals: econazole, fenarimol, nitrofen, and atrazine.

Aromatase Assay
Three types of control samples were included for each run. These included: 1) full enzyme (aromatase) activity controls (consisting of substrate [^{3}H]ASDN, NADPH, propylene glycol, buffer, microsomes, and vehicle [used for preparation of test substance solutions]); 2) background activity controls (all components that are in the full aromatase activity controls except NADPH); and 3) positive control (4-OH ASDN, run at eight concentrations, as indicated in the table above, in duplicate).

Four full activity controls and four background activity controls were included with each run of the assay (two tubes of each at the beginning of the assay and two tubes of each at the end of each assay).

Propylene glycol, [^{3}H] ASDN, NADPH, and assay buffer was combined in test tubes, with or without glyphosate or the positive control substance 4-OH-ASDN, for a total volume of 1 mL. The final concentrations for the major components of the assay are presented in the table below. The test tubes and microsomal suspensions were placed at 37 ± 2 °C in the water bath for five minutes prior to the initiation of the assay by the addition of 1 mL of the diluted microsomal suspension. The total assay volume was 2 mL. Subsequently, the tubes were incubated for 15 minutes at 37 ± 2 °C. The reactions were terminated and the unreacted ASDN extracted. The amount of ^3H_2O in the aqueous fraction was quantified for each assay tube by liquid scintillation counting, and aromatase activity was reported in units of nmol/mg protein/min.

Table 5.8.3-11: Glyphosate: Human Recombinant Aromatase Assay ([redacted] 2012): Summary of aromatase assay components and preparations

Assay Factor	Value

Sodium phosphate buffer (pH 7.4)	0.1 M
Microsomal protein	0.004 mg/mL[1]
NADPH	0.3 mM
[^{3}H]ASDN	100 nM
Propylene glycol	5 %
Temperature	37 ± 2 °C
Incubation time	15 min

[1] The concentration of microsomal protein was optimized for microsomes that produce ~540 pmol product/mg protein/min and 5.7 pmol product/pmol P450/min.

C. DATA ANALYSIS

Statistics

The response curve was fitted by weighted non-linear regression analysis using a four-parameter logistic regression model. For each run, the individual percent of control values were plotted versus logarithm of the test chemical concentration. In order to determine the consistency among runs, the slope and $LogIC_{50}$ for the positive control and test chemical(s) were compared across runs based on one-way random effects analysis of variance (ANOVA), with the runs treated as random effects. The parameters were graphed within each run with associated 95 % confidence intervals based on the within-run standard error, and the average across-run standard error, with the associated 95 % confidence interval incorporating run-to-run variation.

Classification of test material

The test material was classified based on the average of three valid runs of the assay. Each run was individually classified as follows:
Inhibitor = Average curve across runs crossed 50 %.
Non-Inhibitor = Average lowest portion of curve across runs is >75 % activity, or data do not fit the model.
Equivocal = Average lowest portion of curve across runs is between 50 % and 75 % activity.

II. RESULTS AND DISCUSSION

A. AROMATASE ASSAY

Mean aromatase activity in the full activity controls was 0.593 nmol/mg protein/min (range: 0.584 - 0.771 nmol/mg protein/min) for the three assay runs, which was greater than the minimum required aromatase activity of 0.1 nmol/mg protein/min according to guideline. The response of the full activity controls and background controls was acceptable for each run.

Aromatase assay results for glyphosate and the positive control 4-OH ASDN are shown in the table below and illustrated in the figure below.

For the positive control substance 4-OH ASDN, mean aromatase activity was 98.77 ± 1.54 % of vehicle control (VC) value at the lowest tested concentration of 10^{-10} M, and 0.67 ± 0.04 % of VC at the highest tested concentration of 10^{-5} M. Mean $LogIC_{50}$ for 4-OH ASDN was -7.29 M (range: -7.28 to -7.30 M) and mean Hill slope was -0.96 (range: -0.92 to -1.00). Therefore, the aromatase activity results for the positive control substance were within the recommended ranges for the performance criteria.

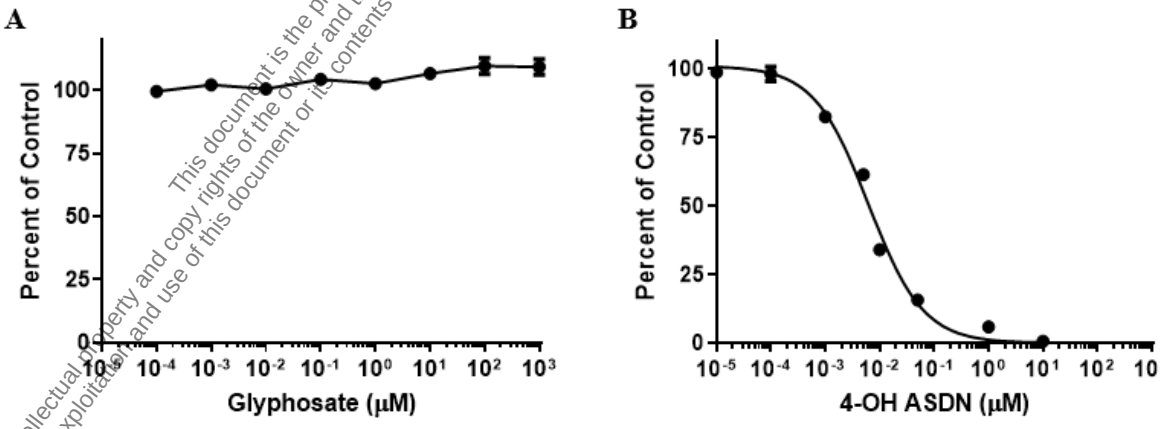
For the test substance glyphosate, mean aromatase activity was 99.67 ± 1.86 % of VC at the lowest tested concentration, 10^{-10} M and 109.3 ± 5.53 % of VC at the highest tested concentration, 10^{-3} M. $LogIC_{50}$. The Hill slope estimates were not determined for glyphosate because it never achieved >25 % inhibition. Based on these results, glyphosate does not inhibit the enzyme aromatase under the conditions of the present aromatase assay.

Table 5.8.3-12: Glyphosate: Human Recombinant Aromatase Assay ([redacted] 2012): Effect of 4-OH ASDN and glyphosate on aromatase activity. Data are presented as mean activity in percent compared to control (mean ± SD).

Substance	Concentration (M)	Run 1	Run 2	Run 3	Overall Mean (% of control)
Positive control 4-OH ASDN	TA	99.90 ± 1.204	106.58 ± 0.255	103.18 ± 1.479	-
	NSB	0.02 ± 0.038	0.01 ± 0.042	0.02 ± 0.041	-
	10^{-5}	0.71 ± 0.011	0.64 ± 0.163	0.66 ± 0.047	0.67
	10^{-6}	6.38 ± 1.015	5.98 ± 0.727	5.49 ± 0.719	5.95
	$10^{-6\,5}$	16.86 ± 0.372	15.59 ± 0.104	14.73 ± 1.378	15.73
	10^{-7}	34.48 ± 0.218	34.06 ± 2.793	33.53 ± 4.675	34.02
	$10^{-7\,5}$	60.36 ± 3.002	61.35 ± 2.708	62.70 ± 0.854	61.47
	10^{-8}	83.62 ± 1.893	81.61 ± 3.938	82.45 ± 3.418	82.56
	10^{-9}	95.72 ± 4.292	97.76 ± 3.312	100.93 ± 1.042	98.14
	10^{-10}	97.99 ± 1.616	100.54 ± 2.450	97.77 ± 2.112	98.77
Test substance Glyphosate	TA	100.10 ± 2.217	93.42 ± 3.574	96.82 ± 0.708	-
	NSB	-0.02 ± 0.015	-0.01 ± 0.019	-0.02 ± 0.023	-
	10^{-3}	105.36 ± 2.440	106.92 ± 1.383	115.62 ± 0.313	109.30
	10^{-4}	107.19 ± 0.887	105.94 ± 5.294	116.07 ± 2.660	109.73
	10^{-5}	106.41 ± 1.959	105.23 ± 1.781	108.52 ± 0.341	106.72
	10^{-6}	103.64 ± 2.538	99.98 ± 4.675	104.63 ± 1.239	102.75
	10^{-7}	104.12 ± 2.743	103.66 ± 1.034	105.67 ± 0.671	104.48
	10^{-8}	100.27 ± 3.510	99.40 ± 6.362	102.33 ± 0.887	100.67
	10^{-9}	101.69 ± 2.054	102.86 ± 2.189	102.11 ± 2.912	102.22
	10^{-10}	100.84 ± 2.476	97.52 ± 3.393	100.64 ± 2.203	99.67

TA = Total activity, i.e. full activity control
NSB = Non-specific binding, i.e. background activity control

Figure 5.8.3-4: Glyphosate: Human Recombinant Aromatase Assay ([redacted] 2012): Mean inhibition response curves for glyphosate (A) and 4-OH ASDN (B, positive control) (mean of three valid assays ± SEM)



III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study is performed in accordance with the current US OPPTS/OCSPP 890.1200 (2009). The study is therefore considered valid. In three independent runs of the present assay, increasing concentrations of glyphosate showed no decrease in aromatase activity. The mean aromatase activity at all tested glyphosate concentrations was ≥ 99.67 % compared to control.
Based on these data, glyphosate did not inhibit aromatase activity under the reported experimental conditions, and was therefore classified as a non-inhibitor of aromatase activity.

Assessment and conclusion by RMS:

CA 5.8.3/005

1. Information on the study

Data point:	CA 5.8.3
Report author	[redacted]
Report year	2012
Report title	A Uterotrophic Assay of Glyphosate Administered Orally in Ovariectomized Rats
Report No	[redacted]-843002
Document No	Not reported
Guidelines followed in study	US EPA OPPTS/OCSPP 890.1600 (2009) OECD 440 (2007)
Deviations from current test guideline (OCSPP 890.1600, 2009) (OECD 440, 2007)	None
Previous evaluation	Yes, accepted in EFSA peer review on endocrine disrupting properties (2017).
GLP/Officially recognised testing facilities	Yes
Comment on GLP	The characterization of the positive control substance, 17α-ethinyl estradiol, was not conducted according to GLP standards. However, the commercial provider characterized 17α-ethinyl estradiol and the certificate of analysis was included in the appendix of the final study report.
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive summary

A uterotrophic assay was conducted in 66 - 67-day old female Crl:CD(SD) Sprague Dawley rats to screen for potential estrogenic activity of glyphosate. Groups of six ovariectomized rats each were administered

glyphosate in 0.5 % methylcellulose (in deionized water) orally via daily gavage at dose levels of 0 (vehicle control), 100, 300, and 1000 mg/kg bw/day for three consecutive days. The high dose level represents the limit dose for the assay. A positive control group of six rats was administered a daily dose of 17α-ethinyl estradiol (EE) in corn oil at 3 µg/kg bw/day by subcutaneous (s.c.) injection. All rats were ovariectomized 17 - 18 days prior to substance administration.

All rats survived to scheduled necropsy 24 hours following the last substance administration. No test substance-related clinical findings were noted at the daily examinations or four hours following dose administration at any dose level. No clinical findings were noted in the positive control group. No macroscopic findings were noted in the uterus at 100, 300, or 1000 mg/kg bw/day glyphosate or in the positive control group.

No test substance-related effects on mean body weights or mean body weight gains were noted in glyphosate treatment groups compared to concurrent control. Mean, absolute and relative, uterine weights (wet and blotted) of treatment groups were similar to control group values.
In positive control group animals, a mean body weight loss (-5.6 g) was observed at the end of the treatment period in contrast to a mean body weight gain (+11.3 g) in control group animals over the same period. Higher mean wet and blotted uterine weights (8.6- and 3.6-fold, respectively) were noted in the positive control group compared to control. These increases in uterine weights demonstrated the expected estrogenic effect of the positive control 17-α-ethinyl estradiol.

Based on the lack of effects on mean uterine weights (wet and blotted), glyphosate did not demonstrate or mimic biological activities consistent with agonism of natural estrogens when administered orally to ovariectomized female rats at dosage levels of 100, 300, and 1000 mg/kg bw/day. The positive control substance (17α-ethynylestradiol) elicited the expected increases in wet and blotted uterine weights.

The absence of effects on uterine weights demonstrated a lack of estrogenicity for glyphosate at all dosage levels evaluated up to and including the limit dose of 1000 mg/kg bw/day.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Identification:	Glyphosate acid
CAS No.:	1071-83-6
Description:	White powder
Lot/Batch No.:	GLP-1103-21149-T
Purity:	95.93 % glyphosate acid 85.14 % calculated glyphosate content
Stability of test compound:	Stored at room temperature and considered stable under this condition.
Vehicle and positive control:	
Solvent/vehicle used:	Methylcellulose Source: Sigma-Aldrich Lot/Batch No.: 060M0123V CAS No.: 9004-67-5 Final concentration: 0.5 % in deionized water

Positive Control (reference estrogen):	17α-ethinyl estradiol (EE) Source: Sigma-Aldrich Lot/Batch No.: 028K1411 Purity: 99 % CAS No.: 57-63-6 Solvent used: corn oil
Test animals:	
Species:	Rat
Strain:	Crl:CD(SD) Sprague Dawley
Source:	[redacted]
Age at ovariectomy:	49 days of age; ovariectomy was performed at Charles River Laboratories
Age at dosing:	66 - 67 days of age; vaginal lavages were performed daily for five consecutive days (beginning at least 12 days after ovariectomy) prior to assignment to study groups, to ensure females were in persistent diestrus
Sex:	Female
Weight at dosing:	245.6 – 301.2 g
Acclimation period:	11 days
Diet/ Food:	2016*CM* Teklad Global 16 % Protein Rodent Diet (mean total isoflavones: 29.0 ppm), *ad libitum*
Water:	Reverse osmosis-purified tap water, *ad libitum*
Housing:	Individually housed in clean, stainless steel wire-mesh cages suspended above cage-board
Environmental conditions:	Temperature: 21.3 - 21.6 °C Humidity: 51.8 - 55.2 % Air changes: 10/hour Photocycle: 12 hours light/ 12 hours dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 02 Jul to 04 Jul 2011 test substance administration; 05 Jul 2011 necropsy

Animal assignment and treatment
Animals were assigned to groups using a WTDMS™ computer program that randomised the animals based on stratification of body weights in a block design. The experimental design consisted of one vehicle control group, three test substance-treated groups, and one positive control group, each composed of six rats.

The vehicle and test substance formulations were administered orally by gavage once daily during study days 0 - 2. The oral dosage volume for all groups was 5 mL/kg bw. The positive control substance, 17α-ethinyl estradiol, was administered by bolus subcutaneous (s.c.) injection in the scapular region at a dosage volume of 5 mL/kg bw. Dosing sites were rotated clockwise between three distinct scapular sites daily. Individual dosages were based on the most recently recorded body weights.

Table 5.8.3-13: A Uterotrophic Assay of Glyphosate Administered Orally in Ovariectomized Rats ([redacted] 2012): Study design of uterotrophic assay with glyphosate, administered orally to female Sprague Dawley rats

Test group	Dose (mg/kg bw/day)[1]	No. of females
Control (vehicle)[2]	0	6

Low dose[2]	100	6
Mid dose[2]	300	6
High dose[2]	1000	6
Positive control 17α-ethinyl estradiol (EE)[3]	0.003	6

[1] Dosing volume: 5 mL/kg bw; a correction factor of 85.14 % was used to account for glyphosate purity.
[2] Exposure route: oral (gavage).
[3] Exposure route: subcutaneous (injection).

Dose Preparation and analysis
The test substance formulations were prepared once as single formulations for each dose level, divided into aliquots for daily dispensing, and stored at room temperature. A stock solution of 17α-ethinyl estradiol (0.05 mg/mL) in corn oil was prepared one day prior to the initiation of dosing. The positive control formulation was prepared by serial dilution of the stock solution in corn oil. The test substance and positive control formulations were prepared at separate stations to avoid cross contamination and were stirred continuously throughout the preparation, sampling and dose administration procedures. Samples for concentration analysis were collected from the middle stratum of each dosing formulation (including the positive control group) prepared during the in-life phase of the study. Homogeneity, resuspension homogeneity, and stability of the test substance formulations were demonstrated in a preceding, seperate study.

Observations
All rats were observed (cage-side) twice daily for clinical signs of toxicity, moribund condition and mortality. Individual clinical observations (hand-held physical examination) were recorded daily (prior to dose administration during the treatment period) through the day of necropsy. Animals were also observed for signs of toxicity approximately four hours following dose administration. Animals of the positive control group were examined for potential erythema, swelling, or other dermal findings at the injection site.

Body weight
Individual body weights were recorded on the day of randomization, daily prior to test substance administration, and on the day of euthanasia. Mean body weight changes were calculated for each corresponding interval and for study days 0 - 3.

Food consumption
Food consumption was not evaluated.

Sacrifice and measurement of uterine weight
All females were euthanized by carbon dioxide inhalation approximately 24 hours following administration of the last dose in the same order of dosing. Macroscopic examination was conducted for the uteri; full necropsies were not conducted. The uterus was carefully dissected and trimmed, being careful to retain the luminal fluid. Each "wet" uterus was weighed intact (with the luminal fluid) to the nearest 0.1 mg, then opened longitudinally and blotted with filter paper to remove the luminal fluid. Subsequently, the blotted uterus was weighed to the nearest 0.1 mg. Any loss in luminal content was reported.

Microscopic Examination
Microscopic examinations were not conducted. However, the uterus and vagina were preserved in 10 % neutral-buffered formalin for possible future histopathologic examination.

Statistics
Each endpoint was tested for homogeneity of variance using Levene's test. Homogeneity of variance was confirmed for each endpoint ($p > 0.01$) and no data transformation was required.

Uterine weights: In the first stage, the 17α-ethinyl estradiol group was compared to the vehicle control

group. An analysis of covariance (ANCOVA) was performed using the body weight at necropsy as the covariate. Both groups were compared to each other in a one-sided test using p = 0.05 to declare significance, looking for significant increases from the vehicle control.

The second stage of the analysis compared the various glyphosate-treated groups to the vehicle control group. An ANCOVA was performed using the body weight at necropsy as the covariate. Comparisons of each treatment group with the vehicle control group were made with a one-sided Dunnett's test using p = 0.05 to declare significance, looking for significant increases from the vehicle control group.

Body weights and cumulative body weight change: The statistical analysis of body weight and body weight change was identical to the uterine weights with the following exception: there was no covariate in the statistical model (ANOVA) and the testing was two-sided rather than one-sided.

II. RESULTS AND DISCUSSION

A. MORTALITY
All animals survived until scheduled necropsy.

B. CLINICAL OBSERVATIONS
No test substance-related clinical findings were noted at the daily examinations at any dose level. No clinical findings were noted four hours following dose administration at any dose level. No clinical observations were noted in the positive control group. Slight and/or very slight edemas were noted at the injection sites of positive control animals.

C. BODY WEIGHT
No test substance-related effects on mean body weights or body weight changes were noted in the 100, 300, and 1000 mg/kg bw/day glyphosate-treated groups. The values in the test substance-treated groups were similar to and not statistically significantly different from the vehicle control group.

In the positive control group, a significant ($p < 0.0001$) mean body weight loss (-5.6 g) was noted when the overall treatment period (study days 0 - 3) was evaluated compared to a mean body weight gain in the vehicle control group (+11.3 g). This resulted in a decreased mean terminal body weight in the positive control group (-5.24 %) compared to the vehicle control group on study day 3. The difference was not statistically significant. Body weights and body weight changes are presented in the table below.

Table 5.8.3-14: A Uterotrophic Assay of Glyphosate Administered Orally in Ovariectomized Rats ([redacted] 2012): Mean group body weight and body weight change in the uterotrophic assay in Sprague Dawley rats. Females were exposed to glyphosate for three days via gavage (n=6).

Dose (mg/kg bw/day)	Body weight (g), mean ± SD				Body weight change (g)
	Study Day 0	Study Day 1	Study Day 2	Study Day 3	
0	277.0 ± 19.18	279.7 ± 19.50	286.4 ± 21.12	288.3 ± 20.96	11.3
100	277.6 ± 14.63	282.4 ± 15.81	288.2 ± 16.34	292.9 ± 15.98	15.3
300	275.2 ± 15.61	279.2 ± 16.88	284.8 ± 15.54	284.3 ± 19.40	9.1
1000	278.3 ± 15.48	282.8 ± 16.64	287.5 ± 16.50	291.6 ± 17.53	13.3
0.003 EE	278.7 ± 18.79	280.2 ± 21.06	278.2 ± 19.36	273.2 ± 20.92	-5.6

SD, standard deviation; EE, 17α-ethinyl estradiol (positive control)

D. NECROPSY
At scheduled necropsy, no macroscopic findings in the uterus were observed at dosage levels of 100, 300,

and 1000 mg/kg bw/day glyphosate or in the positive control group.

E. UTERINE WEIGHTS

Mean wet and blotted uterine weights were unaffected by test substance administration at dose levels of 100, 300, and 1000 mg/kg bw/day. Differences from the vehicle control group were slight and not statistically significant. Mean blotted uterine weight in the vehicle control group was less than 0.04 % of the final body weight, indicating acceptable results.

The positive control substance produced the expected estrogenic response. Mean wet and blotted uterine weights in this group were significantly (p <0.0001) higher (8.6- and 3.6-fold, respectively) than the vehicle control group values. Results of wet and blotted uterine weights are presented in the table below.

Table 5.8.3-15: A Uterotrophic Assay of Glyphosate Administered Orally in Ovariectomized Rats ([redacted] 2012): Uterine weights in the uterotrophic assay Sprague Dawley rats treated orally with glyphosate for three days.

Dose (mg/kg bw/day)	**0**	**100**	**300**	**1000**	**0.003 EE**
Parameter	**Uterine weight (mean ± SD)**				
Terminal body weight	289 ± 20.9	293 ± 16.2	284 ± 19.1	292 ± 17.5	273 ± 21.0
Wet, absolute (mg)	111.0 ± 10.80	110.7 ± 12.45	118.3 ± 16.49	113.6 ± 9.66	953.1* ± 90.44
Wet, relative (%)	0.038 ± 0.0025	0.038 ± 0.0056	0.042 ± 0.0061	0.039 ± 0.0044	0.352* ± 0.0546
Blotted, absolute (mg)	98.2 ± 11.66	98.7 ± 10.62	103.0 ± 11.62	102.4 ± 8.87	349.3* ± 31.14
Blotted, relative (%)	0.034 ± 0.0024	0.034 ± 0.0048	0.036 ± 0.0040	0.035 ± 0.0038	0.129* ± 0.0173

SD, standard deviation; EE, 17α-ethinyl estradiol (positive control); * Significantly different from control at p <0.0001

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study is performed in accordance with the current US EPA OPPTS/OCSPP 890.1600 (2009) and OECD 440 (2007). The study is therefore considered valid. Oral administration of glyphosate at dosage levels up to 1000 mg/kg bw/day did not produce any indication of toxicity. In addition, mean uterine weights (wet and blotted) in the glyphosate-treated groups were similar to control group values. The absence of effects on uterine weights demonstrated a lack of estrogenicity for glyphosate at all dose levels evaluated up to and including the limit dose of 1000 mg/kg bw/day in the present uterotrophic assay. The positive control 17α-ethinyl estradiol elicited expected, significant increase in absolute wet and blotted uterine weights compared to the control group, demonstrating the sensitivity of the assay.

Based on these results, glyphosate did not demonstrate or mimic biological activities consistent with agonism of natural estrogens at all applied doses including the limit dose of 1000 mg/kg bw/day.

Assessment and conclusion by RMS:

CA 5.8.3/006

1. Information on the study

Data point:	CA 5.8.3
Report author	[redacted]
Report year	2012
Report title	A Hershberger Assay of Glyphosate Administered Orally in Peripubertal Orchidoepididymectomized Rats
Report No	[redacted]-843003
Document No	Not reported
Guidelines followed in study	US EPA OPPTS/OCSPP 890.1400 (2009) OECD 441 (2009)
Deviations from current test guideline (OCSPP 890.1400, 2009) (OECD 441, 2009)	None
Previous evaluation	Yes, accepted in EFSA peer review on endocrine disrupting properties (2017).
GLP/Officially recognised testing facilities	Yes
Comment on GLP	The characterisation of the positive control substances, testosterone propionate and flutamide, was not conducted according to GLP standards but were characterized by the commercial provider and documented on the certificate of analysis.
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive summary

The objective of this study was a mechanistic *in vivo* screen for potential androgenic activity of the test substance glyphosate. To screen for potential androgenic activity, glyphosate (in 0.5 % methylcellulose in deionized water) was administered daily for ten days via gavage to 54 - 55-day old, castrated male Crl:CD(SD) Sprague Dawley rats (six per dose group) at dose levels of 0 (vehicle control), 100, 300, or 1000 mg/kg bw/day. The high dose represents the limit dose for this assay. An androgenic positive control group consisted of six castrated rats exposed to 0.2 mg/kg bw/day testosterone propionate (TP) by subcutaneous (s.c.) injection. To screen for potential anti-androgenic activity, glyphosate in 0.5 % methylcellulose (in deionized water) was administered daily for ten days via gavage to 54 - 55-day old, castrated male Crl:CD(SD) Sprague Dawley rats (six per dose group) at dose levels of 0 (vehicle control), 100, 300, or 1000 mg/kg bw/day, in conjunction with a daily dose of the reference androgen TP at 0.2 mg/kg bw/day by s.c. injection. An anti-androgenic positive control group consisted of six castrated rats exposed to 0.2 mg/kg bw/day TP by s.c injection, and to 3 mg/kg bw/day flutamide (FT) by oral gavage. TP alone was used as the anti-androgenic negative control.

All animals were observed twice daily for mortality and moribundity. Clinical observations and body weights were recorded daily. The bulbourethral glands, glans penis, levator ani and bulbocavernosus (LABC) muscle group, seminal vesicles with coagulating glands, and ventral prostate were examined, weighed, and retained in 10 % neutral-buffered formalin.

All males survived to scheduled euthanasia on study day 10. There were no test substance-related clinical or macroscopic findings noted at any dose level. No test substance or control substance-related effects on

mean body weights or body weight change were noted. No test substance-related androgenic effects on the weights of male accessory sex organs (bulbourethral glands, glans penis, LABC muscle group, seminal vesicles with coagulating glands, and ventral prostate) were noted compared to the vehicle control group. Co-administration of TP with the test substance did not reveal an anti-androgenic effect (i.e. decreased mean organ weights) when compared to the TP control group. Administration of the androgenic positive control substance (TP) resulted in the expected higher mean male accessory sex organ weights. Co-administration of the anti-androgenic positive control substance flutamide (FT) with TP inhibited the androgenic response, resulting in lower mean organ weights compared to the TP control group.

Based on the results of the present Hershberger assay, glyphosate was negative for androgenicity and anti-androgenicity under the reported conditions.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Identification:	Glyphosate acid
CAS No.:	1071-83-6
Description:	White powder
Lot/Batch No.:	GLP-1103-21149-T
Purity:	95.93 % glyphosate acid 85.14 % calculated glyphosate content
Stability of test compound:	Stored at room temperature and was considered stable under this condition
Vehicle and positive controls:	
Vehicle:	Methylcellulose Source: Sigma-Aldrich Lot/Batch No.: 060M0123V CAS No.: 9004-67-5 Final concentration: 0.5 % in deionized water
Positive Control (reference androgen):	Testosterone propionate (TP) Source: AK Scientific, Inc., Mountain View, CA, US Lot/Batch No.: 70321J Purity: 98.3 % CAS No.: 57-82-5 Solvent used: minimal amount of 95 % ethanol Vehicle used: corn oil
Positive Control (reference anti-androgen):	Flutamide (FT) Source: Spectrum Chemical Manufacturing Corporation, New Brunswick, NJ, US Lot/Batch No.: 2AC0144 Purity: 100 % CAS No.: 1311-84-7 Solvent used: minimal amount of 95 % ethanol Vehicle used: corn oil

Test animals:

Species:	Rat

Strain:	Crl:CD(SD) Sprague Dawley
Source:	[redacted]
Age at castration:	42 days of age; orchidoepididymectomy was performed at [redacted]
Age at start of dosing:	54 - 55 days of age
Sex:	Male
Weight at dosing:	211.3 – 279.2 g
Acclimation period:	13 days post-castration, 6 days post-arrival at laboratory
Diet/Food:	PMI Nutrition International, LLC Certified Rodent LabDiet 5002, *ad libitum*
Water:	Municipal water filtered by reverse-osmosis, *ad libitum*
Housing:	Individually housed in clean, stainless steel wire-mesh cages suspended above cage-board
Environmental conditions:	Temperature: 21.3 - 21.6 ºC Humidity: 51.8 - 55.2 % Air changes: 10/h Photocycle: 12 hours light/12 hours dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 27 Jun to 06 Jul 2011 test substance administration; 07 Jul 2011 necropsy

Animal assignment and treatment
Males were assigned to groups using a WTDMST™ computer program which randomized the animals based on body weight stratification in a block design. The experimental design consisted of two assays: one for androgen agonist activity and one for anti-androgen activity. In the androgen agonist assay, there was one vehicle control group, one positive control group and three test substance-treated groups, each composed of six rats. In the anti-androgen assay, there was one vehicle control group, one androgen treated negative control group, one androgen and anti-androgen treated positive control group, and three test substance-treated groups (co-treated with the androgen TP), each composed of six rats.

In the Hershberger assay to screen for potential androgenic activity, the test substance glyphosate was administered daily for ten days via oral gavage to castrated male rats at doses of 100, 300, or 1000 mg/kg bw/day (at a dosing volume of 5 mL/kg bw).

In the Hershberger assay to screen for potential anti- androgenic activity, glyphosate was administered daily for ten days via gavage to castrated male rats at the same doses (100, 300, or 1000 mg/kg bw/day) in conjunction with a daily dose of TP (0.2 mg/kg bw/day) by s.c. injection.

A concurrent vehicle control group received the dosing vehicle by gavage for the same duration. In another group, TP was administered via s.c. injection at a dose level of 0.2 mg/kg bw/day and a dosing volume of 0.5 mg/mL. This treatment served as an androgenic positive control group in the androgen agonist assay as well as an anti-androgen negative control in the anti-androgen assay (same animals). In the anti-androgen positive control group, males received 0.2 mg/kg bw/day TP by s.c. injection in conjunction with 3 mg/kg bw/day of flutamide (FT), a known anti-androgenic substance, by oral gavage (at a dosing volume of 5 mL/kg bw) for ten days.

Table 5.8.3-16: A Hershberger Assay of Glyphosate Administered Orally in Peripubertal Orchidoepididymectomized Rats ([redacted] 2012): Study design of Hershberger assay with glyphosate in male Sprague Dawley rats

Test group	Dose[1] (mg/kg bw/day)	No. of males	Dosing volume (mL/kg bw/day)
Androgen agonist assay			
Control (vehicle)	0	6	5
Low	100	6	5
Mid	300	6	5
High	1000	6	5
Positive Control (TP, s.c.)	0.2	6	0.5
Anti-androgen assay			
Control (vehicle)	0	6	5
Negative Control (TP, s.c.)	0.2	6	0.5
Low (+ TP, s.c.)	100 (+ 0.2)	6	5 (+ 0.5)
Mid (+ TP, s.c.)	300 (+ 0.2)	6	5 (+ 0.5)
High (+ TP, s.c.)	1000 (+ 0.2)	6	5 (+ 0.5)
Positive Control (FT, gavage) (+ TP, s.c.)	3 (+ 0.2)	6	5 (+ 0.5)

s.c. = subcutaneous; TP = Testosterone propionate; FT = Flutamide
[1] To calculate the glyphosate dose a correction factor of 85.14 % was used to account for the purity.

Dose Preparation and Analysis
The test substance formulations were prepared approximately weekly as single formulations for each dose level, divided into aliquots for daily dispensation, and stored at room temperature. The test substance formulations were stirred continuously throughout the preparation, sampling, and dose administration procedures.

The positive control substance formulations were prepared once, as follows. The appropriate amount of the positive control substance (TP or FT) was dissolved in a minimal amount of 95 % ethanol and diluted to the final concentration with corn oil. The FT formulation was then divided into aliquots for daily use. Aliquots of the TP formulation were dispensed into sterile septum vials for daily use. The positive control substance formulations were stored at room temperature and were stirred continuously throughout the preparation, sampling, and dose administration procedures.

The vehicle was prepared approximately weekly for administration to the vehicle control group and for preparation of the test substance formulations; aliquots were prepared for daily dispensation to the control group and stored at room temperature. The vehicle was mixed throughout the preparation, sampling, and dose administration procedures.

Clinical observations
All rats were observed (cage-side) twice daily for moribund condition and mortality. Individual clinical observations (hand-held physical examinations) were recorded daily (prior to test substance administration during the treatment period) through the day of scheduled necropsy. Each male was also observed for signs of toxicity at approximately four hours following dose administration. The injection sites for males receiving s.c. injections were examined daily from the initiation of dose administration for erythema, swelling, and other dermal findings. Erythema and swelling were evaluated in accordance with a four-step grading system of very slight, slight, moderate, and severe.

Body weight
Individual male body weights were recorded on the day of randomization, daily prior to test substance administration, and on the day of scheduled necropsy. Mean body weight changes were calculated for each corresponding interval and for study days 0 - 10.

Food consumption
Food consumption was not recorded.

Blood collection
Blood samples (3 mL/animal) were collected from the vena cava following anaesthesia by isoflurane inhalation approximately 24 hours following administration of the last dose. The blood samples were allowed to clot at room temperature, serum was separated by centrifugation, and the samples stored frozen (approximately -70 °C) for possible future hormone analysis.

Sacrifice, measurement of accessory sex organ weights and pathology
Males were euthanized by exsanguination on study day 10. Full necropsies were not conducted. The following tissues and organs were excised, trimmed free of adhering tissue, and weighed fresh (unfixed) and placed in 10 % neutral-buffered formalin:

- Bulbourethral glands (Cowper's gland)[1, 2]
- Glans penis
- Levator ani and bulbocavernosus (LABC) muscle group
- Seminal vesicles with coagulating glands[1, 2]
- Ventral prostate[2]

[1] = Paired organs were weighed together.
[2] = Care was taken to minimize fluid loss from these organs; loss of fluid was documented.

Microscopic examinations were not conducted.

Statistics
Each endpoint was tested for homogeneity of variance using Levene's test. If that test was significant at p = 0.01, then a log transformation was applied and Levene's test conducted on the transformed data. Homogeneity of variance was confirmed for each endpoint (p >0.01).

Androgen agonist assay:
Organ weights (vehicle control vs. TP): The one-sided t-test was performed using p = 0.05 to declare significance, looking for significant increases from the vehicle control group.
Organ weights (vehicle control vs. glyphosate groups): An analysis of variance (ANOVA) was conducted on the raw or log transformed data. A one-sided Dunnett's test was conducted using p = 0.05 to declare significance, regardless of the outcome of the ANOVA, looking for significant increases in the glyphosate groups when compared with the vehicle control group.
Body weight and body weight change: The statistical analysis was identical to that for organ weights with the exception that the testing was two-sided, rather than one-sided, using p = 0.05 to declare significance.

Anti-androgen assay:
Organ weights (TP vs. TP + FT): A one-sided t-test was performed using p = 0.05 to declare significance, looking for significant decreases from the TP group.
Organ weights (TP vs. glyphosate + TP groups): An ANOVA was conducted on the raw or transformed data, as appropriate. A one-sided Dunnett's test was conducted using p = 0.05 to declare significance, regardless of the outcome of the ANOVA, looking for significant decreases in the glyphosate + TP groups when compared with the TP group.
Body weight and body weight change: The statistical analysis was identical to the organ weights with the exception that the testing was two-sided, rather than one-sided, using p = 0.05 to declare significance.

Performance criteria
Because the organ weights of different strains of rats vary, absolute organ weights are not used as the performance criteria in the Hershberger assay. Rather, the performance criteria are based on the coefficient of variance (CV) for each organ. For negative outcomes, the CVs from the control and high dose group were examined to determine if the maximum CV performance was exceeded. To meet the performance criteria, the maximum allowable CV for each organ as set out in the test guideline was applied.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities. All animals survived until scheduled necropsy.

B. CLINICAL OBSERVATIONS

No test substance- or positive control-related clinical findings were noted. Single occurrences of very slight erythema, very slight edema, and slight edema were noted in animals receiving s.c. injection of TP. One animal with desquamation was noted in the TP positive control group.

C. BODY WEIGHT

No test substance-related effects on mean body weights or body weight changes were noted in the 100, 300, and 1000 mg/kg bw/day glyphosate-treated groups compared to the control group (see tables below). In the test substance-treated groups co-administered TP, mean body weights and body weight changes were similar to those of the TP control group. Mean body weights and body weight changes in the TP/FT group were similar to those of the TP control group throughout the study. In the TP control group, mean body weights and body weight changes were similar to those of the vehicle control group. Altogether, no statistically significant differences were noted.

Table 5.8.3-17: A Hershberger Assay of Glyphosate Administered Orally in Peripubertal Orchidoepididymectomized Rats [redacted] 2012): Body weight (bw) and cumulative body weight change in the androgen agonist assay in Sprague Dawley rats administered glyphosate via oral gavage for ten days

Body weight (g)										
Study group	**Control**		**Positive control TP**		**100 mg/kg bw/day**		**300 mg/kg bw/day**		**1000 mg/kg bw/day**	
Study day	**Mean**	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**
0	243.3	14.70	237.7	18.38	240.9	15.72	240.9	13.47	240.9	13.68
1	248.6	15.10	243.5	17.80	245.8	17.41	246.8	16.05	246.1	12.17
2	256.4	14.70	249.3	19.68	252.3	17.10	252.4	15.44	253.1	12.85
3	261.7	14.28	257.0	21.42	258.5	17.32	259.5	17.21	258.2	13.20
4	268.5	14.15	265.5	21.58	265.9	16.03	267.0	17.55	266.9	12.10
5	268.8	13.88	265.9	23.19	266.1	16.54	266.7	18.91	260.4	20.32
6	281.2	13.98	278.2	23.62	276.7	17.63	279.3	20.24	271.7	23.53
7	287.0	13.76	286.1	24.08	283.0	16.89	286.0	19.20	276.3	22.36
8	295.8	14.48	296.7	26.22	293.3	17.17	295.8	21.61	286.5	23.75
9	294.6	12.63	295.4	27.00	292.2	17.19	294.7	19.26	286.0	20.90
10	309.1	13.22	311.1	31.77	306.6	17.48	308.8	23.15	300.5	20.94
BW change Study day 0 - 10	**+65.8**	**6.86**	**+73.4**	**15.16**	**+65.7**	**5.88**	**+67.9**	**13.87**	**+59.6**	**13.21**

SD = standard deviation; TP = Testosterone propionate; n = 6 animals/group

Table 5.8.3-18: A Hershberger Assay of Glyphosate Administered Orally in Peripubertal Orchidoepididymectomized Rats (2012): Body weight and cumulative body weight (bw) change in the anti-androgen assay in Sprague Dawley rats administered glyphosate via oral gavage for ten days

Body weight (g)												
Study group	**Control**		**Negative Control TP**		**Positive Control FT + TP**		**100 mg/kg bw/day + TP**		**300 mg/kg bw/day + TP**		**1000 mg/kg bw/day + TP**	
Study day	**Mean**	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**
0	243.3	14.70	237.7	18.38	240.6	17.78	238.3	16.13	239.6	16.54	242.1	20.44
1	248.6	15.10	243.5	17.80	247.1	18.52	242.3	18.77	248.4	16.91	249.1	19.50
2	256.4	14.70	249.3	19.68	254.1	20.91	250.0	17.74	255.7	17.16	255.6	22.65
3	261.7	14.28	257.0	21.42	262.0	21.14	257.6	16.80	264.9	17.32	265.2	22.70
4	268.5	14.15	265.5	21.58	270.1	23.87	268.2	17.12	274.3	17.85	272.8	22.00
5	268.8	13.88	265.9	23.19	270.3	24.18	268.7	16.59	274.1	18.95	274.4	25.20
6	281.2	13.98	278.2	23.62	281.8	26.67	281.6	17.82	286.5	17.67	286.3	24.10
7	287.0	13.76	286.1	24.08	288.1	25.12	289.7	16.54	295.4	18.16	293.0	22.30
8	295.8	14.48	296.7	26.22	297.8	27.92	302.2	18.63	307.6	17.47	305.3	23.21
9	294.6	12.63	295.4	27.00	296.9	26.89	301.7	17.72	306.0	17.50	305.6	26.43
10	309.1	13.22	311.1	31.77	310.6	28.49	317.2	16.66	322.4	19.47	320.1	26.51
BW change Study day 0 - 10	**65.8**	**6.86**	**73.4**	**15.16**	**70.0**	**12.20**	**78.9**	**6.35**	**82.8**	**6.17**	**78.0**	**10.55**

TP = Testosterone propionate; FT = Flutamide; SD = standard deviation; n = 6 animals/group

D. ACCESSORY SEX ORGAN WEIGHTS

No evidence of androgenic agonism was noted in the 100, 300, or 1000 mg/kg bw/day glyphosate treated groups based on the lack of higher mean weights of the bulbourethral gland, glans penis, LABC muscle group, seminal vesicles/coagulating gland, and ventral prostate (see Table 5.8.3-19). Differences from the vehicle control group were slight and were not statistically significant.

No evidence of androgenic antagonism (anti-androgenic activity) was noted in the 100, 300, or 1000 mg/kg bw/day glyphosate treated groups co-administered TP (see Table 5.8.3-19). Slight differences of glyphosate +TP dose groups from the TP control group were not statistically significant. The androgenic positive control substance (concurrently the anti-androgenic negative control) TP elicited the expected response; statistically significant higher mean bulbourethral gland ($p < 0.0001$), glans penis ($p = 0.0007$), LABC muscle group ($p < 0.0001$), seminal vesicles/coagulating gland ($p < 0.0001$), and ventral prostate ($p < 0.0001$) weights compared to the vehicle control group. The anti-androgenic positive control FT inhibited the androgenic effect of co-administered TP, resulting in statistically significantly ($p \leq 0.0001$) decreased mean bulbourethral gland, glans penis, LABC muscle group, seminal vesicles/coagulating gland, and ventral prostate weights compared to the TP control group. All CV values were within the range of the assay's performance criteria.

Table 5.8.3-19: A Hershberger Assay of Glyphosate Administered Orally in Peripubertal Orchidoepididymectomized Rats (2012): Accessory sex organ weights of the androgen agonist assay in Sprague Dawley rats administered glyphosate via oral gavage for ten days

Organ	Dose (mg/kg bw /day)					
	Control			Positive control TP (0.2)		
	Mean (mg)	± SD	CV	Mean (mg)	± SD	CV
Bulbourethral	6.2	1.33	21.53	28.6**	6.35	22.17
Glans penis	105.0	15.92	15.16	152.1**	20.85	13.71
LABC	157.3	22.02	14.00	471.6**	63.13	13.39
Seminal vesicles with coagulating	86.4	12.06	13.96	463.6**	35.93	7.75
Ventral prostate	16.2	3.73	23.00	134.2**	22.20	16.54

Organ	Dose (mg/kg bw /day)								
	100			300			1000		
	Mean (mg)	± SD	CV	Mean (mg)	± SD	CV	Mean (mg)	± SD	CV
Bulbourethral	6.6	2.00	30.39	7.0	2.01	28.76	6.4	1.46	22.99
Glans penis	102.0	14.36	14.08	103.6	20.14	19.44	100.8	16.36	16.22
LABC	160.0	30.47	19.04	151.8	14.23	9.38	164.3	19.30	11.75
Seminal vesicles with coagulating	89.3	14.03	15.72	93.1	14.66	15.76	84.4	19.34	22.91
Ventral prostate	21.5	9.39	43.74	15.1	5.49	36.45	17.2	4.26	24.79

TP = Testosterone propionate; SD = Standard deviation; CV = Coefficient of variation; LABC = Levator ani and bulbocavernosus;
** = Significantly different from controls at p <0.01; n = 6 animals/ group

Table 5.8.3-20: A Hershberger Assay of Glyphosate Administered Orally in Peripubertal Orchidoepididymectomized Rats ([redacted] 2012): Accessory sex organ weights of the anti-androgen assay in Sprague Dawley rats administered glyphosate via oral gavage for ten days

Organ	Dose (mg/kg bw /day)								
	Control			Negative control TP (0.2)			Positive control FT + TP		
	Mean (mg)	± SD	CV	Mean (mg)	± SD	CV	Mean (mg)	± SD	CV
Bulbourethral	6.2	1.33	21.53	28.6**	6.35	22.17	8.7**	1.92	22.10
Glans penis	105.0	15.92	15.16	152.1*	20.85	13.71	108.3*	5.89	5.44
LABC	157.3	22.02	14.00	471.6*	63.13	13.39	173.9*	31.33	18.02
Seminal vesicles with coagulating	86.4	12.06	13.96	463.6**	35.93	7.75	111.9**	17.50	15.63
Ventral prostate	16.2	3.73	23.00	134.2*	22.20	16.54	27.0**	8.18	30.31

Organ	Dose (mg/kg bw /day)								
	100 + TP			300 + TP			1000 + TP		
	Mean (mg)	± SD	CV	Mean (mg)	± SD	CV	Mean (mg)	± SD	CV
Bulbourethral	31.1	3.12	10.03	28.5	9.22	32.40	29.9	4.95	16.57
Glans penis	150.6	17.86	11.86	148.8	23.69	15.92	168.2	15.42	9.17
LABC	405.4	49.04	12.10	461.0	92.12	19.98	471.8	83.07	17.61
Seminal vesicles with coagulating	411.0	71.92	17.50	417.5	99.47	23.82	391.6	75.25	19.22

Ventral prostate	112.2	18.51	16.50	110.6	26.48	23.95	125.3	25.30	20.19

TP = Testosterone propionate; FT = Flutamide; SD = Standard Deviation; CV = Coefficient of Variation; LABC = Levator ani and bulbocavernosus; ** = Significantly different from controls at p <0.01; n = 6 animals/ group

III. CONCLUSION

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study is performed in accordance with the current US EPA OPPTS/OCSPP 890.1400 (2009) and OECD 441 (2009). The study is therefore considered valid. Glyphosate did not have androgenic or anti-androgenic activity in the Hershberger assay at dose levels of 100, 300, or 1000 mg/kg bw/day. In the androgen agonist assay, the positive control TP elicited the appropriate responses of increased androgen-dependent organ weights. In the anti-androgen assay, the positive control FT inhibited TP induced increases in androgen-dependent organ weights when compared to the TP control group. Organ weights of glyphosate treated animals were not affected when compared to the appropriate control group. Glyphosate alone dose groups were not androgenic compared with the vehicle control group. Glyphosate + TP dose groups were not anti-androgenic compared with the TP alone anti-androgenic negative control group. Based on these results, glyphosate was negative for androgenicity and anti-androgenicity in the Hershberger assay.

Assessment and conclusion by RMS:

CA 5.8.3/007

1. Information on the study

Data point:	CA 5.8.3
Report author	[redacted]
Report year	2012
Report title	A Pubertal Development and Thyroid Function Assay of Glyphosate Administered Orally in Intact Juvenile/Peripubertal Male Rats
Report No	[redacted]-843005
Document No	Not reported
Guidelines followed in study	US EPA OPPTS/OCSPP 890.1500 (2009)
Deviations from current test guideline (OCSPP 890.1500, 2009)	Some values of the control group were outside the guideline performance criteria range (mean body weight at time of weaning (PND 21), CV value for body weight at attainment of PPS, CV value for mean TSH level, and mean thyroid gland and kidney weights). However, CV values of mean body weight (PND 21) and thyroid and kidney weights; mean body weight at the attainment of PPS and the mean/CV value for the age of attainment of PPS; and mean TSH value were in the acceptable guideline performance criteria range. Thus, these deviations were not considered to affect the interpretation of the results.

Previous evaluation	Yes, accepted in EFSA peer review on endocrine disrupting properties (2017).
GLP/Officially recognised testing facilities	Yes
Comment on GLP	None
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive summary

The objective of the present study was to assess the potential effects of glyphosate on the endocrine system, by identifying effects on pubertal development and thyroid function in the juvenile/peripubertal male rat. For this purpose, juvenile/peripubertal male Crl:CD(SD) Sprague Dawley rats (15 per dose group) were treated daily via oral gavage with glyphosate in 0.5 % methylcellulose in deionized water at doses of 0 (vehicle control), 100, 300, or 1000 mg/kg bw/day from postnatal day (PND) 23 to 53. Animals were observed twice daily for mortality and moribundity throughout the study period. Clinical observations and body weights were recorded daily. Animals were examined daily for preputial separation (PPS) beginning on PND 30. Age and weight at day of PPS were recorded. Following sacrifice on PND 53, blood was taken for total thyroxine (T4), testosterone, thyroid stimulating hormone (TSH), and clinical chemistry analysis. A gross necropsy was conducted for all animals and body and organ weights (epididymides, ventral prostate, dorsolateral prostate, adrenal glands, kidneys, liver, levator ani plus bulbocavernosus muscles, testes, pituitary, thyroid, seminal vesicle with coagulating gland) were recorded. Histopathological evaluation was performed on the thyroid, kidneys, testis and epididymides.

One male in the 1000 mg/kg bw/day dose group was found dead prior to dosing on PND 24. This death was considered treatment-related. All other males survived until scheduled sacrifice on PND 53. Rales were noted in a dose-related manner in the 300 and 1000 mg/kg bw/day dose groups approximately four hours post dosing throughout the treatment period. This finding persisted to the daily examinations for the 1000 mg/kg bw/day dose group. Terminal body weight was statistically significantly decreased for 300 and 1000 mg/kg bw/ day dose group animals (-6.63 % and -10.36 %, respectively) compared to control. Body weight change (PND 23 to 53) was significantly decreased in the 300 and 1000 mg/kg bw/day dose group (-7.5 % and -12.4 %, respectively), reaching statistical significance in the highest dose group compared to control. Clinical signs of toxicity together with clearly reduced body weight/ body weight change are indicative of overt systemic toxicity. At an EPA FIFIRA Scientific Advisory Panel in 2013, the panel was asked to comment on the validity of including treatments demonstrated overt systemic toxicity to assess effects of potential endocrine interaction. On the issue of overt systemic toxicity, the panel stated that little, if any, weight should be placed on signs of endocrine disruption in the presence of overt systemic toxicity. All effects in endocrine sensitive tissues should be evaluated in terms of primary interactions with the endocrine system versus secondary effects related to toxicity in non-endocrine organs or overall disruptions in homeostasis. Decreases in body weight gain approaching or above 10 % for the pubertal assays have been shown in the open literature to significantly confound the interpretation of the results of an adverse effect through an endocrine mechanism.

A delay in the mean age of attainment of preputial separation (not statistically significant), decreased reproductive organ weights (epididymides, seminal vesicle with coagulating gland, and ventral prostate) (statistically significant), and a decreased mean testosterone level (not statistically significant) were noted in the 1000 mg/kg bw/day dose group. These observations were considered treatment-related but were considered to be a secondary non-specific consequence of the described systemic toxicity indicated by lower mean body weight in this dose group. No treatment-related effects on organ weights were observed at lower dosages. T4 and TSH levels were lower than those in the control group in all treated groups and

testosterone was lower at 300 and 1000 mg/kg bw/day. However, these changes were not statistically significant and were not associated with any histopathological finding and were therefore not considered as compound-related. At 1000 mg/kg bw/day, the increases in ALT (also at 300 mg/kg bw/day), sodium, albumin, ALP, AST, chloride, phosphorous, and total protein, and the decrease in urea nitrogen were considered to be related to treatment and indicative for systemic toxicity. No adverse effects were noted on clinical pathology parameters at dosage levels of 100, 300, and 1000 mg/kg bw/day. No test substance-related macroscopic or microscopic findings were noted at any dose level.

There was no evidence of any glyphosate-related androgenic or anti-androgenic effects, nor was there any evidence of primary glyphosate-related effects on pubertal development or thyroid function in the juvenile/prepubertal male rat at dose levels of 100, 300, and 1000 mg/kg bw/day.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Identification:	Glyphosate acid
CAS No.:	1071-83-6
Description:	White powder
Lot/Batch No.:	GLP-1103-21149-T
Purity:	95.93 % glyphosate acid 85.14 % calculated glyphosate content
Stability of test compound:	Stable at room temperature
Vehicle:	Methylcellulose Source: Spectrum Chemical Manufacturing Corporation, New Brunswick, NJ, USA Lot/Batch No.: ZQ0344 CAS No.: 9004-67-5 Final concentration: 0.5 % in deionized water

Test animals:

Species:	Rat
Strain:	Crl:CD(SD) Sprague Dawley
Source:	[redacted]
Age at start of dosing:	23 days
Sex:	Male
Weight at dosing:	44.3 - 56.6 g
Acclimation period:	Time-mated female Crl:CD(SD) rats (43 females) were received from Charles River on 23 August 2011. Animals used in the present study were born at the testing facility. Weaning and study group assignment took place on PND 21.
Diet/Food:	Harlan Laboratories basal diet (2016*CM* Teklad Global, 16 % Protein Rodent Diet; 11.0 ppm of total isoflavones (genistein + daidzein + glycitein), *ad libitum*
Water:	Reverse osmosis-purified drinking water, *ad libitum*
Housing:	Dams and littermates in plastic maternity cages with nesting material until weaning on PND 21. After weaning, juveniles were housed two males per cage throughout the study.

Environmental conditions:	Temperature:	21.2 - 22.2 °C
	Humidity:	40.1 - 58.9 %
	Air changes:	10/h
	Photocycle:	14 hours light/ 10 hours dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 23 Aug 2011 delivery of pregnant rats; 29 Sep to 30 Oct 2011 test substance administration to male offspring

Animal assignment and treatment:
Offspring of time-mated female rats were weaned and weighed on PND 21. Animals were assigned using a WTDMS™ computer program which randomized the animals based on stratification of body weights in a block design. Littermates were not placed in the same group. The treatment groups included one vehicle control group and three glyphosate dose groups of 100, 300, and 1000 mg/kg bw/day.

All doses were administered once daily by oral gavage from PND 23 through PND 53, in a volume of 5.0 mL/kg bw. Dosing was performed daily, between 7:00 AM and 9:00 AM.

Table 5.8.3-21: A Pubertal Development and Thyroid Function Assay of Glyphosate Administered Orally in Intact Juvenile/Peripubertal Male Rats ([redacted] 2012): Study design of glyphosate administration to juvenile male Sprague Dawley rats

Test group	Dose (mg/kg bw/day)[1]	No. of males	Dosing volume (mL/kg bw)
Control (vehicle)	0	15	5
Low	100	15	5
Mid	300	15	5
High	1000	15	5

[1] A correction factor of 85.14 % was used to account for the active substance.

Dose Preparation and Analysis
The vehicle (0.5 % methylcellulose in deionized water) was prepared approximately weekly for administration to the control group and for preparation of the test substance formulations; aliquots were prepared for daily dispensation to the control group and stored at room temperature.

The test substance formulations were prepared approximately weekly as single formulations for each dosage level, divided into aliquots for daily dispensation, and stored at room temperature.

Analyses to demonstrate homogeneity, resuspension homogeneity for suspensions, and stability of the test substance formulations for up to 15 days at room temperature storage at concentrations of 1 and 200 mg/mL were conducted prior to the start of dose administration in a separate study ([redacted]-843004). Dosing formulations were analysed five times during the study (September 27, 2011 and October 4, 11, 18 and 25, 2011). Analysed concentrations ranged from 97.5 - 102 % of nominal for the low dose, 99.7 - 104 % of nominal for the mid dose, and 104 - 114 % of nominal for the high dose group. The analytical data indicated that the variation between nominal and actual dose to the animals was acceptable.

Mortality
All animals were examined twice daily, once in the morning and once in the afternoon, for mortality and moribundity.

Clinical observations
Clinical examinations were conducted on the day of randomization and daily through the day of necropsy. Animals were observed for signs of toxicity in the morning prior to test substance administration and

approximately 4 hours following test substance administration.

Body weight
Animals were weighed on the day of randomization, daily prior to test substance administration during the treatment period and on the day of necropsy.

Food consumption
Food consumption was not evaluated.

Preputial separation (PPS)
Beginning on PND 30, all animals were examined daily for onset of PPS. Age and body weight on the day at which complete PPS was observed was recorded for each animal. In addition, the appearance of a partial or complete PPS or a persistent thread of tissue between the glans and prepuce was recorded. If any animal within any group showed incomplete separation (persistent threads) for greater than three days, a separate second analysis was conducted using the age at which incomplete separation was first observed. Examination of the males was continued daily until complete PPS was attained or until euthanasia. When an animal did not attain PPS prior to necropsy, PND 54 (last study day + 1) and body weight at necropsy were used as the age and body weight at PPS, respectively.

Haematology and clinical chemistry
On PND 53 at least two hours post-dosing, rats were removed one at a time from their home cages to a separate room for euthanasia by decapitation without anaesthesia, to not induce stress-related responses in other animals, which may affect hormone measurements. All animals were euthanized by approximately 01:00 PM. Blood from the trunk of the animals was collected immediately into three serum separation tubes. One tube of serum samples was used for serum chemistry evaluation. The remaining two tubes of serum samples for each animal were used for T4, TSH, and testosterone analyses. Samples were analysed fresh or stored frozen (≤ -20 °C).

The following clinical chemistry parameters were examined: calcium, chloride, phosphorus, potassium, sodium, albumin, creatinine, urea nitrogen, total cholesterol, globulin (calculated), albumin/globulin ratio (calculated), glucose, total bilirubin, total protein, triglycerides, bile acids, alkaline phosphatase (ALP), alanine aminotransferase (ALT, also SGPT), aspartate aminotransferase (AST, also SGOT), gamma glutamyltransferase (GGT).

Testosterone and thyroxine (T4) concentrations were analysed using an electrochemiluminescent immunoassay. To determine the concentration of TSH in serum samples, a [^{125}I]rTSH kit was used.

Sacrifice and pathology
On PND 53 at least 2 hours post-dosing, rats were removed one at a time from their home cages to a separate room for euthanasia by decapitation without anaesthesia. Gross necropsy was performed for all animals. The necropsy included examination of the external surface, all orifices, the external surface of the brain and the abdominal, thoracic, and pelvic cavities, including viscera. Any tissues with gross lesions were saved in 10 % neutral-buffered formalin for possible future histopathologic examination. The following organs were collected and weighed: testes (left and right separately), epididymides (left and right separately), seminal vesicle with coagulating glands (with and without fluid), ventral prostate, dorsolateral prostate, levator ani plus bulbocavernosus (LABC) muscle complex, kidneys (paired), thyroid (paired, after fixation), liver, adrenal glands (paired), and pituitary gland. Subsequently, testes and epididymis were fixed in Bouin's solution overnight, then placed in 70 % ethanol until processing. The thyroid and kidneys were fixed in 10 % buffered formalin for at least 24 hours, then placed in 70 % ethanol until histopathological processing. Testes, epididymis, thyroid, and kidneys were routinely, histotechnically processed and haematoxylin and eosin stained slides were microscopically examined.

The thyroid sections (minimum of two sections) were subjectively evaluated for follicular epithelial height and colloid area and any abnormalities/ lesion were noted.

Performance criteria
Performance criteria as set out in the OCSPP test guideline 890.1500 were applied to evaluate measured values of the vehicle control group.

Statistics
Organ weights, organ weight to necropsy body weight ratio (liver, kidneys, pituitary, and adrenal glands), daily body weights (including necropsy body weight), cumulative body weight gain, serum hormones, serum chemistry parameters, and balanopreputial separation data each were tested for homogeneity of variance using Levene's test. For the purposes of this analysis, gamma glutamyltransferase values under range were assigned a value of 0.1 (half the lower limit of quantitation) for statistical analysis and reporting. Except for some daily body weight values and testosterone concentrations, all data were found to be homogeneous. For the non-homogenous data, a nonparametric test, as described below, was used to analyse the data.

When variances were homogeneous, an analysis of variance (ANOVA) was conducted on the raw data. The statistical model contained a factor for treatment group and a blocking factor based on the time of necropsy. A two-sided Dunnett's test was conducted using $p = 0.05$ to detect statistical significance, regardless of the outcome of the ANOVA, looking for significant differences in the test substance- treated groups when compared with the control group. For data that were not homogenous, the nonparametric Kruskal-Wallis test was used, ignoring the blocking factor, followed by Dunn's test, to compare each of the test substance-treated groups with the control group. The tests were two-sided at the 0.05 significance level, looking for significant differences from controls.

In addition, organ weights and balanopreputial separation data were subject to the following analyses: 1) Analysis of covariance (ANCOVA) with Dunnett's test. The model was as described above for ANOVA with the exception that PND 21 body weight was included as a covariate. 2) Linear trend test using the ANOVA model. 3) Linear trend test using the ANCOVA model.

Histopathology findings presented as a dichotomous response were analysed using pairwise Fisher's Exact tests to compare each treated group with the control. The tests were one-sided at the 0.05 significance level (testing for an increase). Histopathology findings presented as a graded response were analysed with pairwise Mann-Whitney U tests to compare each test substance-treated group with the control group. The tests were one-sided at the significance level testing for increased severity. The tests were two-sided for graded responses presented on a Grade 1-5 scale at the 0.05 significance level testing for increased or decreased severity. Exact p-values were calculated for the Mann-Whitney U test.

II. RESULTS AND DISCUSSION

A. MORTALITY
One animal of the 1000 mg/kg bw/day dose group was found dead in the morning on PND 24 (after one dose of glyphosate). Because clinical signs of toxicity and reduced body weight/ body weight change were dose-dependently observed in the 300 and 1000 mg/kg bw/day dose groups already following the first day of dosing, it cannot be excluded that this death was test substance-related. In addition, a death was observed in the 1000 mg/kg bw/day group in the 7-day pilot study. Thus, the unscheduled death observed at 1000 mg/kg bw/day was considered as test substance-related. All other males survived to scheduled necropsy.

B. CLINICAL OBSERVATIONS
A test substance-related clinical finding of rales was noted in a dose-related manner for males in the 300 and 1000 mg/kg bw/day dose groups approximately four hours post dosing (see table below). This condition persisted to the daily examinations primarily in the 1000 mg/kg bw/day dose group. This finding was noted on the first day of test substance administration, and continued sporadically throughout the treatment period until the day prior to necropsy. No other treatment-related clinical signs were noted in the dose groups

compared to control.

C. BODY WEIGHT

Terminal body weight was statistically significantly decreased for 300 and 1000 mg/kg bw/day dose group animals (-6.63 % and -10.36 %, respectively) compared to terminal control group body weight (see table below). Mean body weights were 5.07 % and 8.08 % lower in the 300 and 1000 mg/kg bw/day group than in the control group towards the end of the treatment period (PND 44-53), respectively whereas mean body weights for the 1000 mg/kg bw/day group were 5.09 % to 11.06 % lower generally throughout the treatment period (PND 24 - 53); differences were occasionally statistically significant at 1000 mg/kg bw/day and were statistically significant during PND 50 - 53 at 300 mg/kg bw/day. Body weight gain (PND 23 - 53) was significantly decreased in the 300 and 1000 mg/kg bw/day dose group (-7.5 % and -12.4 %, respectively), reaching statistical significance in the highest dose group, compared to control.

No test substance-related effects on mean body weights, daily body weight gains, or cumulative body weight gain were noted in the 100 mg/kg bw/day dose group.

The mean body weight value at the time of weaning (PND 21) in the control group (44.7 g) was slightly lower than the minimum value in the performance criteria (45.472 g); however, the coefficient of variation (CV) value was within the US EPA OPPTS 890.1500 guideline performance criteria range and the mean body weight value in the control group was similar to those in the test substance-treated groups on this day. Therefore, this out of range value was not considered to have affected interpretation of the body weight data.

D. PREPUTIAL SEPARATION

On the day of attainment of complete PPS, mean body weights in all treatment groups were similar to the control group value. For the mean age of attainment of complete PPS, a not statistically significant delay was observed in the 1000 mg/kg bw/day dose group (48.0 days) when compared to the control group (45.9 days) (see table below). A statistically significant linear trend (p ≤0.0178), which resulted from both the ANOVA (unadjusted value) and ANCOVA (adjusted value) models in the 1000 mg/kg/day dose group, was reported. When a second analysis was performed, adjusting the day of attainment for those males with three or more consecutive days of incomplete separation (persistent threads), no statistically significant delay in the mean age of attainment of preputial separation was noted for the 1000 mg/kg bw/day dose group when compared to the control group. There were 6, 11, 9, and 9 males in the control, 100, 300, and 1000 mg/kg bw/day dose group, respectively, with incomplete PSS for more than three days.

No test substance related effects were observed at doses ≤300 mg/kg bw/day.

The CV value for the body weight at the attainment of PPS in the control group (9.77 %) was above the maximum value in the performance criteria (7.57 %). However, the mean body weight value at the attainment of PPS and the mean and CV value for the age of attainment of PPS in the control group were within the acceptable US EPAOPPTS 890.1500 guideline performance criteria range.

It was previously demonstrated in the literature (Ashby and Lefevre, 2000[40]) that body weight differences of approximately 12 % can result in a delay of PPS of one to two days. The delay observed in the 1000 mg/kg/day group occurred at a dose that produced a 10.36 % lower final body weight. Thus, the observed reduced body weights of the 1000 mg/kg bw/day dose group may account for the delayed age at PPS, which would indicate rather a secondary than a primary treatment-related effect.

E. HAEMATOLOGY AND CLINICAL CHEMISTRY

Blood clinical chemistry

Several serum chemistry parameters were statistically significantly changed (see table below). Higher mean

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[40] Ashby J., & Lefevre, P.A. (2000). The peripubertal male rat assay as an alternative to the Hershberger castrated male rat assay for the detection of anti-androgens, oestrogens and metabolic modulators. *J. Appl. Toxicol*. 20:35-47.

alanine aminotransferase (ALT, 22.0 % and 50.8 %) and sodium (1.4 % and 2.1 %) levels were noted in the 300 and 1000 mg/kg bw/day groups, respectively, compared to control. Increased mean albumin (4.9 %), alkaline phosphatase (ALP, 22.4 %), aspartate aminotransferase (AST, 35.1 %), chloride (4.0 %), phosphorus (6.9 %), and total protein (5.1 %) levels were measured at 1000 mg/kg bw/day. Decreased (-16.9 %) urea nitrogen level was noted in the 1000 mg/kg bw/day group compared to the control group. The changes were of a degree not to be considered toxicologically significant. In addition, the lower urea nitrogen was in a direction opposite to that normally associated with toxicological effects, and therefore was considered to represent normal biologic variability. The elevated albumin, sodium, and chloride may suggest dehydration, which is a nonspecific sign of systemic toxicity.

No other statistically significant differences from the control group were noted.

None of the alterations was regarded to be adverse or indicative for specific (target organ) toxicity in addition to the reported general systemic toxicity (reduced body weight gain).

Hormone Levels

There were no test substance-related alterations in mean serum T4, TSH, and serum testosterone parameters compared to control (see table below). Mean serum T4 and TSH levels were decreased in all treatment groups compared to the control. However, none of the changes reached statistical significance and were not associated with any histological changes. TSH values were not dose-dependently changed. In addition, it was previously reported in literature (Laws *et al.*, 2007[41]; Trentacoste *et al.*, 2001[42]) that lower hormone levels may be due to lower body weights, and the association of lower hormone levels that are secondary to lower body weights have been reported in feed restricted rats during pubertal development.

The CV value for the mean TSH level in the control group (75.059 %) exceeded the maximum value in the performance criteria (58.29 %). However, the mean TSH value in the control group (8.30 ng/mL) was within the acceptable range in the US EPA OPPTS 890.1500 test guideline performance criteria (4.212 ng/mL to 24.112 ng/mL). Thus, this deviation was not considered to affect the final outcome of the study.

G. NECROPSY

Organ weights

Organ weights at necropsy are presented in the table below. Animals receiving 1000 mg/kg bw/ day glyphosate showed statistically reduced absolute liver (-15.1 %), pituitary gland (-15.6 %), LABC muscle (-15.9 %), ventral prostate (-22.6 %), and seminal vesicles with coagulating gland (-18.5 %, with fluid) weights, when compared to control. Animals receiving 300 mg/kg bw/ day glyphosate showed statistically reduced absolute liver (-9.8 %) and dorsolateral prostate (-14.3 %) weights, compared to control. Organ weight changes were considered secondary to reduced body weights, rather than indicative for specific target organ toxicity of the test substance, because no histopathological correlates were identified. Other statistically significant differences from the control group were not observed in a dose-related manner, and therefore, were not considered test substance- related.

The mean thyroid gland (13.73 mg) and kidney (1.93 g) weights in the control group were below the minimum values (14 mg and 2.242 g, respectively) of the US EPA OPPTS 890.1500 test guideline performance criteria; however, the CV values were within the acceptable range. These deviations are not considered to affect the final outcome or validity of the present study.

Gross pathology

At scheduled necropsy, no test substance-related macroscopic findings were noted for treated animals, compared to control.

[41] Laws S.C., Stoker, T.E., Ferrell, J.M., *et al.* (2007). Effects of altered food intake during pubertal development in male and female Wistar rats. *Toxicol. Sci.* 100:194-202.

[42] Trentacoste, S.V., Friedmann, A.S., Youker, R.T., *et al.* (2001). Atrazine effects on testosterone levels and androgen-dependent reproductive organs in peripubertal male rats. *J Androl.* 22:142-148.

Histopathology
There were no test substance-related changes in the thyroid gland, epididymides, testes, or kidney. Specifically, histologic examination of testes and epididymides revealed no alteration in the tissue structure, degree of spermatogenesis (testes), or number of spermatids. With regard to thyroid gland, there was no difference in height of follicular epithelium or amount or nature of colloid within the individual follicles. All histologic changes were considered to be incidental findings.

Table 5.8.3-22: A Pubertal Development and Thyroid Function Assay of Glyphosate Administered Orally in Intact Juvenile/Peripubertal Male Rats [redacted] 2012): Incidence of clinical observations

Observation	Dose level (mg/kg bw/day)			
	Control	100	300	1000
Rales [1]	0 / 15	0 / 15	9 / 15	14 / 14

[1] Number animals with observation / number animals examined

Table 5.8.3-23: A Pubertal Development and Thyroid Function Assay of Glyphosate Administered Orally in Intact Juvenile/Peripubertal Male Rats ([redacted] 2012): Body weight and cumulative body weight (bw) change in male Sprague Dawley rats exposed to glyphosate from PND 23 to PND 53 - selected timepoints

Body weight (g)								
Dose (mg/kg bw/day)	**Control**		**100**		**300**		**1000**	
PND	**Mean**	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**
23	51.7	2.48	50.8	3.38	50.3	3.42	50.8	2.91
30	89.2	4.64	91.1	7.45	88.5	6.79	82.9*	8.36
37	141.6	7.21	145.5	13.56	139.0	9.88	134.2	12.34
44	201.3	11.23	203.1	21.80	191.1	15.67	189.0	15.96
51	259.8	12.99	261.0	25.08	238.8	19.70	232.8*	23.35
53	273.1	14.02	276.3	25.37	255.0*	22.03	244.8*	23.18
BW change PND 23 - 53	-	-	-	-	-7.5 %	-	-12.4* %	-

PND = Postnatal day; SD = Standard deviation; * Significantly different from control at $p < 0.05$; BW = Body weight

Table 5.8.3-24: A Pubertal Development and Thyroid Function Assay of Glyphosate Administered Orally in Intact Juvenile/Peripubertal Male Rats ([redacted] 2012): General growth and preputial separation (PPS)

Parameter evaluated		Vehicle Control				100 mg/kg bw/day				300 mg/kg bw/day				1000 mg/kg bw/day			
		n	Mean	±SD	CV	n	Mean	±SD	CV	n	Mean	±SD	CV	n	Mean	±SD	CV
Initial body weight (PND 23; g)	U	15	51.7	2.48	4.79	15	50.8	3.38	6.66	15	50.3	3.42	6.79	15	50.8	2.91	5.73
Body weight at PPS (g)	U	15	216.8	21.18	9.77	15	227.1	22.63	9.96	15	213.1	18.41	8.64	14	214.6	26.24	12.23
	A	15	216.5	21.18	9.77	15	226.9	22.63	9.96	15	213.1	18.41	8.64	14	214.9	26.24	12.23
Final body weight (g)	U	15	273.1	14.02	5.14	15	276.3	25.37	9.18	15	255.0*	22.03	8.64	14	244.8*	23.18	9.47
Final body weight (% of control)	U	-	-	-	-	-	1.17	-		-	-6.63	-	-	-	-10.36	-	-
Body weight gain (final – initial; g)	U	15	221.4	13.95	6.30	15	225.6	24.35	10.79	15	204.7	19.84	9.69	14	194.0*	21.59	11.13
	A	15	-	-	-	15	-	-	-	15	-	-	-	14	-	-	-
Age at PPS (PND)	U	15	45.9	2.17	4.72	15	46.9	2.42	5.16	15	47.4	3.07	6.47	14	48.0	1.66	3.47
	A	15	45.9	2.17	4.72	15	46.8	2.42	5.16	15	47.5	3.07	6.47	14	47.9	1.66	3.47
Proportion unseparated (#/n)		0/15				0/15				1/15				0/14			

n = number of animals per group; SD = Standard deviation; CV = Coefficient of variation; PND = Postnatal day; U = Unadjusted for body weight on PND 23; A = Adjusted for body weight on PND 23; * Significantly different from control at p <0.05

Table 5.8.3-25: A Pubertal Development and Thyroid Function Assay of Glyphosate Administered Orally in Intact Juvenile/Peripubertal Male Rats ([redacted] 2012): Clinical chemistry – select parameters

	Vehicle (n=15)			100 mg/kg bw/day (n=15)			300 mg/kg bw/day (n=15)			1000 mg/kg bw/day (n=14)		
Parameter	**Mean**	**±SD**	**CV**	**Mean**	**±SD**	**CV**	**Mean**	**±SD**	**CV**	**Mean**	**±SD**	**CV**
Albumin (g/dL)	4.1	0.12	3.05	4.1	0.14	3.27	4.1	0.18	4.35	4.3*	0.22	5.16
ALP (U/L)	277	36.8	13.3	276	34.5	12.5	287	59.6	20.8	339*	63.6	18.7
ALT (U/L)	59	7.7	13.0	66	8.7	13.2	72*	13.4	18.4	89*	23.1	26.0
AST (U/L)	111	17.1	15.4	129	21.7	16.8	131	28.6	21.9	150*	59.7	39.7
Chloride (mEq/L)	100	1.5	1.5	100	1.2	1.2	101	1.1	1.1	104*	2.4	2.3
Phosphorus (mg/dL)	10.1	0.61	6.03	10.3	0.61	5.90	10.4	0.70	6.77	10.8*	0.50	4.64
Sodium (mEq/L)	142	1.3	0.9	143	1.7	1.2	144*	1.3	0.9	145*	2.1	1.5
Total protein (g/dL)	5.9	0.21	3.57	6.0	0.21	3.47	6.0	0.21	3.49	6.2*	0.26	4.15
Urea nitrogen (mg/dL)	15.4	1.57	10.19	14.7	1.98	13.46	14.0	2.27	16.17	12.8*	2.36	18.43

SD = Standard deviation; CV = Coefficient of variation; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase;
* Significantly different from control at $p < 0.05$

Table 5.8.3-26: A Pubertal Development and Thyroid Function Assay of Glyphosate Administered Orally in Intact Juvenile/Peripubertal Male Rats ([redacted] 2012): Serum hormone concentrations

	Vehicle (n=15)			100 mg/kg bw/day (n=15)			300 mg/kg bw/day (n=15)			1000 mg/kg bw/day (n=14)		
Parameter	**Mean**	**±SD**	**CV**	**Mean**	**±SD**	**CV**	**Mean**	**±SD**	**CV**	**Mean**	**±SD**	**CV**
Serum T4, total (µg/dL)	6.22	1.076	17.297	6.02	0.425	7.058	5.92	0.874	14.771	5.68	0.736	12.956
Serum TSH, (ng/mL)	8.30	6.230	75.059	6.77	3.728	55.039	6.91	2.816	40.771	5.37	3.396	63.224
Serum testosterone, total (ng/mL)	2.860	1.5697	54.885 1	3.973	3.2270	81.216 4	2.313	1.5352	66.364 7	1.571	0.7966	50.690 1

n = number animals per group; SD = Standard deviation; CV = Coefficient of variation; T4 = Thyroxine; TSH = Thyroid stimulating hormone

Table 5.8.3-27: A Pubertal Development and Thyroid Function Assay of Glyphosate Administered Orally in Intact Juvenile/Peripubertal Male Rats ([redacted] 2012): Organ weights – selected tissues

Organ		Control (n=15)			100 mg/kg bw/day (n=15)			300 mg/kg bw/day (n=15)			1000 mg/kg bw/day (n=14)		
		Mean	±SD	CV	Mean	±SD	CV	Mean	±SD	CV	Mean	±SD	CV
Adrenal glands (mg)	U	45.6	7.28	15.97	46.8	6.76	14.45	41.7	6.39	15.35	41.4	5.02	12.12
	A	45.4	7.28	15.97	46.9	6.76	14.45	41.5	6.39	15.35	41.7	5.02	12.12
Dorsolateral prostate (mg)	U	122.8	17.59	14.32	115.3	21.53	18.68	105.3*	15.41	14.64	112.2	18.87	16.81
	A	122.4	17.59	14.32	115.6	21.53	18.68	105.0*	15.41	14.64	113.0	18.87	16.81
Epididymis (left) (mg)	U	201.9	19.02	9.42	199.4	18.61	9.33	187.5	29.15	15.54	182.0	23.05	12.67
	A	201.2	19.02	9.42	199.3	18.61	9.33	187.2	29.15	15.54	183.0	23.05	12.67
Epididymis (right) (mg)	U	201.1	21.54	10.71	201.0	15.32	7.62	193.4	30.89	15.97	182.0	17.21	9.45
	A	200.2	21.54	10.71	201.2	15.32	7.62	192.9	30.89	15.97	183.4	17.21	9.45
LABC muscle group (mg)	U	539.6	61.50	11.40	527.8	107.65	20.40	491.4	97.15	19.77	453.9*	67.03	14.77
	A	537.9	61.50	11.40	528.9	107.65	20.40	490.0	97.15	19.77	457.2*	67.03	14.77
Liver (g)	U	12.69	1.064	8.384	13.09	1.563	11.937	11.45*	1.511	13.200	10.77*	1.506	13.985
	A	12.67	1.064	8.384	13.12	1.563	11.937	11.42*	1.511	13.200	10.82*	1.506	13.985
Pituitary (mg)	U	10.9	1.14	10.40	10.5	1.50	14.27	10.0	1.36	13.62	9.2*	1.71	18.59
	A	10.9	1.14	10.40	10.5	1.50	14.27	9.9	1.36	13.62	9.2*	1.71	18.59
Seminal vesicles w/ CG, w/o fluid (mg)	U	387.5	44.74	11.55	387.5	67.84	17.51	344.9	59.80	17.34	323.8*	52.43	16.19
	A	385.9	44.74	11.55	388.0	67.84	17.51	344.9	59.80	17.34	326.5*	52.43	16.19
Seminal vesicles w/ CG, w/ fluid (mg)	U	630.0	106.07	16.84	619.8	140.86	22.73	545.5	136.83	25.08	513.2*	88.22	17.19
	A	627.1	106.07	16.84	621.2	140.86	22.73	543.3	136.83	25.08	518.6*	88.22	17.19
Ventral prostate (mg)	U	257.5	37.75	14.66	264.0	61.11	23.14	236.3	44.33	18.76	199.2*	46.04	23.11
	A	256.5	37.75	14.66	264.8	61.11	23.14	235.4	44.33	18.76	201.2*	46.04	23.11

n = number of animals per group; SD = Standard deviation; CV = Coefficient of Variation; U = Unadjusted for body weight on postnatal day (PND) 23; A = Adjusted for body weight on PND 23; LABC = levator ani plus bulbocavernosus; * Significantly different from control at $p < 0.05$; w/ = with; w/o = without

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is performed in accordance with the current US EPA OPPTS/OCSPP 890.1500 (2009) where the following deviations were noted. Some values of the control group were outside the guideline performance criteria range (mean body weight at time of weaning (PND 21), CV value for body weight at attainment of PPS, CV value for mean TSH level, and mean thyroid gland and kidney weights). However, CV values of mean body weight (PND 21) and thyroid and kidney weights; mean body weight at the attainment of PPS and the mean/CV value for the age of attainment of PPS; and mean TSH value were in the acceptable guideline performance criteria range. Thus, these deviations were not considered to affect the interpretation of the results. The study is therefore considered valid. A delay in the mean age at attainment of balanopreputial separation, lower reproductive organ weights (epididymides, seminal vesicle with coagulating gland, and ventral prostate), and a lower mean testosterone level were noted in the 1000 mg/kg bw/day group. These changes at the limit dose were considered to be a secondary consequence of overt toxicity, noted by 10.36 % lower mean body weights and 12.4 % reduced mean body weight gain during the dosing period in this group versus the control group. Thus, there was no evidence of any direct glyphosate-related androgenic or anti-androgenic effects, nor was there any evidence of direct glyphosate-related effects on pubertal development or thyroid function in the juvenile/ peripubertal male rat administered glyphosate up to the limit dose of 1000 mg/kg bw/day.

Assessment and conclusion by RMS:

CA 5.8.3/008

1. Information on the study

Data point:	CA 5.8.3
Report author	[redacted]
Report year	2012
Report title	A Pubertal Development and Thyroid Function Assay of Glyphosate Administered Orally in Intact Juvenile/Peripubertal Female Rats
Report No	[redacted]-843007
Document No	Not reported
Guidelines followed in study	US EPA OPPTS/OCSPP 890.1450 (2009)
Deviations from current test guideline (OCSPP 890.1450, 2009)	The mean age of vaginal opening attainment in the control group (PND 36.4) was higher than the acceptable range of values in the performance criteria (PND 30.67 to PND 35.62). However, the coefficient of variation (CV) value (6.52) fell within the acceptable range (0.00 - 6.52), and thus, met the acceptance criteria. The weights of adrenal glands of control animals (adjusted and unadjusted: 33.3 and 33.2, respectively) were slightly lower than the acceptable range (38.34 - 48.84 mg). These deviations were not considered to affect the validity or final outcome of the study.

Previous evaluation	Yes, accepted in EFSA peer review on endocrine disrupting properties (2017).
GLP/Officially recognised testing facilities	Yes
Comment on GLP	None
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive summary

The objective of the study was to assess the potential effects of glyphosate on the endocrine system, by identifying effects on pubertal development and thyroid function in the juvenile/peripubertal female rat. For this purpose, 15 juvenile/peripubertal female Crl:CD(SD) Sprague Dawley rats per dose group were treated daily via oral gavage with glyphosate (in 0.5 % methylcellulose in deionized water) at doses of 0 (vehicle control), 100, 300, or the limit dose of 1000 mg/kg bw/day from postnatal day (PND) 22 to 42.

All females were observed twice daily for mortality and moribundity throughout the study. Clinical observations and body weights were recorded daily. All females were observed daily for vaginal opening. Once vaginal opening was observed, daily vaginal lavages were performed for each female to determine the stage of the estrous cycle. A complete necropsy was conducted on all rats euthanized in extremis or that survived to the scheduled euthanasia on PND 42. Adrenal glands, kidneys, liver, ovaries, pituitary gland, and thyroid were weighed and preserved at scheduled necropsy. Hormone (T4 and TSH) and clinical pathology evaluations (serum chemistry) were conducted on all surviving animals on PND 42. In addition, histopathological evaluation of the thyroid, kidney, ovary, and uterus was performed.

All treated females survived until terminal sacrifice. One female in the control group was found to have an impaired right forelimb on PND 27 and was sacrificed *in extremis*. Test substance-related rales were noted in a dose-related manner in the 300 and 1000 mg/kg bw/day dose groups. Rales were not noted in the control or the 100 mg/kg bw/day dose groups. Mean body weight and body weight change were unaffected by treatment. There were no treatment-related effects noted on the mean age of attainment of vaginal opening or body weight on the day of attainment. Mean estrous cycle lengths, estrous cyclicity, and age at the first occurrence of estrus were unaffected by treatment. There were no treatment-related changes in serum chemistry, organ weights or serum hormone levels (T4 and TSH). No test substance-related macroscopic or microscopic findings were noted at any dosage level compared to control.

There was no evidence of any glyphosate-related estrogenic or anti-estrogenic effect, nor was there any evidence of direct glyphosate-related effects on pubertal development or thyroid function in the juvenile/peripubertal female rat following administration of glyphosate at doses of 100, 300, or 1000 mg/kg bw/day.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Identification:	Glyphosate acid
CAS No.:	1071-83-6
Description:	White powder

Lot/Batch No.:	GLP-1103-21149-T
Purity:	95.93 % glyphosate acid 85.14 % calculated glyphosate content
Stability of test compound:	Stable at room temperature
Vehicle:	Methylcellulose Source: Spectrum Chemical Manufacturing Corporation, New Brunswick, NJ, USA Lot/Batch No.: ZQ0344 CAS No.: 9004-67-5 Final concentration: 0.5 % in deionized water
Test animals:	
Species:	Rat
Strain:	Crl:CD(SD) Sprague Dawley
Source:	[redacted]
Age at start of dosing:	22 days
Sex:	Female
Weight at dosing:	41.4 - 51.3 g
Acclimation period:	Time-mated female Crl:CD(SD) Sprague Dawley rats (43 females) were received from Charles River on 23 August 2011. The females were used to obtain the juvenile females for the present study. Hence, animals of the present study were born at the testing facility. Weaning took place on PND 21. Study allocation took place on PND 22.
Diet/Food:	Harlan Laboratories basal diet (2016*CM* Teklad Global, 16 % Protein Rodent Diet; 11.0 ppm of total isoflavones (genistein + daidzein + glycitein), *ad libitum*
Water:	Reverse-osmosis filtered tap water, *ad libitum*
Housing:	Dams and littermates in plastic maternity cages with nesting material until weaning on PND 21. After weaning, juveniles were housed two to three females per cage throughout the study.
Environmental conditions:	Temperature: 21.4 - 22.1 °C Humidity: 41.2 - 58.9 % Air changes: 10/h Photocycle: 14 hours light/ 10 hours dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 23 Aug 2011 delivery of pregnant rats; 28 Sep to 19 Oct 2011 test substance administration to female offspring

Animal assignment and treatment

Offspring of time-mated female rats were weaned and weighed on PND 21. Animals were assigned to study groups using a WTDMS™ computer program, which randomised the animals based on stratification of body weights in a block design. Littermates were not assigned to the same treatment group. The treatment groups included one vehicle control group and three glyphosate treatment groups at doses of 100, 300, and 1000 mg/kg bw/day.

All doses were administered once daily by oral gavage, from PND 22 through PND 42, in a volume of 5 mL/kg bw. Dosing was performed daily between 7:00 AM and 9:00 AM.

Table 5.8.3-28: A Pubertal Development and Thyroid Function Assay of Glyphosate Administered Orally in Intact Juvenile/Peripubertal Female Rats ([redacted] 2012): Study design of pubertal development and thyroid function assay with glyphosate, administered orally to female Sprague Dawley rats

Test group	Dose (mg/kg bw/day)[1]	No. of females	Dosing volume (mL/kg bw/day)
Control (vehicle)	0	15	5
Low	100	15	5
Mid	300	15	5
High	1000	15	5

[1] A correction factor of 85.14 % was used to account for the active substance.

Dose Preparation and Analysis
The vehicle was prepared approximately weekly for administration to the control group and for preparation of the test substance formulations; aliquots were prepared for daily dispensation to the control group and stored at room temperature. The vehicle was mixed throughout the preparation, sampling, and dose administration procedures. The test substance formulations were prepared approximately weekly as single formulations for each dosage level by mixing appropriate amounts of test substance with 0.5 % methylcellulose in deionized water, divided into aliquots for daily dispensation, and stored at room temperature. The test substance formulations were continuously stirred throughout the preparation, sampling, and dose administration procedures.

Analyses to demonstrate homogeneity, resuspension homogeneity for suspensions, and stability of the test substance formulations for up to 15 days of room temperature storage at concentrations of 1 and 200 mg/mL were conducted prior to the start of dose administration in a separate study ([redacted]-843004). During the present study, samples of dose formulations were analysed three times (on September 27, October 4, and October 11, 2011). Analysed concentrations ranged from 98.9 - 100 % of nominal for the low dose, 102 - 105 % of nominal for the mid-dose, and 104 - 112 % of nominal for the high dose. The analytical data indicated that the variation between nominal and actual dose to the animals was acceptable.

Mortality
All animals were examined twice daily, once in the morning and once in the afternoon, for mortality and moribundity.

Clinical observations
Clinical examinations were conducted on the day of randomization and daily (prior to test substance administration) through the day of scheduled necropsy. Animals were also observed for signs of toxicity approximately four hours following dose administration.

Body weight
Animals were weighed on the day of randomization, daily prior to test substance administration during the treatment period, and on the day of scheduled necropsy.

Food consumption
Food consumption was not evaluated.

Vaginal opening
Beginning on PND 22, all animals were examined daily for onset of vaginal opening. In addition, the appearance of a small "pin hole", a vaginal thread, and complete vaginal opening were recorded. The age and body weight on the day at which the vaginal lumen was first observed to open was recorded for each animal. If any animal within any group showed incomplete opening (persistent pin holes and/or threads) for greater than three days, a separate second analysis was conducted using the age at which incomplete

opening was first observed. Examination of the females was continued daily until vaginal opening was present or until euthanasia.

Estrous cyclicity
Beginning on the day of vaginal opening, up to and including the day of necropsy, daily vaginal lavages were performed. Slides were evaluated microscopically to determine the stage of the estrous cycle (proestrus [P], estrus [E], metestrus [M], diestrus [D]) of each female. The age at first vaginal estrus after vaginal opening was recorded. Females that had no E or P during the evaluation period were assigned an age of 43 days (last study day + 1 day). If a female had no E, but had a P during the evaluation period, the age at first estrus was considered the age on the day of P +1 day. The average cycle length was calculated and reported for complete estrous cycles (i.e. the total number of returns to M or D from E or P). Estrous cycle length was determined by counting the number of days from the first M or D in a cycle to the first M or D in a subsequent cycle.

At the end of the study, the overall pattern for each female was characterised as regularly cycling, irregularly cycling, not cycling, or insufficient data; based on predefined established definitions for these terms. For a "complete cycle", an animal must have exhibited at minimum an E from the previous cycle, followed by one cycle (D to E), and a D from the start of the next cycle. The "percent cycling" and "percent cycling regularly" was calculated.

Haematology and clinical chemistry
On PND 42 approximately 2 h post-dosing (approximately 01:00 PM), rats were removed one at a time from their home cages to a separate room for euthanasia by decapitation without anaesthesia so as not to induce stress-related responses or diurnal fluctuations, which may affect hormone measurements. Blood from the trunk of the animals was collected immediately into three serum separation tubes. One tube of serum samples was used for serum chemistry evaluation. The remaining two tubes of serum samples for each animal were used for total thyroxine (T4) and thyroid stimulating hormone (TSH) analyses. Samples were analysed fresh or stored frozen (≤ -20 °C).
The following clinical chemistry parameters were examined: calcium, chloride, phosphorus, potassium, sodium, albumin, creatinine, urea nitrogen, total cholesterol, globulin (calculated), albumin/globulin ratio (calculated), glucose, total bilirubin, total protein, triglycerides, bile acids, alkaline phosphatase (ALP), alanine aminotransferase (ALT, also SGPT), aspartate aminotransferase (AST, also SGOT), gamma glutamyltransferase (GGT).

T4 and TSH levels were analysed using an electrochemiluminescent immunoassay and (^{125}I)rTSH kit, respectively.

Sacrifice and pathology
On PND 42 approximately 2 h post-dosing (at approximately 01:00 PM), rats were removed one at a time from their home cages to a separate room for euthanasia by decapitation without anaesthesia so as not to induce stress-related responses or diurnal fluctuations which may affect hormone measurements. The necropsy was conducted in a randomized manner and included examination of the external surface, all orifices, the external surface of the brain and the abdominal, thoracic, and pelvic cavities, including viscera. Any tissues with gross lesions were saved in 10 % neutral-buffered formalin for possible future histopathologic examination. The following tissues were collected and weighed: ovaries (paired, without oviducts), uterus with cervix (both "wet" with luminal fluid and "blotted" following removal of luminal fluid), thyroid (weighed after fixation), adrenal glands (paired), pituitary, liver, and kidneys (paired). Additionally, the ovaries, uterus, thyroid and kidneys were fixed in 10 % buffered formalin (ovaries and uterus stored in 70 % ethanol prior to embedding) and subjected to histological processing and examination.

The thyroid sections (minimum of two sections) were subjectively evaluated for follicular epithelial height and colloid area and any abnormalities/lesions were noted. Five random sections from both ovaries were evaluated for follicular development and any abnormalities/lesions. The stage of the estrous cycle of a

female at the time of necropsy was taken into account in the final histological assessment.

Statistics
Organ weights, organ weight to necropsy body weight ratio (liver, kidneys, pituitary, and adrenal glands), daily body weights (including necropsy body weight), cumulative body weight gain, serum chemistries, serum hormones and vaginal opening data were each was tested for homogeneity of variance using Levene's test. For the purposes of this analysis, gamma glutamyltransferase (GGT) values under range were assigned a value of 0.1 (half the lower limit of quantitation) for statistical analysis and reporting. Except for GGT concentrations, homogeneity of variance was confirmed for each endpoint ($p > 0.01$); AST concentrations required log transformation to demonstrate homogeneity.

An ANOVA was conducted on the raw or log-transformed homogenous data. The statistical model contained a factor for treatment group and a blocking factor based on the time of necropsy. A two-sided Dunnett's test was conducted, regardless of the outcome of the ANOVA, looking for significant differences in the test substance-treated groups when compared with the control group. For the GGT data, a nonparametric Kruskal-Wallis test was used, ignoring the blocking factor, followed by Dunn's test, to compare each of the test substance-treated groups with the control group. The tests were two-sided at the 0.05 significance level, looking for significant differences from controls.

In addition, organ weights and vaginal opening data were subject to the following analyses: 1) Analysis of covariance (ANCOVA) with Dunnett's test. The model was as described above for ANOVA with the exception that PND 21 body weight was included as a covariate; 2) Linear trend test using the ANOVA model; and 3) Linear trend test using the ANCOVA model.

Chi-square analysis was used to determine significant difference between cycling status (cycling vs. not cycling) and percent of animals cycling regularly of the test substance-treated groups from the control group. Estrous cycle length and the day of first estrus were subjected to a parametric one-way ANOVA to determine intergroup differences. If the ANOVA revealed significant ($p < 0.05$) intergroup variance, Dunnett's test was used to compare the test substance-treated groups to the control group.

Histopathology findings presenting as a dichotomous response were analysed with pairwise Fisher's exact tests to compare each test substance-treated group with the control group. The tests were one-sided at the 0.05 significance level testing for an increase. Histopathology findings presented as a graded response were analysed with pairwise Mann-Whitney U tests to compare each treated group with the control. The tests were one-sided at the 0.05 significance level (testing for an increase). Tests were two-sided for graded responses presented on a grade 1-5 scale at 0.05 significance level testing for increased or decreased severity. Exact p-values were calculated for the Mann-Whitney U test.

II. RESULTS AND DISCUSSION

A. MORTALITY
All treated females survived until scheduled necropsy. Following dosing on PND 27, one control female was noted with impaired use of the right forelimb due to a possible mechanical injury. This female was subsequently euthanized *in extremis*.

B. CLINICAL OBSERVATIONS
Rales were noted for 4 and 13 females in the 300 and 1000 mg/kg bw/day dose group, respectively (see table below). They were observed approximately four hours following dose administration throughout the treatment period, and were considered treatment-related. Rales were neither observed in the control nor in the 100 mg/kg bw/day dose group. No other clinical signs were noted in the treatment groups compared to controls.

Table 5.8.3-29: A Pubertal Development and Thyroid Function Assay of Glyphosate

Administered Orally in Intact Juvenile/Peripubertal Female Rats ([redacted] 2012): Incidence of Clinical Observations

Observation	Dose Level (mg/kg bw/day)			
	Control	100	300	1000
Rales [1]	0 / 15	0 / 15	4 / 15	13 / 15

[1] Number animals with observation / number animals examined

C. BODY WEIGHT

No treatment-related effects on mean body weights or body weight changes were noted in the 100, 300, or 1000 mg/kg bw/day group when compared to the vehicle control group (see table below).

D. VAGINAL OPENING

Mean body weight and mean age at day of attainment of vaginal opening was not affected by treatment with 100, 300, or 1000 mg/kg bw/day glyphosate (see table below).

The mean age of vaginal opening attainment in the control group (PND 36.4) was higher than the acceptable range of values in the performance criteria (PND 30.67 to PND 35.62; US EPA OPPTS 890.1450). However, the coefficient of variation (CV) value (6.52) fell within the acceptable range (0.00 - 6.52), and thus, met the acceptance criteria. The increased mean age of vaginal opening of the control group was not considered to impact the validity or final outcome of the study.

E. ESTROUS CYCLICITY

Mean estrous cycle lengths in the 100, 300, and 1000 mg/kg bw/day treated groups (4.8, 5.0, and 5.0 days, respectively) were similar to control group values (5.0 days) (see table below). No statistically significant differences in the age at the first occurrence of estrus were observed when the glyphosate-treated groups were compared to the control group. The percentage of regularly cycling females in the 300 and 1000 mg/kg bw/day groups was significantly ($p < 0.01$ or $p < 0.05$) lower than the control group. However, a large number of females (up to ten per group) in the 300 and 1000 mg/kg bw/day groups had insufficient estrous cycle data (the animal did not display at least one complete estrous cycle but had at least one E and/or P and a partial cycle of five days or fewer, no E present on any days of estrous cycle determination and four or fewer days of data collected, or at least one E or P present and only one to four days of cycle data collection) that resulted in a limited number of females (five per group) available for evaluation of estrous cyclicity. These lower percentages did not occur in a dose-related manner and the percentage of females cycling in these groups was not statistically significantly different from the control group. Therefore, these lower values were not considered test substance-related.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Blood clinical chemistry

There were no treatment-related changes in serum chemistry parameters that were considered adverse (see table below). The following statistically significant differences from control were observed: decreased AST levels in all three dose groups, decreased potassium levels in the 100 and 300 mg/kg bw/day dose groups, increased chloride and ALP levels in the 1000 mg/kg bw/day dose group, and decreased phosphorus levels in the 100 mg/kg bw/day dose group when compared to concurrent control. The changes of the ALP and chloride values in the 1000 mg/kg bw/day dose group were considered test substance-related because the elevations were limited to the high dose group. However, they were considered of no biological relevance due to the absence of any histological correlate in the organs examined, including liver. In addition, the mean chloride value at 1000 mg/kg bw/day was within the range of historical control data of the testing laboratory for female Sprague Dawley rats at 6 - 8 weeks of age. The changes in AST involved a decrease of normal levels, which is not usually associated with toxicological effects, and therefore the change was considered spurious and unrelated to the test substance. The decreased potassium and phosphorus levels occurred without a dose response-relationship and were considered to be of no biological relevance. Mean

group potassium values, including the control group, were above the historical control data range of 3.97 - 5.94 mEq/L. However, this observation is considered to not influence the final outcome of the study.

Hormone Levels
There were no treatment-related alterations in thyroid hormone parameters at any dose level tested (see table below). The T4 levels in the control group and the corresponding CV were within the US EPA OPPTS 890.1450 performance criteria.

G. NECROPSY
Organ weights
There were no test substance-related changes in weights of the ovaries (paired, without oviducts), uterus, kidneys (paired), thyroid, liver, adrenal glands (paired), and pituitary (see table below). The organ weights of the control animals met the performance criteria outlined in US EPA OPPTS 890.1450, except for adrenal weights (adjusted and unadjusted: 33.3 and 33.2, respectively), which were slightly lower than the acceptable range (38.34 - 48.84 mg). The CVs for organ weights were all within the performance criteria.

Gross pathology
No treatment-related macroscopic findings were observed.

Histopathology
There were no treatment-related microscopic findings in the ovaries, uterus, kidneys, or thyroid gland (see table below). All histologic changes were considered to be incidental findings and consistent with the age and strain of rats used.

Table 5.8.3-30: A Pubertal Development and Thyroid Function Assay of Glyphosate Administered Orally in Intact Juvenile/Peripubertal Female Rats ([redacted] 2012): Body weight and vaginal opening (VO) - selected time points according to test guideline

Parameter Evaluated		Control				100 mg/kg bw/day				300 mg/kg bw/day				1000 mg/kg bw/day			
		n	Mean	±SD	CV	n	Mean	±SD	CV	n	Mean	±SD	CV	n	Mean	±SD	CV
Initial body weight (PND 22; g)	U	15	45.9	1.8	3.93	15	45.3	2.16	4.77	15	45.3	2.13	4.17	15	45.3	2.46	5.43
Body weight at vaginal opening (g)	U	14	119.6	12.62	10.56	15	119.2	16.25	13.64	15	125.1	15.57	12.44	15	117.4	16.80	14.31
	A	14	119.2	12.62	10.56	15	119.4	16.25	13.64	15	124.8	15.57	12.44	15	118.0	16.80	14.31
Final body weight (g)	U	14	147.3	11.68	7.93	15	147	10.3	7.01	15	152.1	6.37	4.19	15	141.3	10.60	7.50
Final body weight (% of control)	U	14	-	-	-	15	-0.20	-	-	15	3.25	-	-	15	-4.07	-	-
Body weight gain (g)	U	14	101.5	10.55	10.40	15	101.7	9.01	8.86	15	106.8	5.87	5.50	15	96.0	9.69	10.09
Age at vaginal opening (PND)	U	14	36.4	2.38	6.52	15	36.1	2.52	6.99	15	36.8	2.51	6.83	15	36.3	2.61	7.18
	A	14	36.5	2.38	6.52	15	36.1	2.52	6.99	15	36.8	2.51	6.83	15	36.3	2.61	7.18
Proportion unopened (animals affected/n)		**1/15**				**0/15**				**1/15**				**0/15**			

U = Unadjusted for body weight on PND 22; A = Adjusted for body weight on PND 22; n = Total number of animals per study group; SD = Standard Deviation; CV = Coefficient of variation; PND = Postnatal day

Table 5.8.3-31: A Pubertal Development and Thyroid Function Assay of Glyphosate Administered Orally in Intact Juvenile/Peripubertal Female Rats ([redacted] 2012): Estrous cyclicity

Dose (mg/kg bw/day)	Number of animals	Mean age at first vaginal estrus (PND)	Mean cycle length (d)	Cycling (%)	Regularly cycling (%)	Cycle status at sacrifice (# females)			
						Diestrus	Proestrus	Estrus	Not Cycling
Control	14	36.5	5.0	100	75	9	1	3	0
100	15	37.5	4.8	100	80	8	1	6	0
300	15	37.8	5.0	100	40*	7	2	5	0
1000	15	38.3	5.0	100	60*	11	0	4	0

PND = Postnatal day; * p <0.05 as compared to control

Table 5.8.3-32: A Pubertal Development and Thyroid Function Assay of Glyphosate Administered Orally in Intact Juvenile/Peripubertal Female Rats ([redacted] 2012): Selected clinical chemistry parameters

Parameter	Control (n=14)			100 mg/kg bw/day (n=15)			300 mg/kg bw/day (n=15)			1000 mg/kg bw/day (n=15)		
	Mean	±SD	CV	Mean	±SD	CV	Mean	±SD	CV	Mean	±SD	CV
ALP (U/L)	252	30.8	12.2	260	51.1	19.7	283	38.1	13.5	303*	50.0	16.5
AST (U/L)	506	164.6	32.5	366*	89.7	24.5	374*	39.7	10.6	383*	109.6	28.6
Chloride (mEq/L)	100	1.6	1.6	101	1.0	1.0	100	1.7	1.7	103*	2.3	2.2
Phosphorus (mg/dL)	11.7	1.00	8.56	10.8*	0.59	5.44	11.4	0.84	7.35	11.8	0.58	4.97
Potassium (mEq/L)	8.08	0.977	12.085	7.00*	0.488	6.973	7.11*	0.558	7.850	7.55	0.638	8.455

SD = Standard deviation; CV = Coefficient of variation; ALP = alkaline phosphatase; AST = aspartate aminotransferase; * p <0.05 as compared to control

Table 5.8.3-33: A Pubertal Development and Thyroid Function Assay of Glyphosate Administered Orally in Intact Juvenile/Peripubertal Female Rats ([redacted] 2012): Hormone levels

Parameter	Control (n=14)			100 mg/kg bw/day (n=15)			300 mg/kg bw/day (n=15)			1000 mg/kg bw/day (n=15)		
	Mean	**±SD**	**CV**	**Mean**	**±SD**	**CV**	**Mean**	**±SD**	**CV**	**Mean**	**±SD**	**CV**
Total T4 (µg/dL)	4.12	0.539	13.069	4.38	0.465	10.613	4.64	0.682	14.685	3.76	0.664	17.664
TSH (ng/mL)	3.74	1.917	51.207	3.86	1.627	42.115	3.85	2.172	56.452	2.69	1.265	47.095

SD = Standard deviation; CV = Coefficient of variation; T4 = Thyroxine; TSG = Thyroid stimulating hormone

Table 5.8.3-34: A Pubertal Development and Thyroid Function Assay of Glyphosate Administered Orally in Intact Juvenile/Peripubertal Female Rats ([redacted] 2012): Organ weights

Organ weight (mg)		Control (n=14)			100 mg/kg bw/day (n=15)			300 mg/kg bw/day (n=15)			1000 mg/kg bw/day (n=15)		
		Mean	±SD	CV	Mean	±SD	CV	Mean	±SD	CV	Mean	±SD	CV
Adrenal glands, abs.	U	33.3	5.66	17.03	33.1	5.19	15.67	33.2	3.84	11.56	31.8	5.63	17.72
Adrenal glands, abs.	A	33.2	5.66	17.03	33.2	5.19	15.67	33.1	3.84	11.56	31.9	5.63	17.72
Adrenal glands, rel.	R	22.5	3.26	14.47	22.5	3.61	15.99	21.8	2.44	11.2	22.5	3.72	16.53
Kidneys, abs.	U	1170	104	8881	1160	119	10259	1170	100	8526	1140	102	8963
Kidneys, abs.	A	1160	104	8881	1160	119	10259	1170	100	8526	1150	102	8963
Kidneys, rel.	R	790	46	5853	790	49	6253	770	49	6340	810	57	7009
Liver, abs.	U	6720	851	12663	6750	649	9613	7030	469	6677	6470	524	8108
Liver, abs.	A	6700	851	12663	6760	649	9613	7010	469	6677	6500	524	8108
Liver, rel.	R	4550	321	7069	4580	219	4779	4620	204	4429	4580	184	4024
Ovaries, abs.	U	68.3	9.32	13.65	67.9	11.01	16.22	67.8	9.31	13.73	69.1	10.78	15.6
Ovaries, abs.	A	67.9	9.32	13.65	68.0	11.01	16.22	67.7	9.31	13.73	69.6	10.78	15.6
Pituitary, abs.	U	8.3	0.92	11.10	9.2	1.68	18.39	8.8	0.98	11.12	8.6	1.29	15.06
Pituitary, abs.	A	8.2	0.92	11.10	9.2	1.68	18.39	8.8	0.98	11.12	8.7	1.29	15.06
Pituitary, rel.	R	5.6	0.59	10.47	6.2	0.98	15.78	5.8	0.69	11.89	6.1	0.9	14.81
Thyroid glands, abs.	U	8.97	2.629	29.311	8.72	1.908	21.872	9.33	1.243	13.32	8.63	1.885	21.83
Thyroid glands, abs.	A	8.88	2.629	29.311	8.76	1.908	21.872	9.27	1.243	13.32	8.76	1.885	21.83
Uterus, blotted, abs.	U	235.7	68.25	28.95	256.8	56.78	22.11	260.2	72.29	27.78	205.9	73.09	35.5
Uterus, blotted, abs.	A	235.2	68.25	28.95	256.8	56.78	22.11	260.5	72.29	27.78	205.9	73.09	35.5
Uterus, wet, abs.	U	263.6	91.13	34.57	316.9	130.03	41.03	342.6	171.33	50.01	238.9	116.84	48.91
Uterus, wet, abs.	A	262.5	91.13	34.57	315.6	130.03	41.03	343.7	171.33	50.01	238.6	116.84	48.91

SD = Standard deviation; CV = Coefficient of variation; abs. = absolute; U = Unadjusted for body weight on PND 22; A = Adjusted for body weight on PND 22; rel. = relative; R = Organ-to-body weight ratio

Table 5.8.3-35: A Pubertal Development and Thyroid Function Assay of Glyphosate Administered Orally in Intact Juvenile/Peripubertal Female Rats ([redacted] 2012): Histopathological lesions – selected parameters

Findings		Control (n=14)		100 mg/kg bw/day (n=15)		300 mg/kg bw/day (n=15)		1000 mg/kg bw/day (n=15)	
		Observed	Examined	Observed	Examined	Observed	Examined	Observed	Examined
Ovaries									
Unremarkable		14	14	15	15	15	15	15	15
Uterus									
Unremarkable		14	14	15	15	15	15	15	15
Thyroid	Grade [1,]								
Colloid area	1	0	14	0	15	0	15	0	15
	2	0	14	0	15	0	15	0	15
	3	1	14	1	15	2	15	2	15
	4	10	14	8	15	8	15	9	15
	5	3	14	6	15	5	15	4	15
Follicular Cell Height	1	5	14	7	15	6	15	7	15
	2	9	14	8	15	9	15	8	15
	3	0	14	0	15	0	15	0	15
	4	0	14	0	15	0	15	0	15
	5	0	14	0	15	0	15	0	15

[1] Colloid area: 1 = most colloid, 5 = least colloid
[2] Follicular cell height: 1 = lowest, 5 = highest

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is performed in accordance with the current US EPA OPPTS/OCSPP 890.1450 (2009) where the following deviations were noted. The mean age of vaginal opening attainment in the control group was higher than the acceptable range of values in the performance criteria. However, the coefficient of variation value fell within the acceptable range, and thus, met the acceptance criteria. The weights of adrenal glands of control animals were slightly lower than the acceptable range. These deviations were not considered to impact the validity or final outcome of the study. The study is therefore considered valid. There was no evidence of any test substance-related estrogenic or anti-estrogenic effects, nor was there any evidence of test substance-related effects on pubertal development or thyroid function in the juvenile/peripubertal female rat following oral administration of glyphosate at dosage levels of 100, 300, and 1000 mg/kg bw/day.

Assessment and conclusion by RMS:

CA 5.8.3/009

1. Information on the study

Data point:	CA 5.8.3
Report author	Hecker, M. *et al.*
Report year	2011
Report title	The OECD validation program of the H295R steroidogenesis assay: Phase 3. Final inter-laboratory validation study
Report No	DOI 10.1007/s11356-010-0396-x
Document No	Not reported
Guidelines followed in study	Not applicable (OECD Test Guideline (TG) was approved in July 2011 as OECD TG 456, after the work of the publication was completed).
Deviations from current test guideline (OCSPP 890.1550, 2009) (OECD 456, 2011)	None (key values provided in the publication are in line with current OECD TG 456).
Previous evaluation	Yes, accepted in EFSA peer review on endocrine disrupting properties (2017).
GLP/Officially recognised testing facilities	Not specified
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary

The purpose of this study was to validate a standardised steroidogenesis assay protocol to develop an OECD

draft test guideline. (Note: The OECD Test Guideline (TG) was approved in July 2011 as OECD TG 456.) Twenty-eight chemicals were selected as model substances to validate the H295R steroidogenesis assay, to identify potential effects of endocrine-disrupting substances on the production of testosterone (T) and 17β-estradiol (E2). These test substances were selected based on their known or suspected endocrine activity, or lack thereof, and included inhibitors and inducers of different potencies as well as positive and negative controls. They were selected and approved by the OECD Validation and Management Group for Non-Animal Testing (VMG NA). Glyphosate was one of the test substances evaluated. A total of seven laboratories from the US, Denmark, Germany, Japan, Hong Kong, and Canada participated in this validation study. Two of these participating laboratories evaluated glyphosate.

H295R cells were cultured under standard cell culture conditions for at least four or five passages prior to use in the assay. The cells were plated into 24-well cell culture plates at a density of approximately 200,000 to 300,000 cells/mL. Cells were allowed to acclimate for 24 hours prior to test substance exposure. Then, cells were exposed for 48 hours to seven concentrations of glyphosate using log intervals between 0.0001 and 100 µM of the test substance in triplicate. A concurrent quality control plate was included in each of the independent runs to demonstrate the correct assay's response to forskolin (a known inducer of testosterone and estradiol production) and prochloraz (a known inhibitor of testosterone and estradiol production). After the 48-hour exposure period, medium was collected from all wells and concentrations of hormones were measured using commercially available hormone detection kits (in one laboratory using enzyme linked immunoassay and liquid chromatography with mass spectroscopy [hormones were extracted with ethyl ether before analysis], in the other laboratory using radio immunoassay without previous extraction of the hormones). After media removal, cell viability was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay or the Live/Dead® variability assay. All concentrations with cell viability ≤80 %, were excluded from the data analysis. One laboratory conducted one experiment without further replicates; the other laboratory conducted three independent experiments with the test substance glyphosate. All laboratories were required to demonstrate proficiency in performing the single procedures that are part of the H295R steroidogenesis assay protocol. For this purpose, predefined performance criteria had to be met. In addition, each test substance was tested for potential interference with the hormone detection system used before exposing cells in the main experiment. This was of particular relevance for antibody-based assays such as the enzyme linked immunoassay and the radioimmunoassay.

To examine the relative changes in hormone production, results were normalized to the mean vehicle control (VC) value, and results were expressed as percent change relative to the VC. Prior to conducting statistical analyses, the assumptions of data normality and variance of homogeneity were evaluated. Normality was evaluated using standard probability plots or the Shapiro–Wilk's test. If the data were normally distributed, differences between chemical treatments and vehicle controls were analysed using one-way analysis of variance (ANOVA) followed by a two-sided Dunnett's test. If data were not normally distributed, the Kruskal–Wallis test followed by the Mann–Whitney U test were used. Data analysis was conducted using pooled replicate experiments. Differences were considered significant at $p < 0.05$.

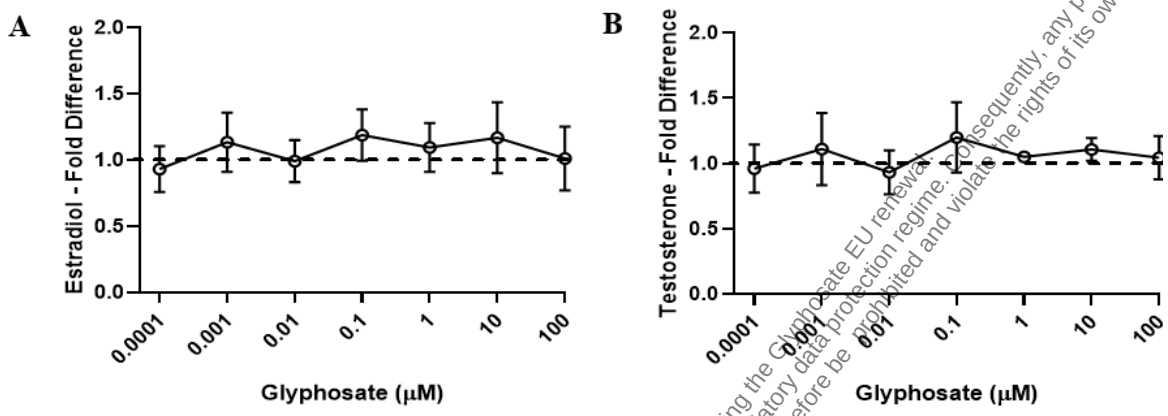
In the present H295R steroidogenesis assay validation study, glyphosate was tested negative for changes in T, as well as E2 production. None of the two laboratories could detect a change in hormone production when compared to the concurrent control (see figure below). The authors stated that test substances that tested negative for T and E2 effects *in vitro* (H295R assay), like glyphosate, did not cause any changes in serum T and E2 concentrations *in vivo*, too (Soso *et al.*, 2007[43]). This result is consistent with an article in the peer-reviewed literature using similar methodology in Leydig cells (Forgacs *et al.*, 2012[44]).

Figure 5.8.3-5: The OECD validation program of the H295R steroidogenesis assay: Phase 3. Final inter-

[43] Soso, A.B, Barcellos, L.J.G., Ranzani-Paiva, M.J. *et al.* (2007). Chronic exposure to sub-lethal concentrations of a glyphosate-based herbicide alters hormone profiles and affects reproduction of female Jundi'a (*Rhamdia quelen*). *Environ Toxicol Pharm*. 23:308-313.

[44] Forgacs A.L., Ding, Q., Jaremba, R.G., *et al.* (2012). BLTK1 murine Leydig cells: A novel steroidogenic model for evaluating the effects of reproductive and developmental toxicants. *Toxicol. Sci*. 127:391-402.

laboratory validation study (Hecker et al., 2011): Glyphosate did not affect production of 17β-estradiol (A) or testosterone (B) in the H295R steroidogenesis assay. Values are shown as mean fold-difference relative to the control ± standard error.



In conclusion, glyphosate was tested negative for changes in E2 and T production in the H295R steroidogenesis assay in two separate laboratories during OECD's Phase 3 validation.

CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:
Key values provided in the publication are in line with current OECD 456 (2011). The study is therefore considered valid. This OECD inter-laboratory validation of the H295R steroidogenesis assay, which includes glyphosate as one of many test materials, demonstrates that glyphosate does not impact 17β-estradiol or testosterone production.

Assessment and conclusion by RMS:

CA 5.8.3/010

1. Information on the study

Data point	CA 5.8.3
Report author	[redacted]
Report year	2020
Report title	Assessment on the endocrine disrupting properties of the active substance glyphosate in accordance with Commission Regulation (EU) 2018/605
Report No	110517-2
Document No	Not reported
Guidelines followed in	Not applicable.

study	Assessment according to *Commission Regulation (EU) 2018/605* and respective guidance document *Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009* (ECHA-EFSA, 2018).
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

According to the ED criteria laid down in Regulation (EU) 2018/605, endocrine mediated adversity as well as activity and the biological link between those two must be apparent to identify a substance as an endocrine disruptor. Therefore, a comprehensive ED assessment was performed according to the guidance document *Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009* (ECHA-EFSA, 2018). For details please refer to the ED assessment report *Assessment on the endocrine disrupting properties of the active substance glyphosate in accordance with Commission Regulation (EU) 2018/605* ([redacted] 2020, CA 5.8.3/010).

Concerning the ED assessment of humans and mammals, potential effects of glyphosate on the HPT and HPG axis were addressed in several repeated dose toxicity studies of subacute to chronic exposure also considering different life stages (level 4 and 5 studies of the OECD conceptual framework). In addition, *in vitro* and *in silico* information are available and considered for the ED assessment of glyphosate. With regard to EATS-mediated adversity, a review of the available mammalian guideline studies in four species (dog, mouse, rabbit, rat), conducted with glyphosate over different exposure periods and considering different life stages (in rat), did not show carcinogenicity or any other EATS-mediated adverse effects based on a sufficient dataset as required in the ECHA/EFSA ED Guidance. Potential EATS-related activity was investigated in the male and female pubertal assay, where hormone measurements were performed, as well as the Uterotrophic and Hershberger Assay providing *in vivo* mechanistic data. Neither the described *in vivo* assays nor *in vitro* and *in silico* information provide any indication on EATS-related endocrine activity of glyphosate.

Hence, the ED criteria for glyphosate with regards to human health and mammals are not met, since neither EATS-mediated adversity nor endocrine activity has been observed.

With regards to non-target organisms, EATS-mediated adversity of glyphosate has not been observed in any of the ecotoxicological studies conducted with glyphosate in birds, fish, amphibians and invertebrates. Regarding the assessment of potential EAS-mediated adversity, only secondary effects as a consequence of systemic toxicity are observed. The effects are ranked as "sensitive to, but not diagnostic of EATS" modalities and "systemic toxicity". Potential EAS-mediated activity has been investigated within a Fish Short-Term Reproduction Assay and is therefore sufficiently investigated. No indication for EAS-related endocrine activity was observed.

T-mediated activity was investigated within an amphibian metamorphosis assay and is therefore sufficiently investigated. No effects on relevant parameters rated as "T-mediated" were found. This result is sufficient to conclude that T-mediated adversity is unlikely, as no T-related endocrine activity has been observed.

Hence, the ED criteria for glyphosate with regards to non-target organisms are therefore not met.

In conclusion, glyphosate does not induce EATS-mediated adversity and no EATS-related endocrine activity was observed *in silico, in vitro,* and *in vivo* for humans and mammals as well as for non-target organisms. This conclusion is in concordance with the current Peer review of the pesticide risk assessment of the potential endocrine disrupting properties of glyphosate (EFSA Journal 2017; 15(9): 4979) as well as

with the conclusion of EPA on the Endocrine Screening Program (EDSP) Tier l (US EPA, 2015).
Since glyphosate has not been shown to induce EATS-mediated adversity or endocrine activity, it is concluded that the ED criteria with regard to EATS-modalities in humans and mammals as well as non-target organisms are not met for glyphosate.

CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:
Since glyphosate has not been shown to induce EATS-mediated adversity or endocrine activity, it is concluded that the ED criteria with regard to EATS-modalities in humans and mammals as well as non-target organisms are not met for glyphosate.
For details please refer to the ED assessment report *Assessment on the endocrine disrupting properties of the active substance glyphosate in accordance with Commission Regulation (EU) 2018/605*.

Assessment and conclusion by RMS:

CA 5.8.3/011

1. Information on the study

Data point	CA 5.8.3
Report author	[redacted]
Report year	2020
Report title	(Q)SAR screening on endocrine disrupting potential of Glyphosate under the Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009
Report No	110517-1
Document No	Not reported
Guidelines followed in study	Not applicable. Assessment performed according to the guidance document *Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009* (ECHA-EFSA, 2018).
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary

(Q)SAR predictions were generated using selected publicly available and commercial models. Five QSAR

tools were applied for predictions of potential endocrine activity of glyphosate: OECD QSAR Toolbox, Vega, Endocrine Disruptome, Danish QSAR database and ToxCast COMPARA/CERAPP consensus models. The list of investigated receptors include: estrogen receptor (ER), androgen receptor (AR), thyroid receptor (TR), glycocorticoid receptor (GR), mineralocorticoid receptor (MR), liver X receptor (LXR), peroxisome proliferator-activated receptor (PPAR), retinoid X receptor (RXR), aryl hydrocarbon receptor (AhR), pregnane X receptor (PXR), and CYP3A4 receptor.
For details please refer to the (Q)SAR ED assessment report *(Q)SAR screening on endocrine disrupting potential of Glyphosate under the Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009*.

Table 5.8.3-36: (Q)SAR screening on endocrine disrupting potential of Glyphosate under the Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009 ([redacted], 2020): Outcome of the *in silico* screening for glyphosate

Modality	Summary outcome of *in silico* screening	Remarks
Estrogen	No indication	Estrogenic activity was predicted negative with all five applied models. Due to the high amount of data available on ER activity, the high quality of CERAPP Consensus predictions and glyphosate being part of the training set for ER binding tests, the assessment of ER activity of glyphosate is considered reliable.
Androgen	No indication	Androgenic activity was predicted negative with all five applied models. Due to the quality of CoMPARA consensus predictions in combination with other models (predicting no androgenic activity), the assessment of androgenic activity based on the available models is considered reliable. This is further strengthened, as glyphosate is part of the testing battery of the Danish QSAR DB and tested negative for antagonistic effect on the human androgen receptor *in vitro*.
Steroid	No indication	There are three results available for steroid receptors: glucocorticoid receptor (GR) and glucocorticoid receptor antagonism and mineralocorticoid receptor (MR). No steroid activity is predicted for all three receptors by the molecular docking method (Endocrine Disruptome).
Thyroid	No indication	TR binding activity is predicted to be low for glyphosate by the molecular docking method (Endocrine Disruptome). Two models available in the Danish QSAR database yield inconclusive and negative results. Both predictions are out of applicability domain and thus of low reliability.
Other	No indication	Overall, there is no indication of activity for endocrine activities other than estrogen, androgen, steroid and thyroid, however due to the general lack of models for the various receptors, the result should be considered with caution.

CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:

Five QSAR tools were applied for predictions of potential endocrine activity of glyphosate: OECD QSAR Toolbox, Vega, Endocrine Disruptome, Danish QSAR database and OPERA ToxCast COMPARA/CERAPP consensus models.
For androgenic and estrogenic activity, the largest number of data and models is available. Specifically due to the available CONSENSUS models (CoMPARA/CERAPP) derived from the extensive high-throughput screening tests as conducted under the EDSP in combination with available literature derived predictions, the overall assessment of androgenic and estrogenic activities is considered reliable and was shown to be negative. This outcome is strengthened because glyphosate is part of the training set of ER binding tests showing negative results as well as tested negative for antagonistic effects on the human

androgen receptor *in vitro*.
For all other modalities, lower amount of data and models are available. Thus, the confidence in the screening assessments for thyroid, steroid or other modalities is lower. However, also here no indication for thyroid, steroid or other modalities activities were observed.
Overall, there is no indication that glyphosate has endocrine disrupting activity.
For details please refer to the (Q)SAR ED assessment report *(Q)SAR screening on endocrine disrupting potential of Glyphosate under the Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009*.

Assessment and conclusion by RMS:

A literature search for the active substance glyphosate was performed in accordance to the provisions of the EFSA Guidance "Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) [redacted] and reliable for this section and the summaries are thus presented below and are part of the general discussion at the beginning of the section.

Table 5.8.3-37: Publications on cellular effects of glyphosate

Annex Point	Study & Substance tested	Study type	Reliability and restriction comments	Result
CA 5.8.3/012	Gigante *et al.*, 2018 Glyphosate (Batch: not specified, Purity: not specified)	*in vitro:* granulosa cells of swine ovaries and adipose stromal cells (ASCs) from swine adipose tissue; parameters examined: cell proliferation; cell viability; steroid, O_2^- and NO production; FRAP assay; cell differentiation; expression of marker genes; and intracellular lipid accumulation	Test substance not characterized No positive control Only one dose tested in adipose stromal cells	Glyphosate decreased cell proliferation, cell viability, estrogen production and ferric reducing capacity and increased progesterone and NO production in granulosa cells when tested at 0.2 to 16 µg/mL. In none of the assays with granulosa cells a concentration-response relationship was established. Glyphosate treatment at 4 µg/mL decreased the viability of proliferating adipose stromal cells. Differentiated cell counts showed a significant inhibition of the adipogenic process by glyphosate.
CA 5.8.3/013	Vanlaeys *et al.*, 2018 Glyphosate (Batch: not specified), Purity: not specified)	*in vitro:* murine TM4 Sertoli cell line, parameters examined: cytotoxicity, glutathione transferase activity, and lipid accumulation	Test substance not characterized No positive controls	Glyphosate had no impact on cell viability after 24 hours of exposure to concentrations from 10 to 10,000 ppm. Glyphosate reduced succinate dehydrogenase to some extent over the entire concentration range from 10 to 10,000 ppm with no concentration-response relationship. It had no impact on glutathione transferase activity. Exposure of TM4 cells to glyphosate for 24 hours at 2,500 or 5,000 ppm induced an increase in cytoplasmic lipid droplets.
CA	Mesnage *et al.*,	*in vitro:* MCF-7, MDA-		Increased cell proliferation was

5.8.3/014	2017 Glyphosate PESTANAL® (Batch: not specified, Purity: ≥98.0 %)	MB-231 and T47D cell lines, parameters examined: estrogen receptor (ER)-mediated cell proliferation, ERE-luciferase reporter gene assay, gene expression profiling		observed in MCF-7 and T47D cells at 10,000 µg/L to 1,000,000 µg/L. Glyphosate stimulated ERE-mediated transcription of the luciferase reporter gene starting at a concentration of 1,000 µg/L. The analysis of gene ontology confirms that genes having their expression altered by treatment of MCF-7 cells with glyphosate were involved in cell cycle regulation, stimulation by steroid hormones and cell death through apoptosis. ONIOM binding energy assessment implies that binding of glyphosate to the ER is weak and unstable.
CA 5.8.3/015	Thongprakaisang *et al.*, 2013 Glyphosate (Batch: not specified, Purity: >98 %)	*in vitro:* T47D, T47D-KBluc, and MDA-MB231 cell lines; parameters examined: cell proliferation of hormone-dependent and - independent cell lines in with/without an ER antagonist; ERE-transcription activity with/without an ER antagonist; and expression of ERs	Results do not corroborate with in vivo Hershberger and Uterotrophic assays	Glyphosate induced cell proliferation in a hormone-dependent cancer cell line but not in a hormone-independent cancer cell line in the absence of estrogen (E2). In the presence of a potent ER antagonist the cell proliferation caused by glyphosate in a hormone-dependent cancer cell line was reduced. The interaction of glyphosate with the ER was confirmed by ERE activation with and without an ER antagonist. When cells were co-incubated with glyphosate and E2, glyphosate suppressed the E2-induced ERE activation suggesting that glyphosate behaves as an antagonist in the presence of an endogenous agonist. Glyphosate altered the expression of ERα and ERβ in human breast cancer cells.
CA 5.8.3/016 CA 5.8.3/017	Brennan *et al.*, 2016 Glyphosate (Batch: not specified, Purity: not specified)	*in vitro:* VM7Luc4E2 and VM7LucERβc9 cell lines, parameters examined: estrogen receptor (ERα/β)-mediated luciferase reporter gene assay	Test substance not characterized	Glyphosate did not show any estrogenic activity at a concentration of 10 µM in two cell lines or via the two human estrogen receptor (hER) subtypes, hERα and hERβ.
CA 5.8.3/018	Defarge *et al.*, 2016 Glyphosate IPA (Batch: not specified, Purity: not specified)	*in vitro:* JEG3 cell lines, parameters examined: aromatase activity, cytotoxicity (MTT, Toxilight bioassay)	Test substance not characterized	An effect on aromatase activity was not observed when cells were treated with glyphosate at non-toxic concentrations. At a higher glyphosate concentration (17.7 mM) a reduction of aromatase activity was seen at unphysiologic conditions and therefore, not considered relevant for human health.

CA 5.8.3/012

2. Information on the study

Data point:	CA 5.8.3
Report author	Gigante, P. *et al.*
Report year	2018
Report title	Glyphosate affects swine ovarian and adipose stromal cell functions
Document No	doi.org/10.1016/j.anireprosci.2018.05.023 E-ISSN: 1873-2232
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
Previous evaluation	No
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Yes/Reliable with restrictions

8. Full summary

Executive Summary

The effect of glyphosate (GLY) at different doses (0.2, 4 and 16 µg/mL) was evaluated on granulosa cells growth (BrDU incorporation and ATP production), steroidogenesis (17-β estradiol and progesterone secretion) and redox status (superoxide and nitric oxide production and non-enzymatic scavenging activity). GLY has been shown to inhibit cell growth, 17-β estradiol and non-enzymatic scavenging activity and to increase progesterone and nitric oxide secretion ($P < 0.05$). In addition, GLY significantly decreased the viability of ASCs ($P < 0.001$), and inhibited their adipogenic differentiation. These data indicate that GLY alters the main features of granulosa cells and ASCs; thus suggesting that GLY could affect both reproductive function and adipose tissues homeostasis.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material

Glyphosate was purchased from Sigma Chemical Co, St Louis, USA. The purity was not reported.

Isolation and culture of granulosa cells

Swine ovaries were collected at a local slaughterhouse, placed in PBS at 4 °C supplemented with penicillin (100 Ul/mL), streptomycin (100 Ul/mL) and amphotericin B (2.5 µg/mL) and transported to the laboratory within 1 hour. The samples were immersed for 1 minute in ethanol 70 % before processing. Cystic or hemorrhagic follicles were discarded and the granulosa cells were harvested by aspiration of the follicles in the later state of maturation. The granulosa cells were then centrifuged at 450xg for 10 minutes and the cell pellet obtained treated with ammonium chloride to remove red blood cells. The cell number was estimated after vital staining with trypan blue whereafter the cells were plated and cultured in a validated serum free system composed of DMEM/Ham's F12 medium supplemented with penicillin (100 µg/mL), amphotericin B (2,5 µg/mL), streptomycin (100 µg/mL), sodium selenite (5 ng/mL) and transferrin (5 µg/mL) (CM). CM medium maintains the characteristics of granulosa cells and avoids luteinization.

B. STUDY DESIGN

Cell proliferation

Cell proliferation is measured using the BrdU cell proliferation ELISA assay. The granulosa cells were plated into 96-well plates (10^4 cells/100 µL CM) and incubated overnight with glyphosate at 0, 0.2, 4 or16 µg/mL. At the end, plates were centrifuged for 10 minutes at 400×g, the supernatants discarded and the cells dried and fixed. The DNA was denatured before the addition of the anti-BrdU antibody, conjugated with horseradish peroxidase (POD). The POD substrate used was tetramethyl-benzidine (TMB). Absorbance at 450 nm of the blue color formed is proportional to the amount of newly synthesized DNA. To quantify the viable cell number, the absorbance of each sample was related to a standard curve prepared by culturing, in quintuplicate, granulosa cells at different plating densities for 48 hours. The curve was repeated in four different experiments. The cell number/well was estimated from the resulting linear regression equation and was used to correct experimental data. The assay detection limit was 10^3 cell/well and the variation coeffcient was less than 5 %.

Cell viability

Granulosa cell viability was assessed using the ATP-lite bioluminescent assay based on the reaction of ATP luciferase and luciferin. The light emitted is proportional to the ATP concentration in the cells. The test was validated by plating different viable cell numbers (from 2.5×10^3 to 4×10^5/100 µL) three times. The relationship between cell number and luminescence was linear ($r = 0.95$). 2×10^5 cells/100 µL CM were seeded in 96-well plates and treated with glyphosate at 0, 0.2, 4 or 16 µg/mL for 48 hours. Kit reagents were added according to the instructions and luminescence was measured.

Granulosa cell steroid production - Granulosa cells at 10^4/200 µL in CM supplemented with androstenedione at 28 ng/mL were seeded in 96-well plates and treated with glyphosate at 0.2, 4 or16 µg/mL. After 48 hours incubation, culture media were collected, frozen and stored at −20 °C until determination of progesterone (P4) and 17-β estradiol (E2) by a validated RIA. P4 assay sensitivity and ED_{50} were 0.24 and 1 nmol/L, respectively. E2 assay sensitivity and ED_{50} were 0.05 and 0.2 nmol/L. The intra- and inter-assay coefficients of variation were less than 12 % for both assays.

Granulosa cell superoxide (O_2^-) production

O_2^--production was measured using the WST-1 (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolium]-1,3-benzene disulfonate) test which is based on the cleavage of the water soluble tetrazolium salt, WST-1, to the water soluble formazan. A total of 10^4 cells/200 µL CM were seeded in 96-well plates and incubated with glyphosate at 0, 0.2, 4 or 16 µg/mL for 48 hours. During the last 4 hours of incubation, 20 µL of WST-1 was added to the cells, and the absorbance determined at 450 nm against 620 nm. The coefficients of variation were less than 3 %.

Granulosa cell nitric oxide (NO) production

10^5 viable cells/200 µL CM were seeded in 96-well plates and treated with glyphosate at 0.2, 4 or 16 µg/mL for 48 hours. At the end, plates were centrifuged for 10 minutes at 400×g, the supernatants were collected and NO levels assessed by measuring nitrite after incubation with Greiss reagent (1 % sulfanilamide, 5 % phosphoric acid and 0.1 % N-[naphtyl] ethylenediaminedihydrochloride). The absorbance was measured at 540 nm against 620 nm. A calibration curve ranging from 25 to 0.39 µM was prepared by diluting sodium nitrite in CM.

Non-enzymatic scavenging activity

The Ferric Reducing Activity of Plasma (FRAP) assay is a colorimetric method based on the ability of the antioxidant molecules present in a biological matrix to reduce ferric-tripiridyltriazine (Fe^{3+} TPTZ) to a ferrous form (Fe^{2+} TPTZ). 2×10^5 cells/200 µL CM were seeded in 96-well plates and treated with glyphosate at 0, 0.2, 4 or 16 µg/mL. At the end, the plates were centrifuged for 10 minutes at 400×g, the supernatants discarded and the cells lysed by adding, Triton 0.5 % + PMSF in PBS at 200 µL/well in an ice bath for 30 minutes. The test was performed on 40 µL of cell lysates added to Fe^{3+} TPTZ reagent. After 30 minutes incubation at 37 °C, absorbance of Fe^{2+} TPTZ was determined at 595 nm. The ferric reducing ability of cell lysates was calculated by plotting a standard curve of absorbance against $FeSO_4 .7H_2O$ standard solution.

Isolation and culture of adipose stromal cells (ASCs) from swine adipose tissue

Samples of about 5 g of subcutaneous abdominal adipose tissue were collected from pigs at a local slaughterhouse and placed in 20 mL of PBS supplemented with penicillin (100 Ul/mL), streptomycin (100 Ul / mL) and amphotericin B (2.5 µg/mL), transferred to Petri dishes in sterile conditions and shredded in fragments of about 3 mm^3. Thereafter, the adipose tissue fragments were distributed in 6-well plates, at a density of 10 fragments/well and then carefully covered with a minimal quantity (1 mL) of culture medium

(CM_{ASC}) to avoid floating. CM_{ASC} was composed of low glucose DMEM + GlutaMax, supplemented with 10 % FBS, penicillin (100 UI/mL), streptomycin (100 UI/mL) and amphotericin B (2.5 µg/mL). The plates were maintained at 37 °C in a humidified atmosphere at 5 % CO_2 and 19 % O_2. Every 48 hours CM_{ASC} was replaced. After 5 to 7 days, the adipose tissue fragments were removed from the culture plates and the remaining adherent cells, growing in monolayer, were cultured in fresh CM_{ASC} for 5 to 7 days, until 80 % confluence was reached. The ASCs were the trypsinized and cultured in 25 cm^2 flasks with 5 mL CM_{ASC}. The ASCs obtained were subsequently either used to evaluate the expression of adipogenesis-related marker genes or plated to study the effects of glyphosate at 4 µg/mL on cell viability and adipogenic differentiation.

Adipose stromal cells (ASCs) viability

Cell viability was evaluated using the MTT assay. ASCs were seeded in 96-well plates at 10^4 cells/well in 200 µL CM_{ASC} and incubated at 37 °C in humidified atmosphere at 5 % CO_2 and 19 % O_2 for 48 and 72 hours with glyphosate at 0 or 4 µg/mL. At the end of each incubation period, 20 µL of 5 mg/mL of MTT were added and incubated for 4 hours. Subsequently, media were discarded and 100 µL of lysis solution (SDS10 % in HCl 0.01 N) were added to the wells and left overnight at 37 °C. Absorbance was measured at 540 nm.

Adipose stromal cells (ASCs) adipogenic differentiation

ASCs were seeded in CM_{ASC} and incubated for 48 hours at 37 °C in a humidified atmosphere at 5 % CO_2 and 19 % O_2 to permit cell adhesion. Adipogenic differentiation was performed by exposing cells to 3 cycles of 4 days each, of the following treatments:

- 3 days with induction medium: CM_{ASC} supplemented with dexamethasone (DEX) at 1 µM, insulin (INS) at 1.7 µM, 3-iso-butyl-1-methylxanthine (IBMX) at 0.5 mM and indomethacin at 250 µM.
- 1 day with differentiation maintaining medium: CM_{ASC} supplemented with insulin (INS) at 1.7 µM.

Negative controls were performed using ASCs cultured in CM_{ASC}. Cell differentiation was evaluated by Oil Red O Staining to detect cytoplasmic lipid vacuoles as markers of adipogenesis.

Expression of adipogenic marker genes

In order to evaluate the expression of adipogenic marker genes crucial for the function of adipose tissue, PPARγ and Leptin total RNA was extracted from ASCs pellets (7×10^5 cells), subjected or not to adipogenic differentiation, using NucleoSpin RNA II. cDNA was then obtained by reverse transcription of 2 µg of the extracted RNA using the High-capacity cDNA reverse transcription kit. The expression of the genes was evaluated by PCR (Polymerase Chain Reaction) using the primers for PPARγ, leptin and GADPH. The occurrence of the amplification was verified by 2.5 % agarose gel electrophoresis in TAE buffer (Tris base, glacial acetic acid, EDTA 0,5 M, pH 8), where the correct length of the amplicons was verified by comparison with Gene Ruler™ 100 bp DNA LaddeR markers. DEPC treated water was used instead of cDNA as a negative control for all reactions. The DNA patterns were displayed under UV light and the images were captured with a PowerShot A610 Canon camera.

Solubilisation and quantification of Oil Red O production

To evaluate intracellular lipid accumulation, 20×10^4 ASCs were plated in 24 wells plates and subjected to a differentiation process with glyphosate at 0 or 4 µg/mL. After 12 days the cells were washed 3 times with PBS, fixed with 4 % formaldehyde for 1 hour and washed with 60 % isopropanol. The cells were then dried and stained with Oil Red O staining solution for 30 minutes. After multiple washings, Oil Red O was solubilized in each well with 60 % isopropanol for 2 hours and the absorbance measured at 540 nm. Absorbance was considered to be proportional to the amount of differentiated cells.

Adipose cell counting

5×10^4 MSCs were seeded on a cover slip in 6-well plates and subjected to the adipose differentiation process with glyphosate at 0 or 4 µg/mL. After 12 days, cover slips were fixed with formalin (4 %) and stained with Oil Red O. Then, cells were stained in Mayer haematoxylin for 3 minutes and images made by light microscopy (total magnification 10x) and quantified by counting the stained cells in 10 different fields.

Statistical analysis

The experiments were performed five times on granulosa cells and three times on ASCs and six replicate wells were used for the assessment of the effect of glyphosate. Every time the adipose tissue was collected from 3 pigs. Data are presented as the mean ± SEM. Statistical differences were calculated by ANOVA

using Statgraphics software. In the presence of a significant difference ($p < 0.05$), the means were subjected to Scheffè' F test for multiple comparisons.

II. RESULTS AND DISCUSSION

The effect of glyphosate on swine granulosa cells

Glyphosate statistically significantly decreased cell proliferation ($p < 0.001$) as evaluated by BrdU incorporation and cell viability ($p < 0.05$) as measured by ATP production without a concentration-response relationship. Granulosa cell steroidogenesis was affected by glyphosate. E2 secretion was statistically significantly inhibited ($p < 0.05$) whereas P4 secretion was statistically significantly increased ($p < 0.05$) both at all tested concentrations but without a concentration-response relationship. While O_2^--production was not statistically significantly modified by glyphosate at any of the concentrations tested, NO production was significantly stimulated ($p < 0.001$) at all the tested concentrations although with no statistically significant differences amongst them. Scavenging activity, represented by non-enzymatic antioxidant power, was significantly inhibited ($p < 0.05$) by glyphosate at all the concentrations tested but also without significant differences among them.

The effect of glyphosate on swine adipose stromal cells (ASCs)

The histological analysis of the isolated fragments obtained from pig fat showed typical characteristics of adipose tissue. In particular, adipocytes containing a single large lipid droplet with thin cytoplasm and flattened peripheral nucleus are surrounded by a microvasculature dispersed in the stromal tissue. After 3 to 4 days of *in vitro* culture of the fragmented explants, there was a migration of cells characterized by typical mesenchymal fibroblast-like morphology, which proliferated in adhesion to the bottom of the well. After 5 to 7 days from fragment removal, ASCs reached 80 % confluence in monolayer, maintaining their morphological characteristics. The proliferation of ASCs under basic culture conditions was statistically significantly increased ($p < 0.001$) (454 ± 12 vs 476 ± 7), while glyphosate treatment at 4 µg/mL significantly decreased ($p < 0.001$) the viability of proliferating ASCs both after 48 (432 ± 10) and 72 hours (451 ± 7). The expression of PPARγ and LEP, adipogenic markers was not observed in undifferentiated ASCs, but was detected following adipogenic differentiation. Adipogenic differentiation of ASCs was achieved, as shown by the appearance of red lipid droplets in the differentiated cell cytoplasm. Differentiated cell counts showed a significant inhibition ($p < 0.05$) of the adipogenic process by glyphosate at 4 µg/mL. These data were confirmed by spectrophotometric evaluation of solubilized Oil RedO.

Discussion

This study shows that glyphosate is able to significantly stimulate P4 production. This is a critical effect for granulosa cells because P4 is a hallmark of luteinization. Ovarian steroidogenesis is a key function for the survival of GCs, and is essential for follicular development and growth. To verify the potential effects of glyphosate on follicular physiology, the present study also analyzed the possible effect of glyphosate on granulosa cell proliferation and metabolic activity. The data show an inhibition of DNA replication activity and ATP production at all concentrations analyzed suggesting a disruption of follicular development in vivo. Since endothelial cell characteristics have been recently attributed to granulosa cells, it is interesting to evaluate the increased NO production in granulosa cells following glyphosate exposure. It can be hypothesized that glyphosate exerts direct pro-angiogenic effects on pigs GCs at all tested concentrations as a result of a significant increase in NO, which is a key molecule of this process. The stimulation of NO production can also be associated with an increase in oxidative stress which seems to be supported by the glyphosate-induced reduction of the non-enzymatic antioxidant power in GCs at all concentrations tested. Adipose stromal cells (ASCs) were isolated from abdominal subcutaneous fat tissue of pig and characterized. Their ability to differentiate into adipocytes under appropriate stimuli has been evaluated on the basis of the expression of PPARγ and leptin genes that was not detected in undifferentiated ASCs. These results were essential to define an adequate experimental model to investigate potential effects of glyphosate on the expression of these adipogenic markers. The inhibitory effect of glyphosate during induction of ASCs adipogenic differentiation suggests that this substance, once accumulated as a consequence of environmental exposure, can interfere with adipose tissue biology *in vivo*. Moreover, glyphosate was found to significantly decrease the viability of ASCs after 48 and 72 hours of exposure. Such evidence appears to be critical since it suggests a direct interference of glyphosate with adipose tissue function.

Study conclusion

In this *in vitro* study it is shown that glyphosate inhibits cell growth, 17-β estradiol production and non-enzymatic scavenging activity and increased progesterone production and nitric oxide secretion in granulosa cells. In addition, glyphosate significantly decreased the viability of adipose stromal cells (ASCs) and inhibited their adipogenic differentiation. These data are indicative of the interference of glyphosate with the main functional parameters of granulosa cells and ASCs and could affect both reproductive function and adipogenic processes *in vivo*.

III. CONCLUSIONS

9. Assessment and conclusion

Assessment and conclusion by applicant:

The effects of glyphosate on functional parameters of granulosa cells and adipose stromal cells from swine were investigated *in vitro*. In granulosa cells the effect of glyphosate was studied on cell proliferation, cell viability, steroid production, superoxide anion production, NO production and ferric reducing activity. In adipose stromal cells the effect of glyphosate was studied on cell viability, adipogenic differentiation, adipogenic marker genes (PPARγ and leptin), intracellular lipid accumulation and adipose cell count. Glyphosate was found to significantly decrease cell proliferation, cell viability, estrogen production and ferric reducing capacity and increase progesterone and NO production in granulosa cells when tested at concentrations ranging from 0.2 to 16 µg/mL. However, in none of the assays with granulosa cells a concentration-response relationship was established. Glyphosate treatment at 4 µg/mL significantly decreased ($p < 0.001$) the viability of proliferating adipose stromal cells after 48 and 72 hours. Differentiated cell counts showed a significant inhibition ($p < 0.05$) of the adipogenic process by glyphosate at 4 µg/mL. Since only one concentration of glyphosate was tested it was not possible to establish a concentration-response relationship. In this publication it is suggested that glyphosate interferes with the main functional parameters of the granulosa cell which could affect reproductive function. No effects on female reproductive function were reported in the rat in regulatory reproductive toxicology tests at doses beyond 2,000 mg/kg bw/day producing systemic glyphosate concentrations that are higher than those tested in this study.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate tested was not sufficiently characterized, no positive controls were included in the assays and only one dose level was used for the testing of adipose stromal cells.

Reliability criteria for *in vitro* toxicology studies

Publication: Gigante *et al.*, 2018.	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	N	Purity is not reported. Source: Sigma Chemical Co, St Louis, USA.
Only glyphosate acid or one of its salts is the tested substance	Y	
AMPA is the tested substance	N	
Study		

Test system clearly and completely described	Y	Granulosa cells from swine ovaries, adipose stromal cells from swine adipose tissue.
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	N	
Test concentrations in physiologically acceptable range (< 1 mM)	Y	0.2, 4 or 16 µg/mL.
Cytotoxicity tests reported	Y	
Positive and negative controls	N	No positive control.
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate tested was not sufficiently characterized, no positive controls were included in the assays and only one dose level was used for the testing of adipose stromal cells.		

Assessment and conclusion by RMS:

CA 5.8.3/013

1. Information on the study

Data point:	CA 5.8.3
Report author	Vanlaeys, A. *et al.*
Report year	2018
Report title	Formulants of glyphosate-based herbicides have more deleterious impact than glyphosate on TM4 Sertoli cells
Document No	doi.org/10.1016/j.tiv.2018.01.002 E-ISSN: 1879-3177
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
Previous evaluation	No
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary

Executive Summary

Roundup and Glyphogan are glyphosate-based herbicides containing the same concentration of glyphosate

and confidential formulants. Formulants are declared as inert diluents but some are more toxic than glyphosate, such as the family of polyethoxylated alkylamines (POEA). In this study glyphosate alone, glyphosate-based herbicide formulations and POEA on the immature mouse Sertoli cell line (TM4) was tested, at concentrations ranging from environmental to agricultural-use levels.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material
Glyphosate was purchased from Sigma-Aldrich, St Louis, USA. The purity was not reported.

Culture of TM4 Sertoli cells and treatments
The murine TM4 Sertoli cell line was obtained from the American Type Culture Collection (ATCC Manassas, USA). Cells were maintained in DMEM/HamF12 medium containing 0.2 % glutamine, 1.2 g/L $NaHCO_3$, 15mM Hepes, 5 % horse serum and 2.5 % foetal calf serum, 100 U/mL of antibiotics and fungizone (complemented DMEM/Ham F12 medium) at 37 °C (5 % CO2, 95 % air) during 24 hours to 80 % confluence in 24-well plates or in 6-well plates (for measurement of GST activity). Cells were then exposed to various concentrations of glyphosate.

B. STUDY DESIGN

Crystal violet cell viability assay
After incubation with glyphosate at different concentrations, culture supernatants were discarded and cells incubated in medium containing crystal violet solution (0.1 % w/v in PBS 0.01 M, pH 7.4) for 30 minutes at 20 °C with gentle rocking. Excess dye and non-adherent dead cells were removed through 3 washing steps with PBS. Diluted acetic acid solution (10 %) was then added to release the crystal violet taken up by cells, and the optical density reflecting living adherent cells was determined by absorption at 600 nm using a plate reader.

MTT cytotoxicity assay
This enzymatic test is based on the cleavage of MTT (tetrazolium dye, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) into a blue-colored product (formazan) by the mitochondrial enzyme succinate dehydrogenase. Activity of mitochondrial dehydrogenase enzymes indirectly measures activity of mitochondrial respiration and antioxidant defense systems. Culture medium was removed, and cells were washed once with PBS and then incubated with 500 µL MTT solution per well after each treatment. The plates were incubated for 3 hours at 37 °C. The reaction was stopped by placing the plates for 10 minutes at 4 °C followed by adding to each well 500 µL 0.04 N hydrochloric acid-containing isopropanol. The plates were then vigorously shaken for 40 min to solubilize the blue formazan crystals formed. Absorbance was measured by spectrophotometry at 570 nm.

Glutathione-S-transferase (GST) activity
For preparation of S9 fractions enriched in GST, the medium was removed, and cells were detached by treatment with trypsin-EDTA and washed twice with PBS at room temperature. Cells were then resuspended in 500 µL of 50 mM phosphate buffer at pH 7.2 containing 0.25 M sucrose and 1mM DTT. Then, they were homogenized and centrifuged at 9000g at 4 °C for 30 minutes. Supernatants corresponding to the S9 fraction were collected and stored at -80 °C. The protein concentration of each S9 fraction was determined using the Bradford assay. For the determination of GST activity 250 µg of S9 cell fraction was mixed with 10 µL of 100 mM reduced L-glutathione and 990 µL phosphate buffer (pH 6.5). The reaction was initiated by the addition of 10 µL of 100 mM 1-chloro-2,4-dinitrobenzene (CDNB) solution in 95 % ethanol. After incubation for 90 seconds at 37 °C absorbance was measured at 340 nm every 60 seconds for 3 minutes.

Lipid Sudan Black B staining
Sudan Black B stains lipids, including phospholipids and sterols. Culture medium was removed and cells were washed once with PBS. Cells were then incubated for 5 minutes at room temperature with 500 µL of

Sudan Black solution with gentle shaking. This solution was removed, and cells were washed 3 times or more with 70 % ethanol to remove excess stain and then at least 3 times with PBS. Then, intracellular Sudan Black B was extracted by incubation with DMSO for 30 minutes with gentle rocking and absorbance measured at 600 nm using a microplate reader.

Statistical analysis

The experiments were repeated at least in triplicate in different weeks on three independent cultures on each occasion (n=9). All data are presented as the mean ± standard error (SEM). Statistical differences from controls were determined by an ANOVA test. Results were statistically significantly different from controls when $p < 0.05$.

II. RESULTS AND DISCUSSION

Viability of TM4 cells

Glyphosate was found to have no impact on cell viability of TM4 cells after 24 hours of exposure at concentrations ranging from 10 to 10,000 ppm.

Mitochondrial succinate dehydrogenase activity

The measurement of succinate dehydrogenase (SD) activity was used to assess the effect of glyphosate on mitochondrial function and viability after 24 hours of exposure. Reduced SD activity was seen for glyphosate over the entire concentration range from 0.001 % (approx. 85 %) to 1 % (approx. 75 %) as derived from the graph.

Inhibition of glutathione-S-transferase (GST) activity

The effect of glyphosate on GST activity involved in the anti-xenobiotic defense system was evaluated. At an LC_{50} concentration glyphosate was found to have no impact on GST activity.

Lipid droplet accumulation

After staining with Sudan Black B exposure of TM4 cells to glyphosate for 24 hours at 2,500 or 5,000 ppm induces an increase in cytoplasmic lipid droplets as assessed microscopically.

Discussion

Measurements of cell viability, respiratory chain activity, detoxification system and lipid accumulation were undertaken in immature murine TM4 Sertoli cell line following 24 hours of exposure to glyphosate at concentrations ranging from environmental levels to agricultural use concentrations (1 %, 10,000 ppm). This study demonstrated that at sub-agricultural use levels (10-10,000 ppm) TM4 cell viability is not affected by glyphosate. Exposure of TM4 cells to glyphosate reduces mitochondrial succinate dehydrogenase (SD) activity at the lowest concentrations tested in TM4 cells, as compared with other cell types. The formulants present in commercial herbicides are known to increase glyphosate penetration into cells by membrane disruption and thus probably potentiate perturbation of mitochondrial permeability induced by glyphosate. This mechanism may explain the higher toxicity of formulations on mitochondrial activity. In this study, it was also demonstrated that 24 hours exposure of TM4 cells to glyphosate induced lipid droplet accumulation.

III. CONCLUSIONS

9. Assessment and conclusion

Assessment and conclusion by applicant:

In this study the effect of glyphosate on murine TM4 Sertoli cells was investigated *in vitro*. The endpoints were cytotoxicity, glutathione transferase activity and lipid accumulation. In contrast to the glyphosate-based formulations and co-formulants tested glyphosate was found to have no impact on cell viability after 24 hours of exposure at concentrations ranging from 10 ppm to 10,000 ppm. Glyphosate reduced succinate dehydrogenase to some extent over the entire concentration range from 10 (approx. 85 % of control) to 10,000 ppm (approx.75 % of control) with no dose-effect relationship and was found to have no impact on glutathione transferase activity. Exposure of TM4 cells to glyphosate for 24 hours at 2,500 or 5,000 ppm induces an increase in cytoplasmic lipid droplets. These concentrations are far beyond what is physiologically feasible *in vivo*.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate used is not sufficiently characterized and no positive controls were used in any of the assays conducted.

Reliability criteria for *in vitro* toxicology studies

Publication: Vanlaeys *et al*., 2018	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y?	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	N	Purity not reported. Source: Sigma-Aldrich, St Louis, USA.
Only glyphosate acid or one of its salts is the tested substance	N	Also co-formulants and formulations were tested.
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	Y	
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	N	
Test concentrations in physiologically acceptable range (<1 mM)	Y (partly)	
Cytotoxicity tests reported	Y	
Positive and negative controls	N	No positive controls used.
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Reliability not assignable		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate used is not sufficiently characterized and no positive controls were used in any of the assays conducted.		

Assessment and conclusion by RMS:

CA 5.8.3/014

1. Information on the study

Data point:	CA 5.8.3
Report author	Mesnage, R. *et al.*
Report year	2017
Report title	Evaluation of estrogen receptor alpha activation by glyphosate-based herbicide constituents
Document No	doi.org/10.1016/j.fct.2017.07.025 E-ISSN: 1873-6351
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Yes/Reliable without restrictions

2. Full summary

Executive Summary

In this study, the estrogenic potential of glyphosate, commercial GBHs and polyethoxylated tallowamine adjuvants present as co-formulants in GBHs was evaluated. Glyphosate (≥10,000 µg/L or 59 µM) promoted proliferation of estrogen-dependent MCF-7 human breast cancer cells. Glyphosate also increased the expression of an estrogen response element-luciferase reporter gene (ERE-luc) in T47DKBluc cells, which was blocked by the estrogen antagonist ICI 182,780. Commercial GBH formulations or their adjuvants alone did not exhibit estrogenic effects in either assay. Transcriptomics analysis of MCF-7 cells treated with glyphosate revealed changes in gene expression reflective of hormone-induced cell proliferation but did not overlap with an ERα gene expression biomarker. Calculation of glyphosate binding energy to ERα predicts a weak and unstable interaction (-4.10 CAl mol-1) compared to estradiol (-25.79 CAl mol-1), which suggests that activation of this receptor by glyphosate is via a ligan- independent mechanism. Induction of ERE-luc expression by the PKA signaling activator IBMX shows that ERE-luc is responsive to ligand-independent activation, suggesting a possible mechanism of glyphosate-mediated activation.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material

Glyphosate used was the PESTANAL® analytical standard (≥98.0 %) obtained from Sigma-Aldrich (UK). The batch of glyphosate (≥98.0 %) purchased from AccuStandard (New Haven, CT, USA) was tested exclusively in the ERE transcription luciferase reporter gene assay. Glyphosate-based formulations available on the market were Glyphogan (France, 39-43 % iso-propylamine salt of glyphosate, 13-18 % of POEA), Roundup Grand Travaux Plus (France, 450 g/L of glyphosate, 90 g/L of ethoxylated etheralkylamine), Roundup Original DI (Brazil, 445 g/L of glyphosate diammonium salt, 751 g/L of other ingredients) and Roundup Probio (UK, 441 g/L of the potassium salt of glyphosate, and other ingredients). POEA was purchased from ChemService (West Chester, PA, USA). The agricultural spray adjuvant was Gen-amin T200 (France, 60-80 % of POE-15).

Cell culture

MCF-7, MDA-MB-231 and T47D cell lines were obtained from Prof Joy Burchell (Research Oncology Department, King's College London). T47D-KBluc cells were purchased from the American Type Culture

Collection (ATCC, Teddington, UK) and harbour a stably integrated copy of a luciferase reporter gene under control of a promoter containing ERE. All cells were grown in a maintenance medium. Stock solutions of glyphosate, glyphosate-based herbicide formulations, POEA and Genamin T200 surfactant formulation were prepared in serum-free medium and adjusted to pH 7.2. Stock solutions of with estradiol, 3-isobutyl-1-methylxanthine (IBMX) and BPA were prepared by dissolution in ethanol. The solutions for testing were prepared by dilution of the stock solutions in test medium taking care that the solvent concentrations were always kept below 0.5 % for the cell assays and below 0.0005 % for transcriptome profiling. Cells were released from the flask substrate with trypsin and counted with a hemocytometer prior to seeding. A 24-hour recovery period was allowed for cell adherence in DMEM maintenance medium before cultures were subjected to the desired tests.

B. STUDY DESIGN

E-screen assay

The E-screen allows the determination of estrogenic effects by measuring ER-mediated cell proliferation in hormone responsive cells (MCF-7, MDA-MB-231, T47D). Cells were seeded into 48-well plates at a density of 20,000 cells per well in 250 µL maintenance medium. Following a 24-hour incubation to allow cell attachment, the medium was changed to the medium containing the test compounds. The test medium was refreshed after 3 days. Following another 3-day period of incubation, an MTT assay was performed. The MTT test allows the measurement of cytotoxic effects since the activity of mitochondrial dehydrogenase enzymes indirectly reflects cellular mitochondrial respiration. Cells were incubated with 250 µL of MTT solution for 2 hours. The test was terminated by lysing the cells with dimethyl sulfoxide (DMSO) and optical density was measured at 570 nm using the SPECTROstar Nano plate reader. The proliferative effect was expressed as a percentage of the control cell culture receiving no treatment.

ERE-luciferase reporter gene assay

The ERE-mediated transcription of a luciferase reporter gene was determined in T47D-KBluc cells using the Steady-Glo® luciferase assay system following the manufacturer's instructions. T47D-KBluc cells were seeded in 96-well plates at a density of 20,000 cells per well in 50 µL of maintenance medium and allowed to attach overnight. Prior to adding the test substance an initial 24-hour incubation was performed in the absence of test substance to improve residual estrogen clearance and assay sensitivity. After incubation, Steady-Glo® luciferase reagent was added. The plates were left to stand for 10 minutes in the dark at room temperature to allow cell lysis. Bioluminescence was measured using the Orion II microplate luminometer. ER-mediated gene activation was confirmed by ascertaining if the observed effects were subject to inhibition by addition of the estrogen antagonist ICI 182,780.

Microarray gene expression profiling

MCF-7 cells were seeded into 96-well plates with maintenance medium at a density of 20,000 cells per well. After steroid deprivation in hormone free medium, the cells were treated with test substance in triplicate in three independent experiments. RNA extraction was performed using the Agencourt RNAdvance Cell V2 kit according to the manufacturer's instructions. The samples were checked for RNA quality and quantified. Subsequently, technical replicates of samples, were pooled appropriately such that the final input amount of each biological replicate was 3 ng. Transcriptome gene expression profiles were determined using the Affymetrix Human Transcriptome 2.0 Array. Data were imported and normalized together in Omics Explorer 3.0 using the Robust Multi-array Average (RMA) sketch algorithm. These microarray data were submitted to Gene Expression Omnibus (NCBI) and are accessible through accession number GSE86472.

RNA-sequencing gene expression profiling

Gene expression profiling was performed by applying Illumina sequencing by synthesis technology. The RNA-seq data were submitted to Gene Expression Omnibus (NCBI) and are accessible through accession number GSE8770.

Statistical analysis

The ERE transcription luciferase reporter gene assay and the E-Screen assay were performed 4 and 6 times in triplicate, respectively. The concentrations required to elicit a 50 % response (AC_{50}) were determined using a nonlinear regression fit. For the transcriptome analysis, pair-wise comparisons were performed using a t-test controlling for batch effects in Omics Explorer 3.0. Affymetrix microarray and RNA-seq gene expression profiling were performed 3 times in triplicate. Data used for the functional analysis were selected at cut off p-values of <0.05 with fold change >1.2 to evaluate the ER activation signature. Gene and disease ontology were analyzed using the Thomson Reuters MetaCore Analytical Suite recognizing network objects (proteins, protein complexes or groups, peptides, RNA species, compounds among others). The p-values are determined by hypergeometric calculation and adjusted using the method of Benjamini and Hochberg.

II. RESULTS AND DISCUSSION

E-screen assay

The AC_{50} of 17β-estradiol and BPA in MCF-7 human breast cancer cells was 0.0013 µg/L and 46 µg/L, respectively. Glyphosate induced cell proliferation starting between 1,000 and 10,000 µg/L and peaking around 1,000,000 µg/L. Similar but less pronounced results were observed with the T47D cell line with cells retaining a response to glyphosate but not to Roundup Probio. No proliferative effects were observed in the ER-negative, hormone-independent MDA-MB-231 cell line, suggesting that the proliferative effects were mediated via the ER. Roundup Probio, tested in MCF-7 cells induced a non-statistically significant trend of cell proliferation.

ERE-luciferase reporter gene assay

Glyphosate stimulated ERE-mediated transcription of the luciferase reporter gene starting at a concentration of 1,000 µg/L. Roundup Probio did not show ER activity at a glyphosate equivalent concentration, which induced a cell proliferative effect when glyphosate is tested alone. This may be explained by the potentially higher toxicity of the glyphosate-based formulation, which could have resulted in cell death at the higher concentrations tested. Two other commercial formulations, Roundup Original DI and Roundup Grand Travaux Plus, also gave negative results. To evaluate whether the luciferase reporter gene stimulatory effects observed with glyphosate were mediated through the ER, experiments were performed with the addition of the potent ER antagonist ICI 182,780. This antagonist was effective at suppressing ER activation induced by 0.001 and 0.01 µg/L but not at 0.1 µg/L of 17β-estradiol. The addition of ICI 182,780 effectively blocked the stimulatory effects of glyphosate at 2,000-20,000 µg/L, confirming its agonist-like mode of action.

Microarray gene expression profiling

MCF-7 cells treated for 48 hours with glyphosate, Roundup Probio, POEA, or bisphenol A, and 17β-estradiol were subjected to full transcriptome profiling. The analysis of gene ontology confirms that genes having their expression altered by treatment of MCF-7 cells with 10,000 µg/L glyphosate were involved in cell cycle regulation, as well as in stimulation by steroid hormones. The transcriptome of glyphosate-treated cells was also reflective of cell death through apoptosis. Roundup ProBio was assessed at the glyphosate equivalent concentrations of 1 µg/L (environmental level), 100 µg/L and 1000 µg/L (showing a cell proliferative trend). The statistical analysis of differential expression showed that genes having their function altered by Roundup or POEA had low fold changes). No genes whose expression was increased or repressed by POEA showed fold changes higher than 2. The study of transcriptome profiles shows that POEA alone is unlikely to have estrogenic effects. This was confirmed in that none of the treatments exhibited statistically significant correlations to the ER biomarker

RNA-sequencing gene expression profiling

In order to confirm endocrine disturbances provoked by glyphosate, RNA extracted from MCF-7 cells treated with glyphosate (10,000 µg/L), estradiol (0.27 µg /L) or bisphenol A (80 µg/L) for 48 hours were subjected to a full RNA-Seq analysis using the Illumina sequencing platform. Although their gene expression profile were different, MCF-7 cells treated by these 3 chemicals presented alterations reflecting

a response to steroid hormones and a modulation of cell proliferation, although the significance of the overlapping genes and those in these pathways was lower for glyphosate than the other compounds. Overall, the RNA-seq method was more sensitive and identified 2-3 times more genes whose expression was significantly altered compared to the microarray approach. A total of 5102, 2939 and 1083 genes had their expression significantly disturbed by estradiol, BPA and glyphosate, respectively. Afterwards the ER gene expression biomarker was applied to see if ERα agonist effects could be detected after transcriptome profiling using RNA-Seq platforms. The results were similar to those obtained using the microarray data. Glyphosate failed to pass the threshold of significance for ERα activation. Although the RNA-seq platform was able to identify more statistically significant genes than microarrays, the genes altered differed between the two methods.

Molecular dynamics simulation and ONIOM binding energy calculations
The ability of glyphosate to bind to ERα was evaluated by molecular dynamics simulations and ONIOM calculations. Results from the molecular dynamics simulations of glyphosate-ER interactions reveal that glyphosate enters the active site with a large number of water molecules. The glyphosate phosphonate group interacts with ARG 394 by creating hydrogen bonds. It was noted that glyphosate is unlikely to interact with HIS 524, a residue having a pivotal role in maintaining protein structure in the biologically active agonist conformation. The results of the ONIOM binding energy assessment strongly imply that the binding of glyphosate at the active site of the receptor is weak and unstable, suggesting that glyphosate is unlikely to bind to ERα.

Discussion
The results of this study suggest that glyphosate is a weak activator of ERα in hormone-dependent hu-man breast cancer cells. The glyphosate intake necessary to reach a systemic concentration representative of the estrogenic effects shown in this study would only be encountered in cases of extreme exposures (incidental ingestion, mishandling). An evaluation of the glyphosate binding energy confirmed that this compound is unlikely to activate the ER. The presence of glyphosate-associated cytotoxic effects could explain the discrepancies between the results we obtained with the ERα biomarker and those from the cellular assays. It is thus plausible that glyphosate is activating ERα through a ligand-independent mechanism albeit at high concentrations. To determine any estrogenic potential of adjuvant co-formulants, a number of glyphosate-based formulations were tested and no estrogenic effects could be demonstrated. However, cytotoxicity was observed at glyphosate-equivalent concentrations lower than those required to elicit a proliferative response to glyphosate alone.

Study conclusion
This study has demonstrated that glyphosate activates ERα in breast cancer cells but only at relatively high concentrations, and that this activation happens through a ligand-independent pathway. These results suggest that humans exposed to glyphosate would not exhibit ER activation at typical exposure levels.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant: The objective of this study was to evaluate the possible estrogenicity of glyphosate and glyphosate-based formulations and their adjuvants. The tests performed were the E-screen using different cell lines, the ERE-luciferase reporter gene assay, mmicroarray gene expression profiling and RNA-sequencing gene expression profiling. An increase in cell proliferation was observed in human breast cancer cells (MCF-7) at 10,000 µg/L and reached a maximum response at 1,000,000 µg/L. Similar but less pronounced results were observed with the T47D cell line. Glyphosate stimulated ERE-mediated transcription of the luciferase reporter gene starting at a concentration of 1,000 µg/L. The analysis of gene ontology confirms that genes having their expression altered by treatment of MCF-7 cells with glyphosate were involved in cell cycle regulation, stimulation by steroid hormones and cell death through apoptosis. ONIOM binding energy assessment strongly implies that the binding of glyphosate at the active site of the

estrogen receptor is weak and unstable, suggesting that glyphosate is unlikely to bind to ERα. This study has demonstrated that glyphosate activates ERα through a ligand-independent pathway only at high concentrations that are not encountered at typical exposure levels. This publication is considered relevant for glyphosate risk assessment and reliable without restrictions.

Reliability criteria for *in vitro* toxicology studies

Publication: Mesnage *et al*., 2017	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity of 98.0 %. Source: Sigma-Aldrich (UK).
Only glyphosate acid or one of its salts is the tested substance	Y	Also glyphosate based formulations and surfactants were tested.
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	Y	
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	NA	
Test concentrations in physiologically acceptable range (< 1 mM)	Y	
Cytotoxicity tests reported	Y	
Positive and negative controls	Y	
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions	Y	
Reliable with restrictions		
Not reliable		
This study has demonstrated that glyphosate activates ERα through a ligand-independent pathway only at high concentrations that are not encountered at typical exposure levels. This publication is considered relevant for glyphosate risk assessment and reliable without restrictions.		

<u>Assessment and conclusion by RMS</u>:

CA 5.8.3/015

1. Information on the study

Data point:	CA 5.8.3
Report author	Thongprakaisang, S. *et al.*
Report year	2013
Report title	Glyphosate induces human breast cancer cells growth via estrogen receptors
Document No	doi.org/10.1016/j.fct.2013.05.057 E-ISSN: 1873-6351. L-ISSN: 0278-6915
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary

Executive Summary

This study focuses on the effects of pure glyphosate on estrogen receptors (ERs) mediated transcriptional activity and their expressions. Glyphosate exerted proliferative effects only in human hormone-dependent breast cancer, T47D cells, but not in hormone-independent breast cancer, MDA-MB231 cells, at 10^{-12} to 10^{-6} M in estrogen withdrawal condition. The proliferative concentrations of glyphosate that induced the activation of estrogen response element (ERE) transcription activity were 5-13 fold of control in T47D-KBluc cells and this activation was inhibited by an estrogen antagonist, ICI 182780, indicating that the estrogenic activity of glyphosate was mediated via ERs. Furthermore, glyphosate also altered both ERα and β expression. These results indicated that low and environmentally relevant concentrations of glyphosate possessed estrogenic activity.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material

Glyphosate (purity >98 %) was obtained from AccuStandard (New Haven, CT, USA).

Cell lines and culture conditions

A hormone-dependent human breast cancer cell line (T47D), a stably ERE-luc construct transfected hormone-dependent breast cancer cell line (T47D-KBluc) and a hormone-independent human breast cancer cell line (MDA-MB231) were obtained from the American Type Culture Collection (ATCC).

B. STUDY DESIGN

In vitro estrogen receptor activation-reporter assay

To study the estrogenicity and/or anti-estrogenicity of glyphosate, the T47D-KBluc cell line, stably transfected with a triplet ERE (estrogen response element)-promoter-luciferase reporter gene construct was used in this study. To minimize the effect of estrogen in the medium, 5 days prior to the start of the assay, cells were incubated in a non-phenol red RPMI modified medium with replacement of 10 % FBS by 10 %

dextran-charcoal treated FBS (CSS). One day prior to the assay, cells were seeded at 10^4 cells/100 µL/well and were allowed to attach overnight. The dosing medium was further modified by reduction to 5 % CSS and then replaced with 100 µL/well of dosing medium containing glyphosate at concentrations ranging from 10^{-12} to 10^{-6} M. Estradiol (E2) in the same range of concentrations was used as the positive control. Dosing medium without glyphosate was used as the negative control and wells without cells were used as the blank. After 24 hours of incubation, cells were washed with 100 µL PBS and harvested in 25 µL lysis buffer. The luciferase assay was performed by injecting 50 µL of reaction buffer and 50 µL of 1 mM D-luciferin and fluorescent intensity was measured by means of the microplate luminometer. Luciferase activity was quantified as relative light units (RLU).

Cell viability MTT assay

Cell growth and cell viability were tested using the 3-(4,5-dimetylthiazol, 2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent assay. Cells were seeded at 10^4 cells/100 µL/well in 96-well microtiter plates. For the E2 withdrawal condition, cells were cultured in 10 % CSS and a non-phenol red RPMI medium for 4 days before seeding. After 24 hours the cells were treated with E2 or glyphosate at concentrations ranging from 10^{-12} to 10^{-6} M. For E2 receptor antagonist conditions, E2- or glyphosate-treated cells were co-incubated with ICI 182780 at 1 and 10 nM. Cell sensitivity to a chemical was expressed as the % cell viability compared to control cells.

Cell number counting

T47D Cells were prepared in E2 withdrawal conditions 4 days before the start of the assay. Cells were placed in a 24 well culture plate with 10^4 cells/mL/well and incubated overnight. The medium was then replaced with 1 mL of treatment solution and incubated for 72 hours. Afterwards, the cells were washed with 1 mL PBS and then 100 µL of a trypsin–EDTA solution was added to detach. An aliquot of the cells was taken for counting using a counter analyzer.

Western blot analysis

Whole-cell extracts were prepared from cells treated for 6 and 24 hours with 10^{-12}, 10^{-9}, and 10^{-7} M glyphosate and non-treated control. The cells were lysed, incubated on ice and centrifuged. The supernatants were collected and either processed or stored at -80 °C until use. The protein concentration was measured using Bradford reagent and each lysate was aliquot for an equal amount of protein, 30 µg, before mixing with Laemmli loading buffer and then boiled at 95 °C for 5 minutes. The samples were resolved over 7.5 % polyacrylamide-SDS gels and transferred to a nitrocellulose membrane using a Mini Trans-Blot Electrophoretic Transfer Cell. The membrane was treated with blocking solution for one hour at room temperature and subsequently probed overnight with primary antibody (ERα, ERβ or β-actin) and then rinsed. HRP-conjugated secondary antibodies were added to the membrane for 2 hours and the membranes rinsed with TBS-T. Protein visualization was achieved by using enhanced chemiluminescence and the emitted light was captured on film. The signals on the films were quantified using densitometry.

Statistical analysis

Data are presented as the means ± SE. Statistical significance was determined using the Student's t-test. A two-tailed $p<0.05$ was evaluated as a statistically significant difference.

II. RESULTS AND DISCUSSION

T47D, hormone-dependent breast cancer cell growth

The hormone-dependent T47D and hormone-independent MDA-MB231 cell lines were studied both in completed medium and estrogen withdrawal medium to differentiate the effect of glyphosate from that of endogenous estrogen. Estrogen in a concentration range of 10^{-12} to 10^{-6} M was used as the positive control. Cell growth was assessed using the MTT cell viability assay. The results showed that T47D and MDA-MB231 cells exhibited different patterns of responses to glyphosate. In the absence of E2, glyphosate produced cell proliferation in T47D cells of approximately 15–30 %. This effect was about half of the E2 response which is the most potent agonist in hormone dependent ER-positive breast cancer cells. No effect

was observed on cell proliferation in MDA-MB231 cells both in the absence or presence of E2.

Cell proliferation via estrogen receptors
Due to the fact that the proliferative effect of glyphosate occurred only in T47D cells in the absence of E2, it was hypothesized that ER signaling may be involved in glyphosate-induced cell proliferation. Therefore, the effect of glyphosate on T47D cells was investigated in the presence of an ER antagonist, ICI 182780, to inhibit the estrogen receptor mediated response. The effective concentration of 1 nM of ICI 182780 was added to varying concentrations of glyphosate and E2 to observe its antagonistic activity. The results showed that ICI 182780 at 1 nM mitigated the proliferative effects of both glyphosate and E2. A higher concentration of ICI 182780 (10 nM) completely inhibited the growth promoting effects of glyphosate. These results suggest that glyphosate may produce the proliferative effect via the ER.

ERE-transcription activity via estrogen receptors
T47D-KBluc cells, stably transfected with a triplet ERE (estrogen response element)-promoter-luciferase reporter gene construct, were treated with the concentrations of glyphosate that produced cell proliferation. The results showed that glyphosate in a concentration range of 10^{-12} to 10^{-6} M induced ERE activation 5- to 13-fold of control and these effects were less than about half of that induced by E2. Glyphosate co-incubation with the ER antagonist, ICI 182780, exhibited a significant reduction in responses. ICI 182780 at 10 nM completely inhibited the ERE transcriptional activity of glyphosate. Since glyphosate was shown to induce cell proliferation and ERE activation via the ER, the potential effects of glyphosate on endogenous E2 signaling was investigated. Cells were co-incubated with glyphosate and E2 and the results revealed that glyphosate suppressed E2-induced ERE activation suggesting that glyphosate behaves as an ER antagonist in the presence of E2.

Expression of ERα and ERβ in human breast cancer cells
The expression of proteins involved in ERs including ERα and ERβ, was studied with the Western blot technique. After 6 hours of exposure, glyphosate increased the levels of both ERα and ERβ in a concentration-dependent manner while after 24 hours only ERα showed a significant induction at the highest glyphosate concentration tested (10^{-5} M). This result suggests that glyphosate alters the expression of both ERα and ERβ in human breast cancer cells.

Discussion
In this study, glyphosate was found to increase cell proliferation of a hormone dependent breast cancer T47D cell line at concentrations ranging from 10^{-12} to 10^{-6} M while this effect was not observed in a hormone independent breast cancer MDA-MB231 cell line. The results from the ERE luciferase assay confirmed ER activation because the responses seen could be blocked by the ER antagonist ICI 182780. Glyphosate induced rapid activation of ERβ while activation of ERα was slower but prolonged. It is suggested that glyphosate may behave like a weak xenoestrogen which can activate both subtypes of ER but with a different time course. Although the nature of the binding of glyphosate to the ER is still unknown, the ability of glyphosate to stimulate the ERE-gene transcription activity and upregulation of ERα protein expression suggests that glyphosate may exert the stimulatory effects via an ER-dependent mechanism.

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:
The objective of this study was to investigate the possible estrogenic effect of glyphosate and its mode of action. The endpoints explored were the cell proliferation of hormone-dependent and hormone-independent cell lines with and without an ER antagonist, ERE-transcription activity with and without an ER antagonist, and expression of ERs. Glyphosate was found to produce cell proliferation in a hormone-dependent cancer cell line but not in a hormone-independent cancer cell line in the absence of E2. In the presence of a potent ER antagonist the cell proliferation caused by glyphosate in a hormone-dependent cancer cell line was reduced. The interaction of glyphosate with the ER was confirmed by

ERE activation with and without an ER antagonist. When cells were co-incubated with glyphosate and E2, glyphosate suppressed the E2-induced ERE activation suggesting that glyphosate behaves as an antagonist in the presence of an endogenous agonist. It was demonstrated that glyphosate alters the expression of both ERα and ERβ in human breast cancer cells.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the test results are not corroborated by *in vivo* regulatory ED toxicology studies such as the uterotrophic assay and the female pubertal assay (U.S. EPA Endocrine Disruptor Screening Program).

Reliability criteria for *in vitro* toxicology studies

Publication: Thongprakaisang *et al*., 2013	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity of >98 %. Source: AccuStandard, New Haven, USA.
Only glyphosate acid or one of its salts is the tested substance	Y	
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	Y	
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	NA	
Test concentrations in physiologically acceptable range (< 1 mM)	Y	
Cytotoxicity tests reported	Y	
Positive and negative controls	Y	
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	Y	The slight estrogenic effect of glyphosate reported was not confirmed in *in vivo* studies such as the uterotrophic assay and the female pubertal assay.
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	Results not consistent with other publications on ED.
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the test results are not corroborated by *in vivo* regulatory ED toxicology studies such as the uterotrophic assay and the female pubertal assay.		

Assessment and conclusion by RMS:

CA 5.8.3/016 and CA 5.8.3/017

1. Information on the study

Data point:	CA 5.8.3/016
Report author	Brennan, J.C. *et al.*
Report year	2016
Report title	Development of a recombinant human ovarian (BG1) cell line containing estrogen receptor alpha and beta for improved detection of estrogenic/antiestrogenic chemicals. and its corrigendum
Reference	Environmental Toxicology and Chemistry, 2016, Vol. 35 (1): pp. 91-100
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Yes/Reliable with restrictions

Data point:	CA 5.8.3/017
Report author	Brennan, J.C. *et al.*
Report year	2017
Report title	Corrigendum of: Development of a recombinant human ovarian (BG1) cell line containing estrogen receptor alpha and beta for improved detection of estrogenic/antiestrogenic chemicals.
Reference	Environmental Toxicology and Chemistry, 2017, Vol. 36 (5): p. 1405
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Not applicable

2. Full summary

Executive Summary

The study was based on an estrogen-responsive recombinant human breast cancer cell line (VM7Luc4E2) that was accepted by the US Environmental Protection Agency (USEPA) and Organisation for Economic Co-operation and Development (OECD) as a bioanalytical method to detect estrogen receptor (ER) agonists/antagonists. This cell line contains only 1 of the 2 known ER isoforms, ERα but not ERβ, and the

differential ligand selectivity of these ERs indicates that the currently accepted screening method only detects a subset of total estrogenic chemicals. To improve the estrogen screening bioassay, VM7Luc4E2 cells, which are derived from a human breast cancer cell line (MCF7 cells), were stably transfected with an ERβ expression plasmid and positive clones identified using ERβ-selective ligands. A highly responsive clone (VM7LucERβc9) was identified that exhibited greater sensitivity and responsiveness to ERβ-selective ligands than VM7Luc4E2 cells, and quantitative reverse-transcription polymerase chain reaction confirmed the presence of ERβ expression in these cells. Screening of pesticides and industrial chemicals identified chemicals that preferentially stimulated ERβ-dependent reporter gene expression. Together, these results demonstrate the utility of this dual-ER recombinant cell line for detecting a broader range of estrogenic chemicals than the current VM7Luc4E2 cell line. Additionally, screening with both cell lines allows identification of ERα- and ERβ-selective chemicals.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material
Glyphosate (no data on purity or CAS number) (vehicle control: Dimethyl sulphoxide (DMSO, no data on purity); positive control: 17β-Estradiol (E2; no data on purity))

Cell lines and culture conditions
VM7Luc4E2 and VM7LucERβc9 cell lines were used in the studies with glyphosate. The VM7Luc4E2 cell line was developed by the authors and is used and accepted by USEPA and OECD for determination of ER agonists/antagonists. The VM7LucERβc9 was produced by stable transfection of the VM7LucERβc9 cells with ERβ/pcDNA3.1+Zeo. For routine maintenance, cells were grown in α-minimal essential medium containing 10% fetal bovine serum and additional 600 mg/L Zeocin for the VM7LucERβc9 cells.

B. STUDY DESIGN

Stable transfection
To produce the stable VM7LucERβ cell line, human ovarian carcinoma (VM7Luc4E2) cells containing a stably transfected estrogen-responsive luciferase plasmid were transfected with ERβ/pcDNA3.1+Zeo using FuGene6 transfection reagent according to the manufacturer's recommendation. After 24 h incubation in regular medium, the transfected cells were split 1 to 15 and replated into selective medium containing the antibiotic Zeocin (600 mg/L) which was replaced every 3 d with fresh Zeocin-containing medium. After approximately 3 weeks of growth, 28 individual cell colonies were isolated, their ERβ responsiveness was determined, and those clones exhibiting the greatest induction by ERβ-selective ligands (Br-ERβ-04 [31.6 nM] and genistein [10 nM]), were selected for further evaluation.

RNA isolation and RT-PCR
Cells in 10 cm plates (4 replicates per cell line) were grown in maintenance medium with the addition of Zeocin for the VM7LucERβc9 cells. Total RNA was isolated using RNeasy (Qiagen) according to the manufacturer's instructions (β-mercaptoethanol was added to the lysis buffer immediately before use, and cells were homogenized using QIAshredder homogenizer). Complementary DNA (cDNA) was generated from 2 mg RNA using a high-capacity cDNA Reverse Transcription kit (Applied Biosystems) with random primers followed by a 1:10 dilution with RNAse/DNAse-free water. Real-time quantitative polymerase chain reactions (20 mL) were performed with TaqMan Fast Universal PCR Master Mix and TaqMan Gene Expression assays (Applied Biosystems). The ERα and ERβ messenger RNA (mRNA) levels were quantitated, normalized to β-actin (internal control), and results presented relative to levels in VM7Luc4E2 cells (set to value of 1.0). Primers or probes for human ERβ (ESR2, Hs01100353_m1), human ERα (ESR1, Hs00174860_m1), and human b-actin (ACTB, Hs999999031_m1) were obtained from Applied Biosystems.

Incubation and luciferase analysis

VM7LucERβ cells were switched from maintenance medium to estrogen-stripped medium, containing 10% charcoal-stripped fetal bovine serum and incubated 3 d before plating into white, clear-bottomed 96-well tissue culture plates at a density of 750 000 cells/mL. Cells were allowed to attach for 24 h and then were incubated with carrier solvent DMSO (1% final solvent concentration) or 10 µM glyphosate for 24 h at 37°C with triplicate wells per chemical and controls. After incubation, cells were rinsed twice with phosphate-buffered saline, lysed with Promega cell lysis buffer, and shaken for 20 min at room temperature to allow complete cell lysis. Luciferase activity in each well was measured by a microplate luminometer. For comparative purposes, luciferase induction values were normalized to maximal luciferase induction obtained with 1 nM E2 in each plate (set at 100%). Values represented the mean ± standard deviation (SD) of triplicate incubations in the single screening analysis of the chemical compound.

Statistical analysis

Significant differences between results were determined using one-way analysis of variance (i.e., Student's t test, 2-tailed, type 2, $p<0.05$). Luciferase activity of control (solvent-treated) cells was substracted from that of treated cells to obtain final induced activity (relative light units [RLU]). Final RLU values less than 0 were set at 0 RLUs. Half-maximal concentrations induced (50% effective concentration [EC50]) or repressed (50% inhibitory concentration [IC50]) by chemical or extract were determined using SigmaPlot (Ver 12) by the concentration of chemical that induced exactly 50% of maximal E2-induced luciferase activity.

II. RESULTS AND DISCUSSION

Estrogenic effects of glyphosate

Glyphosate had no estrogenic effect in both VM7LucERβ and VM7LucERβ cells. The induction of luciferase activity was expressed as the mean ± SD of three replicate analyses of one exposure experiments. Values are presented as a percent of maximum E2 induction (set at 100%), and the mean result for glyphosate had been in the range of the solvent control. In the table below the solvent control, DMSO was set to 0% and E2 was set to 100%:

Compound	Activity [%] VM7Luc4E2	Activity [%] VM7LucERβc9
DMSO	0	0
Glyphosate	6 ± 2	2 ± 4
E2	100	100

III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:

Glyphosate did not show any estrogenic activity at a concentration of 10 µM in two cell lines or via the two human estrogen receptor (hER) subtypes, hERα and hERβ. Based on the OECD 455 guideline for the VM7 assay, relative activity for the test substance that is < 10% of the response of a maximally inducing concentration of E2 is considered to be negative. In addition, when the VM7Luc4E2 cell line was validated[45] (previously known as BG1 cell line) the threshold for positive agonist activity was set at 20% of the response of a maximally inducing dose of E2. Therefore, glyphosate is concluded to have no ERα, ERβ agonistic activities, in vitro.

The estrogenic effect of glyphosate has been investigated in VM7LucERβ and VM7LucERβ cells. In

[45] Interagency Coordinating Committee on the Validation of Alternative Methods. 2011. Independent Scientific Peer Review Panel Report: Evaluation of the LUMI-CELL® ER BG1Luc ER TA Test Method. Research Triangle Park, NC:National Institute of Environmental Health Sciences

the assay the induction of luciferase activity as a parameter for receptor activation was measured. The induction of luciferase activity by glyphosate was found to be in the range of the solvent control.

The publication on in vitro estrogen receptor agonistic activity of pesticides indicates that, even though it does not follow a defined guideline, the study is well documented and is scientifically sound. Therefore, the study is reliable with restriction and is relevant for risk assessment.

Reliability criteria for *in vitro* toxicology studies

Publication: Brennan, J.C. *et al.*, 2016	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	Development a new cell line to improve the detection of estrogenic and antiestrogenic chemicals. The OECD TG 455 is partially respected.
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	N	
Only glyphosate acid or one of its salts is the tested substance	Y	
AMPA is the tested substance	NA	
Study		
Test system clearly and completely described	Y	
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	NA	
Test concentrations in physiologically acceptable range (< 1 mM)	Y	
Cytotoxicity tests reported	N	
Positive and negative controls	Y	
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	NA	
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate used was not sufficiently characterized.		

Assessment and conclusion by RMS:

CA 5.8.3/018

1. Information on the study

Data point:	CA 5.8.3/018
Report author	Defarge, N. *et al.*
Report year	2016
Report title	Co-Formulants in Glyphosate-Based Herbicides Disrupt Aromatase Activity in Human Cells below Toxic Levels
Document No	International Journal of Environmental Research and Public Health, 2016, Vol. 13 (264): pp. 1-17
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary

Executive Summary

In this study endocrine disruption of co-formulants in six glyphosate-based herbicides were tested. All co-formulants and formulations were comparably cytotoxic well below the agricultural dilution of 1% (18–2000 times for co-formulants, 8–141 times for formulations), and not the declared active ingredient glyphosate alone. The endocrine-disrupting effects of all these compounds were measured on aromatase activity below the toxicity threshold. Aromatase activity was decreased both by the co-formulants alone (polyethoxylated tallow amine—POEA and alkyl polyglucoside—APG) and by the formulations, from concentrations 800 times lower than the agricultural dilutions; while glyphosate exerted an effect only at 1/3 of the agricultural dilution. It was demonstrated that endocrine disruption by glyphosate-based herbicides could not only be based on the active ingredient but also to co-formulants. These results could explain numerous *in vivo* results with glyphosate-based herbicides that were not seen with glyphosate alone.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material

This study tested formulations, co-formulants and glyphosate alone. The focus of this assessment is on the glyphosate alone data. Glyphosate (isopropylamine salt of n-phosphonomethylglycine) (no data on purity, CAS number 1071-83-6) (vehicle control: not further specified, positive control: Formestan (4-hydroxyandrost-4-ene-3,17-dione; no data on purity))

Cell lines and culture conditions

JEG3 cells (human placental choriocarcinoma cells) used in these assays are well-characterized and validated as useful models to test toxicities of pesticides, corresponding to what is observed in fresh tissue or primary cells. They were reported to be partly less sensitive than primary cells and therefore do not overestimate cellular toxicity.

JEG3 cells were grown in phenol red-free Eagle's minimum essential medium (EMEM) containing 2 mM glutamine, 1% non-essential amino acid, 100 U/mL of antibiotics (a mixture of penicillin, streptomycin and fungizone), 10 mg/mL of liquid kanamycin and 10% fetal bovine serum. JEG3 cells were supplemented

with 1 mM sodium pyruvate. Cells were grown with this medium at 37°C (5% CO2, 95% air) during 48 h to 80% confluence, then washed and exposed 24 h with serum-free EMEM to glyphosate, glyphosate-based herbicide formulations and their co-formulants. Before treatment, all the glyphosate, glyphosate-based herbicide and co-formulants were diluted in serum-free medium and adjusted to a similar pH.

B. STUDY DESIGN

Cell treatment and cytotoxicity biomarkers

Confluent cells (80% of confluence) were washed with serum-free EMEM and then exposed to various concentrations of glyphosate, glyphosate-based herbicide and co-formulants in EMEM serum-free medium for 24 h. After treatments, cytotoxicity was determined by an MTT assay (optical density measured at 570 nm). The bioluminescent Toxilight bioassay (Lonza, Saint Beauzire, France) was applied for the membrane degradation assessment by intracellular adenylate kinase (AK) release in the medium that is described as a necrosis marker. Glyphosate was tested from 1 to 10000 mg/L.

Determination of aromatase activity

Aromatase activity was evaluated according to the tritiated water release assay. This method is based on the stereo-specific release of 1β-hydrogen from the androstenedione substrate, which forms tritiated water during aromatization.

JEG3 cells were exposed for 22 h at 37°C (5% CO2, 95% air) to 700 µL of non-toxic doses of different xenobiotics. Formestane, a well-known aromatase inhibitor, was used as a positive control. Then 50 µL of 200 nM [1β-3H] androstenedione was added, and incubation went on for 120 min more. The reaction was stopped by placing the plates at 4°C for 10 min. Cell fragments were removed by 5 min centrifugation at 2000 rpm at 4°C and by addition of 1 mL of chloroform to the 500 µL supernatant. After 5 min centrifugation at 4000 rpm at 4°C, 0.5 mL of charcoal/dextran (0.25%/0.025%) was added. The mixture was gently agitated, rested at 4°C for 10 min, and centrifuged at 4000 rpm for 10 min at 4°C. Supernatant fractions (500 µL) were harvested in 6 mL vials and 4 mL Ultima Gold LLT was added. The mixture was assessed for radioactivity by a double 5 min scintillation counting. Glyphosate was tested at non-toxic doses in the aromatase assay (16 mg/L).

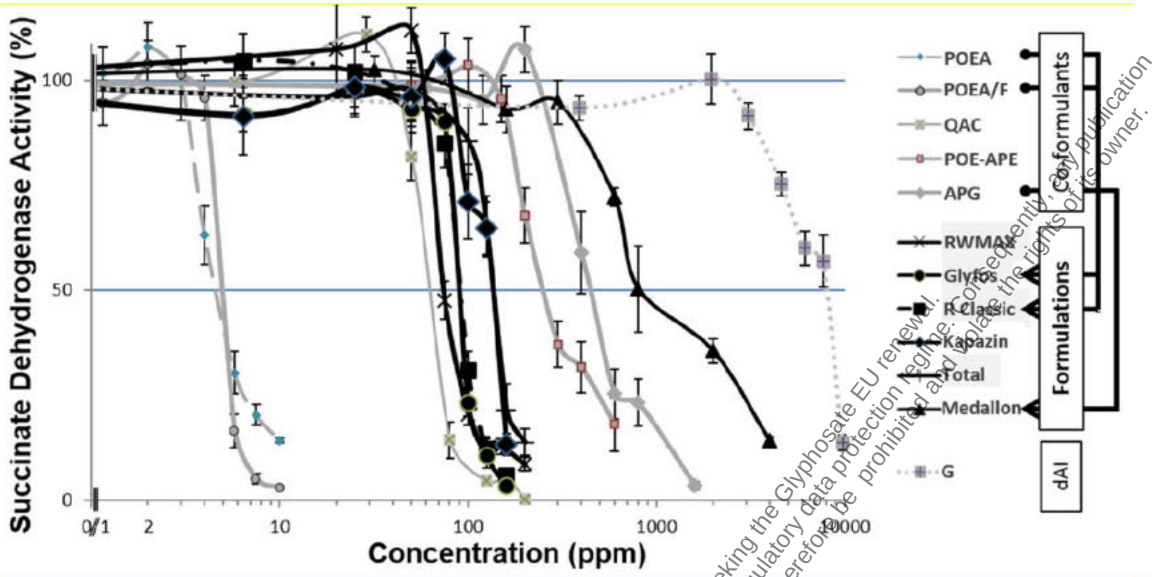
Statistical analysis

All results are means ± SEM. Three independent experiments were performed using triplicate cultures each. In MTT assays, LC50 values were the best-fitted value of a non-linear regression using asymmetric (5-parameters) equation with GraphPad Prism 5 (GraphPad software, La Jolla, CA, USA). Statistical differences were determined by a non-parametric Wilcoxon (Mann–Whitney) rank-sum test or, in case of more than two samples, a non-parametric Kruskal–Wallis test followed by a Dunn's post hoc test for multiple comparisons, using GraphPad Prism 5 (GraphPad software, La Jolla, CA, USA). Significant levels were reported with $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***).

II. RESULTS AND DISCUSSION

Toxicity tests

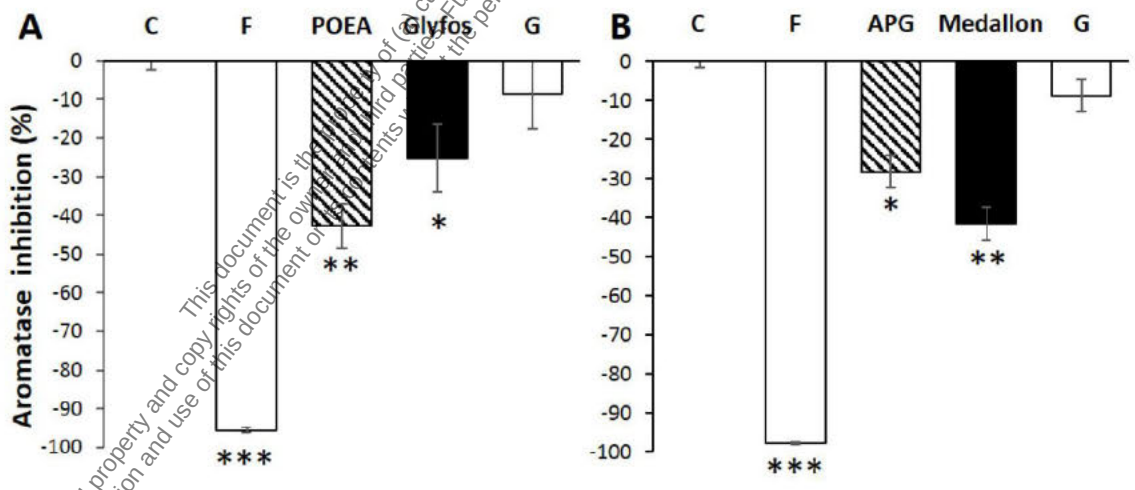
The lowest concentration exerting a significant toxic effect (LOEC) was considered to be the toxicity threshold. The highest concentration without significant cytotoxic effect (NOEC) was also reported. LC_{50}, NOEC, and LOEC values of 7878, 3100, and 4600 mg/L were determined for glyphosate. Glyphosate showed cytotoxic effects at high doses only, whereas the co-formulants or formulations were more toxic by a factor 10-1000 (see the following graph):



The adenylate kinase activity, which was determined as a marker for necrosis, was not influenced by glyphosate when compared to the negative control.

Aromatase activity

Glyphosate had no effect on the aromatase activity when tested at concentrations used in the formulations (16 – 146 mg/L). At very high doses of 3000 ppm a significant aromatase inhibition was observed by glyphosate (-51%; data not shown in article for 3000 ppm which is equivalent to 17.7 mM glyphosate). Nevertheless, according to the authors this is considered not relevant because of the high concentration used. In the following figure the effects of glyphosate (G) on the aromatase activity is exemplary shown (A: POEA, 2.5 ppm; Glyfos, 25 ppm; glyphosate, 16 ppm; B: APG, 120 ppm; Medallon, 300 ppm; glyphosate, 146 ppm):



III. CONCLUSIONS

3. Assessment and conclusion

Assessment and conclusion by applicant:
Toxicity in JEG3 cells as well as aromatase activity have been investigated. Cells were treated with glyphosate alone, formulations containing glyphosate as active ingredient and co-formulants alone. Glyphosate showed generally low cytotoxicity. An effect on aromatase activity was not observed when cells were treated with glyphosate at non-toxic concentrations. At a higher glyphosate concentration (17.7 mM) a reduction of aromatase activity was seen, which is in accordance with the authors not considered relevant at this high concentration in the test system used. Moreover, such a high concentration is not to be reached at physiologic conditions and therefore, not considered relevant for human health.

The publication on in vitro aromatase activity in human cells shows that, even though it does not follow a defined guideline, the study is well documented and is scientifically sound. Therefore, the study is reliable with restrictions and is relevant for risk assessment.

Reliability criteria for *in vitro* toxicology studies

Publication: Defarge *et al*., 2016	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	N	
Only glyphosate acid or one of its salts is the tested substance	Y	
AMPA is the tested substance	NA	
Study		
Test system clearly and completely described	Y	
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	NA	
Test concentrations in physiologically acceptable range (< 1 mM)	N	
Cytotoxicity tests reported	N	
Positive and negative controls	Y	
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	NA	
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate and reliable with restrictions because the glyphosate used was not sufficiently characterized and as a too high, non-physiological concentration had been considered for glyphosate exposure.		

Assessment and conclusion by RMS:

CA 5.9 Medical Data

CA 5.9.1 Medical surveillance on manufacturing plant personnel and monitoring studies

Monsanto Glyphosate Manufacturing Industrial Hygiene Monitoring Data, Luling, Louisiana, USA

Industrial hygiene air monitoring data for glyphosate with workers at the Monsanto Luling, Louisiana manufacturing facility are available for the years 1981-1998 and are presented below. No such data are available from a Monsanto European manufacturing facility. Based on the measured low exposures to glyphosate in the manufacturing setting (well below the ADI) and low toxicological concern, glyphosate specific medical monitoring is not considered necessary. These data are air concentration measurements which are conservatively applied as 100% bioavailable to calculations of mean and maximum daily exposures.

Table 5.8-38: Particulate exposures from glyphosate technical acid operations involving wetcake, e.g., supersack or container filling operations. Values are time weighted averages.

Glyphosate Technical Dust (mg/m^3)					Mean Daily Exposure* (mg/kg/day)	Maximum Daily Exposure* (mg/kg/day)
Sample Type	# Samples	Range	Mean	SD		
All	179	0.0003-0.2594	0.00647	0.0218	0.00108	0.04323
Personal	176	0.0003-0.2549	0.00655	0.022	0.00109	0.04248
Area	3	0.0008-0.024	0.00153	0.0008 1	0.00026	0.00400
Operator	158	0.0008-0.2594	0.00727	0.0235	0.00121	0.00393
Maintenance	16	0.0005-0.0053	0.00206	0.0014 4	0.00034	0.00088
Lab	2	0.0003-0.0004	0.00035	N/A	0.00006	0.00007

* based on breathing 10 m^3 air/shift and 60 kg worker

Table 5.8-39: glyphosate isopropylamine salt liquid formulation bottling, drumming and tote filling operations. Values are time weighted averages.

Glyphosate IPA Salt- Liquid Formulations (mg/m^3)					Mean Daily Exposure** (mg/kg/day)	Maximum Daily Exposure** (mg/kg/day)
Sample Type	# Samples	Range	Mean	SD		
All	72	0.0001-0.47	0.085	0.105	0.01050	0.05804
Personal	58	0.0001-0.47	0.0251	0.106	0.00310	0.05804
Area	14	0.004-0.28	0.0932	0.105	0.01151	0.03458
Operator	54	0.0001-0.47	0.0966	0.11	0.01193	0.05804
Maintenance	4	0.0041-0.0088	0.00792	0.00187	0.00098	0.00099

** based on breathing 10 m^3 air/shift and 60 kg worker and divided by 1.3496 to convert IPA salt to technical acid

Improvements in manufacturing facility containment and ventilation systems over recent years further reduce the likelihood of operator exposures within glyphosate manufacturing facilities.

CA 5.9.2 Data collected on humans

Please refer to CA 5.9.3 and 5.9.4 were information available on direct observations and epidemiology is referenced.
Further references with regards to biomonitoring or other exposure information is provided at the end of the section under "Literature summaries". The full summaries of these publications are also provided at the end of the section under "Literature summaries".

CA 5.9.3 Direct observations

The summary in this section is based on well over 30 years of experience with numerous formulations of glyphosate in a wide range of situations. The extensive use of glyphosate has encouraged clinical assessment of various interventions and has resulted in reporting of alleged associations of symptoms with exposures to glyphosate products. The clinical toxicology of glyphosate and of glyphosate-surfactant formulations have been the subject of an extensive review (Bradberry et al 2004), and a review of cases with assessment of clinical prognostic factors was more recently published (Lee et al. 2008).

GENERAL:
Glyphosate does not inhibit cholinesterase, and has no cholinergic effect. Animals do not have the shikimic acid pathway; and no direct target-mediated action in mammalian systems has been clearly identified to date (Bradberry et al. 2004). While incidental exposure in glyphosate-surfactant herbicide mixtures is common, review of available case reports (AAPCC 2003-2011) indicates that the vast majority of reported non-suicidal exposures involve skin and/or eye irritation or irritation of the respiratory tract by inhalation of spray mist, and that systemic symptoms are rare following non-suicidal exposures to glyphosate products. Based upon human experience and animal data, even those systemic symptoms reported following incidental exposure appear unlikely to be causally related to exposure (Goldstein et al. 2002).

CLASSIFICATION OF EXPOSURES:
The following clinical effects are divided into those expected following minor and significant exposures for each category based upon expected severity of systemic symptoms. The factors which determine if the exposure is minor or significant include:

- The route of exposure. Dermal, eye and mist inhalation exposures to any commercially formulated glyphosate products of any dilution are minor exposures for purposes of the symptom descriptions below. Ingestions more than 50 ml (one mouthful if amount unknown) of a product with >10% glyphosate concentration may be significant.
- The concentration of the product. Glyphosate concentrations of less than 10% rarely if ever produce significant toxicity. Most serious illness has historically resulted from ingestion of the 41% (glyphosate IPA) concentrate. In the absence of extensive clinical experience for the 11-40% concentration range, any ingestion of greater than 50 ml of a glyphosate preparation having a greater than 10% concentration of glyphosate salts should be considered potentially significant for purposes of the symptom descriptions below.
- The intent of the exposure. Accidental ingestion rarely involves large quantities of concentrated formulations. Intentional ingestion cases may not present with a reliable history and may require observation if the amount ingested cannot be reliably determined.
- Clinical condition of the patient.
- Known or suspected co-ingestants (if any).
- Professional judgment.

ROUTE AND ORGAN SYSTEM SPECIFIC SYMPTOMS OF EXPOSURE:

DERMAL

MINOR EXPOSURES:

- Contact with skin may produce a dermatitis similar to that of detergents (Bradberry et al. 2004)
- It is expected that the severity of injury following skin exposure will be significantly decreased with a less concentrated product and with a reduced duration of contact.
- Phototoxic reactions (sunlight or ultraviolet (UV) light induced skin reactions) have been reported. This is believed due to an antimicrobial additive (benzisothiazolone) which is present in selected residential use (i.e. non- agricultural) products containing 10% glyphosate or less (Bradberry et al. 2004).
- Significant absorption through the skin does not occur (<0.2% for concentrates and <0.01% for dilute formulations; see section 5.9.9)
- Studies in farmers and farm family members during the machine spray application of glyphosate products indicates that farmer exposure is generally far below recommended maximal daily intakes and that urinary levels in children and spouses are largely non-detectable (limit of urinary detection 1 µg/L) (Acquavella et al. 2004). These studies do not provide a quantitative measure of dermal exposure, but are consistent with the primate data noted above.

SIGNIFICANT EXPOSURES:

- Skin exposures are not expected to cause systemic effects or serious cutaneous effects. Symptoms as noted in the minor exposure may occur.

OCULAR

MINOR EXPOSURES:

- A review of ocular exposures to US glyphosate-surfactant formulations (1513 exposures over a 5-year period), showed no permanent eye injury (Acquavella et al. 1999).
- Human eye exposures have generally resulted in temporary conjunctival irritation, clearing after irrigation or in 1-2 days and permanent eye damage is said to be “most unlikely” (Bradberry 2004).
- It is expected that the severity of injury following eye exposure will be significantly decreased with a less concentrated product or with a reduced contact time.

SIGNIFICANT EXPOSURES:

- Eye exposures are not expected to cause systemic effects or serious ocular injury (Acquavella et al. 1999; Bradberry et al. et al. 2004).

SYSTEMIC EXPOSURE- INGESTION OR INHALATION

NEUROLOGIC:

MINOR EXPOSURES:

- There is no clinical or experimental evidence that glyphosate or glyphosate-surfactant formulations cause neurological symptoms or injury after exposure by any route.

SIGNIFICANT EXPOSURES:

- There have been no reports of primary convulsions after ingestion.
- One author reports most patients present with a clear sensorium unless another substance, such as alcohol, has been co-ingested or severe hypoxemia has occurred (Tominack 1989); however "moderate disorders of consciousness" have been reported within 48 hours of suicidal ingestions of the concentrate (Sawada and Nagai 1987; Sawada et al. 1988). This has occurred in patients with significant systemic illness and is not believed to be the result of reduced organ perfusion (Bradberry et al. 2004) or perhaps other factors such as metabolic disturbance, but the possibility of a direct toxicological effect cannot be excluded (Bradberry et al. 2004).

- There are three isolated case report of Parkinson's disease developing in individuals with a history of glyphosate product exposure. In one case, Parkinson's disease of relatively acute onset was diagnosed 6 months following incidental dermal exposure to a glyphosate-surfactant product (Barbosa et al. 2001). It appears that the same case was reported as part of a case series by daCosta et at (2003) [Similar list of authors on both publications, case descriptions and ages match (52 years old at diagnosis vs 54 year old with a 2 year history of Parkinsons) and the T2- weighted Axial MRI images shown appear to be identical]. The second case (Wang et al. 2011) reports the development of Parkinson's of a 44 year old woman who had been employed in a glyphosate manufacturing facility. The third case described a woman who developed transient Parkinsonism that was reportedly reversed by the administration of atropine and pralidoxime. (Zheng et al 2018). In all instances, there is no evidence for causation other than a history of prior exposure. In the last case, it is notable that the patient recovered with the treatment for organophosphate exposure, which suggests a completely different etiology as glyphosate does not require treatment with anticholinergic agents. No other human or animal data support the contention that Parkinson's disease results from exposure to glyphosate, even following massive ingestion or prolonged exposure.

GASTROINTESTINAL:

MINOR EXPOSURES:

- Minor exposures are likely to be asymptomatic, but the patient may experience an unpleasant taste, tingling, mild self-limited nausea and vomiting.
- Self-limited diarrhoea may also occur, which is thought to be due to the surfactant.

SIGNIFICANT EXPOSURES:

- A burning sensation in the mouth and throat, salivation, oral erythema, sore throat, dysphonia, dysphagia, epigastric pain, nausea, spontaneous vomiting, abdominal pain and diarrhoea are common and may last up to a week.
- Serum amylase may be elevated; isoenzyme analysis done in a few cases identified a salivary gland origin (Tominack et al. 1989).
- In severe cases with large ingested doses, hematemesis, GI bleeding, melena and hematochezia may occur. Paralytic ileus has been reported as a rare event.
- Endoscopy has noted erosions of the pharynx and larynx, esophagitis and gastritis with mucosal oedema, erosions and haemorrhage. Transmural injury and perforation have not been noted on panendoscopy (Chang et al. 1999).
- In fatal cases, autopsy notes mucosal or transmural oedema and necrosis throughout the small bowel with erosion and haemorrhage; in the large bowel, mucosal oedema and focal haemorrhage was noted (Tominack et al. 1989).
- Clinical, autopsy and experimental evidence ([redacted] 1987) indicate a potential for gastrointestinal damage from glyphosate components of glyphosate formulations, but the frequency of severe injury appears to be low and early endoscopy is probably not indicated (see below).

CARDIOVASCULAR:

MINOR EXPOSURES:

- Dermal, eye and mist inhalation exposures to any commercially formulated glyphosate products of any dilution are minor exposures. Cardiovascular effects are not expected from minor exposures. A recent case report describes a patient who presented with syncope and a wide-complex tachycardia where the authors claim that there is a prolonged QTc while measuring a wide complex beat. This is not how a QT interval is determined, it should be derived from the measurement of a narrow complex as a prolonged QRS interval actually makes it impossible to determine the QTc. The patient, when pressed repeatedly about pesticide exposure, said that she had spilled a small amount of formulated glyphosate on her hand the previous evening. The authors attribute her syncope and arrythmia to this minor exposure. Since significant absorption through the skin does not occur (<0.2% for concentrates and <0.01% for dilute formulations; see section 5.9.9), it is inaccurate to claim that a small dermal

exposure can trigger a malignant arrhythmia, furthermore, glyphosate is not cardiotoxic and this patient appears to have early and late afterdepolarisations on her EKG, suggestive of a channelopathy.(Brunetti et al, 2020)

SIGNIFICANT EXPOSURES:

- Hypotension is common after ingestions of a mouthful or more of the concentrated product (not the diluted forms) and usually responds to IV fluids and pressor amines. Shock as manifested by oliguria, anuria and hypotension which was unresponsive to fluids and pressors, ultimately resulting in death, has been reported. (Tominack et al. 1989, Bradberry et al. 2004). Transient hypertension may be noted.

UPPER RESPIRATORY:

MINOR EXPOSURES:

- Dermal, eye and minor ingestions of dilute solution exposures to any commercially formulated glyphosate products of any dilution are minor exposures. Significant upper respiratory effects are not expected from minor exposures, but minor irritation or discomfort may occur (Bradberry et al. 2004).

SIGNIFICANT EXPOSURES:

- Significant systemic exposures are not anticipated to occur via the inhalational route, see minor exposures within this subheading.

LOWER RESPIRATORY:

MINOR EXPOSURES:

- Because of the non-volatile nature of glyphosate and the surfactant, there are no vapour exposures possible. The spray equipment commonly used with the product produces particles that are non-respirable.

SIGNIFICANT EXPOSURES:

- Tachypnea, dyspnea, cough and bronchospasm including cyanosis have been seen in severe ingestions (more than a mouthful of concentrated product). These effects appear to be the result of systemic toxicity.
- Aspiration pneumonia, pulmonary oedema and respiratory failure have been seen although the exact role of aspiration has not been fully investigated.
- An isolated case report suggests the development of acute pneumonitis in a worker following his performing maintenance on non-operating spray equipment used to apply a glyphosate-surfactant formulation (Pushnoy et al. 1998). However, the registrants do not believe that a credible mechanism of exposure was documented in this case, and the occurrence of pneumonitis in this individual was more likely coincidental in nature (Goldstein et al. 1999).
- There is also a case report out of Germany in which a glyphosate-surfactant product (tallowamine or "POEA" based) was applied by knapsack spayer in a 0.5ha forestry application at the registered application rate at 25° C for approximately 3 hours. About 7 hours after application he developed chest pain with rapidly increasing severe respiratory distress and fever up to approximately 38° C. On hospital admission, radiographic changes of lungs could be demonstrated. To further assess possible causes, bronchoscopy and closed lung biopsy was performed. Histopathology revealed "toxic inflammation of the lungs" (significantly different than bacterial infection). After 7-days of drug treatments, changes in lung reversed. Six months after the incident the patient still experienced moderate respiratory complaints on exertion. In the X-ray findings lungs showed improved results, but still detectable changes. While it is possible to differentiate acute bacterial infections on histopathology (microorganisms and polymorphonuclear leucocytic inflammatory changes should be visible), characteristics of viral, mycoplasmal, or autoimmune (vasculitic, Wegoner's granulomatosis) induced pneumonitis or Bronchiolitis Obliterans with Organizing Pneumonia (BOOP, which closely mirrors the limited case information available) are not clinically distinguishable from "toxic" etiologies. Many cases occur, most being idiopathic (no identifiable cause). Agricultural aerosols are far larger than 10 microns (generally 200 microns or so in size) and

not respirable to lung, and POEA is not volatile. Contrary to this isolated case, backpack applications of glyphosate-surfactant products occur regularly in forestry and in agriculture in the developing world, without known occurrence of serious lower respiratory disease.

RENAL:

MINOR EXPOSURES:

- Dermal, eye, mist inhalation and minor ingestions of dilute solution exposures to any commercially formulated glyphosate products of any dilution are minor exposures. Renal effects are not expected from minor exposures.

SIGNIFICANT EXPOSURES:

- Hypotension and hypovolemic shock may result in oliguria and anuria, following severe ingestions (Bradberry et al. 2004). Abrupt rises in BUN and serum creatinine may be seen.

METABOLIC:

MINOR EXPOSURES:

- Dermal, eye, mist inhalation and minor ingestions of dilute solution exposures to any commercially formulated glyphosate products of any dilution should be considered minor exposures. Metabolic effects are not expected following minor exposures.

SIGNIFICANT EXPOSURES:

- Mild fever may be noted even in the absence of infection (Bradberry et al. 2004)
- Metabolic acidosis is often seen in a severely poisoned patient (Bradberry et al. 2004) and the acidosis may fail to respond to bicarbonate therapy. Although the exact cause of the acidosis is unknown, a lactic acidosis is suspected.

HEMATOLOGIC:

MINOR EXPOSURES:

- Dermal, eye, mist inhalation and minor ingestions of dilute solution exposures to any commercially formulated glyphosate products of any dilution should be considered minor exposures. Haematological effects are not expected from minor exposures.

SIGNIFICANT EXPOSURES:

- Leukocytosis without evidence of bacterial infection has been noted in peripheral blood after ingestion of the concentrate (Bradberry et al. 2004).
- Hemoconcentration has been seen as a result of intravascular volume depletion (possibly indicating severe capillary fluid leakage) (Tominack et al. 1989).
- No primary toxic effects on bone marrow or formed elements have been seen to date.

HEPATIC:

MINOR EXPOSURES:

- Dermal, eye, mist inhalation and minor ingestions of dilute solution exposures to any commercially formulated glyphosate products of any dilution should be considered minor exposures. Hepatic effects are not expected from minor exposures.

SIGNIFICANT EXPOSURES:

- No direct hepatotoxic effects have been noted; however, minor elevations in transaminases and bilirubin are reported (Tominack et al. 1989; Bradberry et al. 2004).
- A 2018 case report describes a patient who developed fulminant hepatic failure after opening a bottle of formulated glyphosate with his mouth and accidentally ingesting a mouthful of the product. There is not a mechanism by which a small ingestion would cause fulminant hepatic failure nor do any GLP studies demonstrate hepatotoxicity; (Khot et al. 2018)

ELECTROLYTES:

MINOR EXPOSURES:

- Severe or prolonged vomiting and diarrhoea may induce fluid and electrolyte imbalance. This degree of illness is not generally expected from a minor exposure.

SIGNIFICANT EXPOSURES:

- Electrolytes (Na, K, Cl and Ca) in the absence of renal failure generally remain normal. Severe or prolonged vomiting and diarrhoea may induce fluid and electrolyte imbalance.
- POTASSIUM SALTS: While potentially toxic ingestions of all glyphosate products may result in fluid and electrolyte disturbances, particular attention to potassium may be important following ingestion of the potassium salt products. Close monitoring of serum potassium levels and/or electro-cardiographic monitoring (for peaked T-waves or rhythm disturbances) is recommended following significant ingestion of potassium salt products, particularly for high-risk individuals. Individuals with the following may be at elevated risk following acute potassium exposure: known hyperkalemia, renal failure / renal dysfunction, use of potassium sparing diuretics, hypoaldosteronism, co-ingestion of other K+ containing materials, underlying heart disease, use of digoxin, digitoxin, oabain, or exposure to other cardiac glycosides. The quantity of potassium ingested from a glyphosate potassium salt product can be estimated from the weight percent of glyphosate potassium as:

  Percent K+ salt x 5.3 = mEq potassium per 100 cc of product

- Several case reports do indicate that with large ingestions of glyphosate-potassium salt concentrate solutions, clinically significant hyperkalemia may occur. Bando et al (2001) report a 65 year old female who ingested a glyphosate-potassium salt (350 ml Roundup Maxload missing from container, in addition to 250 ml of another glyphosate formulation which was not a potassium salt- but amount actually ingested unclear) in an attempt at suicide. On admission, serum potassium level was 9.3mEq/L (typical normal value < 5) with electrocardiographic changes consistent with hyperkalemia. The patient did have a concomitant acidosis (pH 7.272) which may account for some portion of the elevation in potassium (acidosis displaces intracellular potassium). The patient responded to medical management and survived.
- Kamijo et al (2012) report a 69 year old female who ingested approximately 500 ml of the same product. On arrival in the hospital, the patient had hyperkalemia (10.7 mEq/l), pulseless ventricular tachycardia, and a severe metabolic acidosis (pH 7.005, will elevate potassium.) The patient required aggressive cardiopulmonary resuscitation and hemodialysis but did recover.
- Monsanto is aware of one additional inquiry (unpublished) of a similar ingestion with a dramatically elevated potassium level in which the patient was moribund when medical care was instituted. The patient could not be resuscitated. Because serum potassium levels rise rapidly following death (due to redistribution of intracellular potassium) , it is not possible to know how much of the observed hyperkalemia was the result of the ingestion versus profound acidosis and post-mortem redistribution (which is partially due to acidosis).
- It should be noted that the issue of hyperkalemia is limited to cases involving the suicidal ingestion of glyphosate-potassium concentrates. Potassium is a normal component of the human diet, and potassium intake attributable to occupational glyphosate-surfactant herbicide exposure will be negligible compared to typical dietary intake. While the concentrate formulations may contain up to approximately 250 mEq of potassium per 100 ml, product diluted for use (1% glyphosate concentration) will contain about 6 mEq potassium per 100 ml. By way of reference, a medium size banana contains about 10 mEq (425 mg) of potassium.
- Finally, it should be noted that the apparently very large (>150 ml) ingestions of glyphosate-surfactant concentrates observed in these cases are well within the range isopropylamine salt products reported to produce fatalities, and that elevations in potassium concentrations are reported (probably due to acidosis) following ingestions of glyphosate IPA salt products. While the cases do suggest that potassium salt products likely contribute to the risk of hyperkalemia, it is not clear at this time that the use of postassium salts will increase the overall clinical severity and/or mortality associated with

glyphosate concentrate product ingestions.

SPECIFIC DIAGNOSTIC TESTING AND PROGNOSTIC CONSIDERATIONS

Serum or other body fluid measurements of glyphosate are generally not available in a time frame useful for acute clinical diagnosis. As the management of symptoms associated with glyphosate-surfactant product ingestion is symptom-driven in any event, the lack of rapidly available concentrations of glyphosate will generally not impair clinical care. Levels may be helpful in addressing forensic issues following clinical recovery or in the event of a fatality of unclear cause.

Attention should be paid to electrolyte concentrations in individuals with significant ingestion exposures, particularly to glyphosate-potassium concentrate solutions.

Respiratory distress requiring intubation, pulmonary oedema, shock (systolic BP < 90 mmHg), altered consciousness, abnormal chest X-ray, ingestion of over 200 cc concentrate (41%), or renal failure necessitating dialysis have been associated with a higher risk of poor clinical outcomes including mortality (Lee 2008). These authors also developed a prognostic index based upon these factors. The use of prognostic criteria does not appear to add significantly to patient care. As symptom onset may be delayed, early use of such prognostic indicators may lead to an under-estimate of clinical severity.

National Chemical Emergency Centre (NCEC)

Reports from the National Chemical Emergency Centre (NCEC) in EU on any emergency calls received during the timeframe of 2017-2019 are submitted under CA 5.9/012 and CA 5.9/013.

A list of case reports is added in Appendix 1 of this document and available in the literature search report (CA 9-01).

CA 5.9.4 Epidemiological studies

The epidemiological publications found relevant and reliable were discussed in detail in section CA 5.5 and summaries of these publications were also prepared and provide in CA 5.5 (CA 5.5/030, CA 5.5/033, CA 5.5/035, CA 5.5/036, CA 5.5/037).

CA 5.9.5 Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical tests

Any relevant information on poisoning is provided in CA 5.9.7.

CA 5.9.6 Proposed treatment: first aid measures, antidotes, medical treatment

First aid measures

SKIN EXPOSURE:

- Remove all contaminated clothing and flood the skin surface with water.
- Wash the exposed skin twice with soap and water.
- A close examination of the skin may be required if pain or irritation exist after decontamination.
- All clothing that are contaminated should be laundered before they are worn again

EYE EXPOSURE:

- Remove contact lens from the affected eye(s) if appropriate.
- Exposed eyes should be irrigated with copious amounts of water or saline for at least 15 minutes. Pour the water from a cup or glass held 3 inches from the eye.
- A close examination of the eyes may be needed if pain or irritation persists after 15 minutes of irrigation with water or saline. If symptoms persist, seek medical evaluation, preferably with an eye specialist.

INGESTION EXPOSURE:

- DILUTE PREPARATIONS (Glyphosate <10%): An ingestion of a dilute preparation of glyphosate (<10%) probably does not require treatment other than dilution with milk or water, and symptomatic care. Further gastrointestinal decontamination is not needed, even if spontaneous emesis has not occurred.
- Concentrated (> 10%) preparations: Irrigate and dilute: irrigate the mouth with water. Immediate therapy should include dilution with milk or water if the patient is able to swallow. Do not exceed 5 ml/kg in a child or 250 ml in an adult.

INHALATION EXPOSURE:

- No pulmonary treatment is necessary for occasional, accidental breathing of mist.
- Severe, acute pulmonary injury has not been reported following inhalation exposure. Individuals with respiratory distress from any cause should be relocated (if medically stable) to fresh air and receive supplemental oxygen if available.
- In the event of respiratory failure or lack of respiration, administer artificial respiration (or if pulse not detectable, cardiopulmonary resuscitation).

Therapeutic regimes

The registrants believe that the following represent general best practices for medical management of serious ingestions of glyphosate-surfactant products.

1. Establish respiration and assure adequacy of ventilation.

2. Eye exposure:
   A) Remove contact lens from the affected eye(s) if appropriate.
   B) Exposed eyes should be irrigated with copious amounts of water or saline for at least 15 minutes. Pour the water from a cup or glass held 3 inches from the eye.
   C) A close examination of the eyes may be needed if pain or irritation persists after 15 minutes of irrigation with water or saline. If symptoms persist, seek medical evaluation, preferably by an eye specialist.

3. Ingestion exposure:
   A) Irrigate and dilute: irrigate the mouth with water. Immediate therapy should include dilution with milk or water if the patient is able to swallow. Do not exceed 5 ml/kg in a child or 250 ml in an adult.
   B) patient disposition:
   Concentrated preparations (Glyphosate 41% or greater):
      1) Any person ingesting greater than a large mouthful (50 ml in an adult, 0.5 ml/kg in a child) of a 41 % or greater glyphosate concentrate product should be admitted to a hospital and observed for 24 hours.
      2) Any adult ingesting greater than 100 ml of a 41% or greater glyphosate concentrate product (>1.4 ml/kg in a child) should be admitted to the intensive care unit.
      3) Any suicide attempt by person ingesting a concentrated product should be evaluated for psychological status and should be admitted if necessary for observation with suicide precautions.

Concentrated preparations (Glyphosate 10%-40%):

An ingestion of concentrated glyphosate (10%-40%) will usually result in spontaneous emesis. There is limited experience with glyphosate formulations in this concentration range. In view of this limited information, the registrants currently recommend managing these ingestions in a manner similar to the management of the 41% concentrate.

4. Prevention of absorption *(This lists various methods for "Prevention of Absorption". These should NOT be construed as being in order of preference. Consult with Poison Center or medical personnel to determine the need for and preferred method for decontamination. In many instances, no intervention is required.):*

A) Gastric aspiration: If no significant spontaneous vomiting has occurred gastric aspiration may be considered. If performed soon after ingestion, gastric emptying by aspirating liquid gastric content with a lavage or standard NG tube may possibly remove some of the ingested glyphosate. The intent is to remove unabsorbed liquid by aspiration not to use lavage fluid. As absorption of liquids is likely to be relatively rapid, gastric aspiration after 1 to 2 hours is unlikely to be effective.

B) Emesis: Emesis is controversial at this time. Glyphosate/surfactant products are irritants. The registrants do not recommend the routine use of syrup of ipecac for glyphosate/ surfactant ingestions because of the risk of exacerbating the irritant effects on the GI tract.

C) Activated charcoal: There are no data to support or refute the use of activated charcoal in glyphosate/surfactant product ingestions. Low molecular weight, amphoteric compounds and detergents do not always bind well to activated charcoal. In the event of a mixed ingestion, activated charcoal may be advisable.

5. Assessment of gastro-intestinal injury:

Injury to the upper gastrointestinal tract may occur following ingestion of glyphosate concentrates. A study of upper gastrointestinal endoscopy following glyphosate–surfactant ingestions suggested that Zarger grade 2 lesions (erosions) were associated with longer hospital stay and with a higher incidence of serious complications (Chang 1999). However, no major esophageal or gastrointestinal injury was observed, and strictures have not been reported following uncomplicated glyphosate-surfactant ingestion.

Because no serious gastrointestinal injury is reported, and because the need for hospitalization and/or treatment of complications can be determined without endoscopic evaluation, the registrants recommend that endoscopy be reserved for patients with co-ingestions suggesting a need for endoscopy or for patients with signs and symptoms suggestive of more serious injury (serious oral burns, inability to handle secretions, clinical obstruction) regardless of clinical history.

6. Monitor blood pressure:

Monitor the patient closely for signs of hemodynamic instability.

7. Hypotension:

If the patient is hypotensive, administer IV fluid boluses and place in Trendelenburg position. If the patient is unresponsive to these measures, administer a vasopressor (dopamine, epinephrine, norepinephrine, phenylephrine.) if needed.

8. Monitor blood gases and obtain chest radiograph:

Consider the use of repeat blood gases and a peripheral pulse oximeter to monitor hypoxemia. Observe closely for sign of acidosis.

9. Pulmonary oedema:

Closely monitor arterial blood gases. If PO2 cannot be maintained above 50 mm Hg with inspiration of 60% oxygen by face mask or mechanical ventilation, then positive end expiratory pressure (PEEP) or continuous positive airway pressure (CPAP) may be needed. Avoid a positive

fluid balance by careful administration of crystalloid solutions. Monitor fluid status through a central venous line or Swan Ganz catheter as needed.

10. Acidosis:
Correction of acidosis should be guided by blood gases, electrolytes and clinical judgment. Attention should be directed to volume status and correction of poor perfusion in mild cases. Sodium bicarbonate may be used to correct the acidosis in severe cases.

11. Hyperkalemia (from ingestion of Potassium salt formulations):
For moderate hyperkalemia (K+ of 6.0-7.0 mEq/L), administer sodium polystyrene sulfonate For more severe hyperkalemia (K+ > 7 mEq/L) or serious complications of hyperkalemia, correct metabolic or respiratory acidosis if present to allow potassium to enter the intracellular space. Additional management may include a glucose/insulin drip, intravenous sodium bicarbonate or calcium, and dialysis to remove excess potassium.

12. Monitor renal function closely:
Assure adequate urine output. Catheterize severely ill patients. Hemodialysis may be needed in the event of renal failure or electrolyte disturbances.

11. Enhanced elimination:
A) Forced diuresis: Glyphosate is excreted very well by the kidneys. Adequate urine flow will ensure the rapid elimination of glyphosate. Although elimination may perhaps be enhanced by forced diuresis, there is no clinical evidence that this is necessary, and fluid overload may precipitate pulmonary oedema.
B) Hemodialysis: Hemodialysis may be useful to correct fluid, electrolyte and metabolic disturbances in the patient with renal failure. The institution of hemodialysis solely to enhance the removal of glyphosate or other product components is not of proven benefit. Nevertheless, it is reasonable to consider the initiation of hemodialysis in the significantly ill patient who fails to respond to routine supportive management.

12. Serious exposure via inhalation is not expected:
Inhalation exposures are not expected due to the aerodynamics of droplet size from sprayers and because the product is not volatile. Monitor the patient for signs of respiratory compromise. Create an artificial airway if necessary. Check adequacy of tidal volume. Monitor the patient for respiratory distress; if a cough or dyspnea develop, evaluate the patient for respiratory irritation, bronchitis and/or pneumonia, but these are not expected.

13. Serious exposure via skin is not expected:
Significant skin exposures are not expected; however, the patient should be treated empirically if a dermal exposure is suspected. Remove all contaminated clothing and flood the skin surface with water. Wash the exposed skin twice with soap and water. A close examination of the skin may be required if pain or irritation exist after decontamination. All contaminated clothing should be laundered before wearing.

14. Laboratory:
Monitor electrolytes, especially if the patient is experiencing vomiting and diarrhea.15 Patients ingesting concentrated products based on the potassium salt of glyphosate may ingest large amounts of potassium (see calculations above). Observe serum potassium and/or electrocardiogram carefully. Patients experiencing pulmonary symptoms or having chest radiograph changes should have arterial blood gas monitoring. A peripheral pulse oximeter and a Swan Ganz catheter may be needed.

CA 5.9.7 Expected effects of poisoning

Expected effects and duration of poisoning as a function of varying time periods between exposure or ingestion and commencement of treatment

The outcome of eye, dermal, and inhalational exposures, which are not expected to result in serious injury in any event, will not be significantly altered by delays in medical management. Similarly, minor oral exposures are symptomatically managed and unlikely to result in severe gastrointestinal symptoms. Medical management with intravenous fluids may provide some symptomatic relief in the event of dehydration, but recovery is anticipated in any event.

For serious ingestions having major electrolyte disturbances or life threatening alterations of cardiovascular performance, medical intervention may be life saving. Fortunately, as noted above, the onset of serious symptoms following ingestion is generally delayed by at least several hours, allowing for medical transport in all but the most remote or extreme circumstances. The availability (or lack) of acute field management does not appear likely to impact severity of survival of most serious ingestions.

Expected effects and duration of poisoning as a function of the type, level and duration of exposure or ingestion

Dermal exposure:

Skin irritation following exposure to glyphosate-only or glyphosate-surfactant materials is generally limited to topical irritation which will resolve within 3 days to 1 week following exposure. If exposure is aggravated by occluded conditions or physical abrasion, more severe skin injury with open skin injury may rarely result and may take longer to fully resolve.

Eye exposure:

Irritant symptoms generally resolve within 3-7 days of exposure. Most irritation is minor, but exposure to concentrate or the occurrence of a foreign body or of abrasions (from rubbing the eye) may result in corneal abrasion requiring topical antimicrobial therapy, often given in conjunction with topical corticosteroids and temporary eye patching to provide symptomatic relief. As noted above, a large study of (U.S.) ocular exposures to glyphosate-surfactant products demonstrated no long term eye injury.

Inhalation exposure:

Glyphosate-surfactant products generally do not contain readily volatile ingredients and thus inhalation exposure is limited to inhalation of agricultural droplets, which will deposit primarily in the upper airway. Resulting irritant symptoms will generally resolve within hours to a few days following exposure.

Ingestion:

Following minor or incidental ingestions, or ingestion of fully diluted formulations, gastrointestinal upset with nausea, vomiting, and diarrhoea may occur. Nausea and vomiting usually resolve within a few hours of ingestion. Diarrhoea may last for several days but is generally not severe. Following a major ingestion, the onset of systemic symptoms may be delayed by several hours. Fatalities due to cardiovascular failure are generally delayed by 12 – 36 hours. For serious but non-fatal cases, primary clinical injury generally is manifest within 72 hours but secondary complications such as infection or respiratory distress syndrome may supervene. The majority of serious but surviving cases will be fully recovered within 7-10 days of ingestion. Individuals with complicated hospital courses can require a more extended and highly variable time to recover.

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Literature summaries

Table 5.9- 6: Overview on literature found relevant for evaluation of glyphosate in section 5.9

Annex Point	Study	Title	Reliability
CA 5.9/001	Connolly A. *et al.*, 2018	Characterising glyphosate exposures among amenity horticulturists using multiple spot urine samples	Reliable with restrictions
CA 5.9/002	Connolly A. *et al.*, 2019	Exploring the half-life of glyphosate in human urine samples	Reliable with restrictions
CA 5.9/003	Connolly A. *et al.*, 2017	Exposure assessment using human biomonitoring for glyphosate and fluroxypyr users in amenity horticulture	Reliable without restrictions
CA 5.9/004	Connolly A. *et al.*, 2018	Glyphosate in Irish adults – A pilot study in 2017	Reliable without restrictions
CA 5.9/005	Connolly A. *et al.*, 2019	Evaluating glyphosate exposure routes and their contribution to total body burden: a study among amenity horticulturalists	Reliable without restrictions
CA 5.9/006	Conrad U. *et al.*	Glyphosate in German adults – Time trend (2001 to 2015) of human exposure to a widely used herbicide	Reliable without restrictions
CA 5.9/007	Kongtip P. *et al.,* 2017	Glyphosate and Paraquat in Maternal and Fetal Serums in Thai Women	Reliable with restrictions
CA 5.9/008	McGuire M.K.. *et al.,* 2016	Glyphosate and aminomethylphosphonic acid are not detectable in human milk	Reliable without restrictions
CA 5.9/009	Sierra-Diaz E.. *et al.,* 2019	Urinary pesticide levels in children and adolescents residing in two agricultural communities in Mexico	Reliable with restrictions
CA 5.9/010	Steinborn A. *et al.*, 2016	Determination of Glyphosate Levels in Breast Milk Samples from Germany by LC-MS/MS and GC-MS/MS	Reliable with restrictions
CA 5.9/011	Trasande L. *et al.*, 2020	Glyphosate exposures and kidney injury biomarkers in infants and young children	Reliable without restrictions

3. Information on the study

Data point:	CA 5.9/001
Report author	Connolly, A. *et al.*
Report year	2018
Report title	Characterising glyphosate exposures among amenity horticulturists using multiple spot urine samples
Document No	doi.org/10.1016/j.ijheh.2018.06.007 E-ISSN: 1618-131X
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Yes/Reliable with restrictions

1. Full summary of the study according to OECD format

The study aimed to characterise occupational exposures to glyphosate among amenity horticulturists through the collection and analysis of urine samples following pesticide application. The impact of work practices on personal exposure, as well as suitability of collecting multiple spot urine samples as a sampling strategy for the assessment of occupational exposure for glyphosate were also examined.

Materials and methods

Site description and study population - The monitoring campaign took place from September 2016 to September 2017. In this study 3 similar exposure groups (SEGs) were considered for glyphosate applications: (1) manual knapsack (Roundup Biactive XL, 360 g/L; Clinic Ace, 360 g/L; Roundup Biactive, 360 g/L; Pistol, 250 g/L; Roundup XL, 360 g/L), (2) pressurized handheld lance (Roundup Biactive XL, 360 g/L; Pistol, 250 g/L; Glyfos, 360 g/L; Rambo, 360 g/L; Roundup Gold, 450 g/L), (3) controlled droplet applicator (Nomix Dual, 120 g/L; Roundup XL, 360 g/L). Recruitment was completed in coordination with the OPW Health and Safety Unit. The lead researcher explained the project background and objectives and circulated project information leaflets to potential study participants. Participation in the study was voluntary. After having given informed consent the participants completed a self-administered questionnaire providing relevant personal and work related details, as well as information on their use of pesticides outside of work and dietary habits. Biomonitoring protocols were developed based on established protocols. Project ethical approval was received from the National University of Ireland, Galway Research Ethics Committee.

Urine collection - Biomonitoring exposure assessments took only place on the days workers were using glyphosate based pesticide products. The researcher observed all work tasks on site, and collected information such as SEG, personal protective equipment (PPE) worn, climatic conditions, and any activities or work duties performed between samples and the duration of these activities. Individual full urinary void spot samples were collected over the exposure assessment period. The void spot samples were kept and analysed separately. Time and date were mentioned on each sample container which was stored in a cooler box until collection by the researcher the morning following each task. A minimum of 3 spot urine samples were provided: a pre-task sample, a post-task sample taken within one hour of task completion and a sample taken on the following morning after the first morning void. Participants were also given the option to provide urine samples for all additional voids over the exposure assessment period (pre-task to the following first morning void sample). Participants provided more than the minimum required 3 spot samples for 59% (17) of the tasks sampled ranging between 3 and 7 samples per task. Of the spot urine

samples provided, a peak urinary sample was identified for each task and defined as the highest urinary glyphosate concentration measured during the sampling period. Following sample collection, the researcher measured and recorded the volume of each urinary spot sample. A 20 mL aliquot of the well mixed spot urine sample was then transferred into a 20 mL Sterilin™ pot and labelled with a unique identifier number, date and time. Care was taken to avoid any contamination. All samples were stored at −18°C within 24 hours pending analysis.

Analysis of glyphosate in urine samples - Glyphosate was extracted from diluted urine samples (200 µL urine added to 800 µL deionised water) using anion exchange solid phase extraction (SPE) with 10% formic acid in methanol as the eluent. The eluent was evaporated under a stream of nitrogen and reconstituted in 100 µL of 0.1% formic acid for quantitative analysis by LC-MS/MS. Chromatographic separation was achieved on a Zorbax XDB-C8, 150×4.6 mm, 5 µm (Agilent, Stockport, UK) column with 0.1% formic acid and acetonitrile with a gradient elution as the mobile phase. The method was linear over the range of 0–20 µg/L and the limit of quantification (LOQ) was 0.5 µg/L. Creatinine was determined in all urine samples with a Pentra C400 clinical analyser using the alkaline picrate method.

Statistical analysis - Prior to analysis, all glyphosate concentration levels below the LOQ were imputed, using SAS v 9.4. Differences in urinary glyphosate concentrations between the pre-task samples versus the post-task and the peak urinary samples were both analysed using paired Student t-tests. Determinants of exposure on glyphosate urine concentrations were evaluated using Pearson's correlation coefficients and linear regression. A multivariate mixed effect model was elaborated to compare the glyphosate concentrations between post-task and following first morning void samples. In these models, worker identity was entered as a random effect to account for the presence of correlations between repeated measurements from the same individuals.

Results

Descriptive *and summary statistics of demographic and task characteristics* - 18 male and 2 female amenity horticultural workers applying pesticides as part of their work duties consented to participate in the study. Urine samples were collected for 29 work tasks involving glyphosate based pesticides. The concentration of glyphosate in 14 (48%) of the pre-task samples could have been influenced by work tasks performed in the days prior to this study and by starting the work task before giving the pre-task sample. For 6 (21%) of the pre-task samples, workers reported performing work tasks involving handling of glyphosate based products on the previous day to the measurement period. Similarly, for 10 (35%) of the pre-task samples (including 2 who were also involved with spraying the previous day), workers reported collecting, checking or handling potentially contaminated spraying equipment or other work equipment prior to providing the first sample (pre-task sample). A large proportion of workers reported using pesticides outside of their job (90%), corresponding to 27 (93%) of the 29 tasks included in this study. However, none reported using glyphosate based pesticides at home on the days before the sampling. The majority of the workers (60%) reported using pesticides at work for 6–7 months per year. 100% of the workers wore gloves, 90% a Tyvec suit and 97% RPE.

Urinary glyphosate concentrations - 125 spot urinary samples were collected and analysed for 29 work tasks. Participants provided between 3 and 7 individual spot samples per exposure assessment period. Participants giving more than 3 spot urine samples over the exposure assessment period (n=17) allowed for a more accurate estimation of the urinary concentrations over time. There was no statistically significant difference in the average log transformed peak urinary concentrations where only the minimum of 3 samples were provided versus those tasks where multiple samples were provided (Student t-test; $p = 0.14$). For 13 (45%) of the 29 tasks, the peak concentrations were identified within the samples that were collected in addition to the minimum required (pre-task, post-task and following first morning void) samples. Another 31% of the peak samples were identified in post-task samples, whereas 24% comprised of following first morning void samples. The geometric mean of the glyphosate concentrations measured in urine samples of the combined glyphosate SEGs were 0.68 µg/L for pre-task samples, 1.17 µg/L for post-task samples and 0.83 µg/L for following morning void samples. The geometric mean of the peak samples was 1.9 µg/L. Glyphosate concentrations were less than the LOQ in 34 (27%) urinary samples, of which 11 (38%) were

pre-task samples and a further 11 (38%) were following first morning void samples. Two (7%) of the 29 work tasks had peak samples with urinary glyphosate concentrations below the LOQ, both belonging to the manual knapsack SEG.

Conclusion

This study provides information on occupational exposures to glyphosate among amenity horticulturalists and suggests that the collection and analysis of urine samples given up to 3 hours after task completion can be a suitable sampling strategy for estimating potential occupational exposures to glyphosate. The findings suggest that amenity horticulturists, largely complying with workplace exposure controls, have low levels of glyphosate exposures.

1. Assessment and conclusion

Assessment and conclusion by applicant:

In this study the exposure of amenity horticulturalists to glyphosate was assessed. Three similar exposure groups (SEGs) were considered for the application of various glyphosate based herbicides: one using a manual knapsack, one using a pressurized handheld lance and one using a controlled droplet applicator. Urine samples were taken pre-task, post-task and the morning after the task and analysed for glyphosate using and LC-MS/MS method with an LOQ of 0.5 µg/L. Glyphosate concentrations were found to be less than the LOQ in 27% of the urinary samples, of which 38% were pre-task samples and 38% were following morning void samples. Two of the 29 work tasks had peak samples with urinary glyphosate concentrations below the LOQ, both belonging to the manual knapsack SEG. The geometric means of the glyphosate concentrations measured in urine samples of the combined glyphosate SEGs were 0.68 µg/L for pre-task samples, 1.17 µg/L for post-task samples and 0.83 µg/L for following morning void samples. 100% of the workers wore gloves, 90% a Tyvec suit and 97% RPE.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because more detail could have been provided on the validation of the analytical method. Also the number of participants per exposure scenario was rather limited.

Assessment and conclusion by RMS:

Reliability criteria of exposure studies

Publication: Connolly *et al.,* 2018	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines/practices.	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Exposure to formulations with only glyphosate as a.s.	Y	
Exposure to formulations with glyphosate combined with other a.s.	N	
Exposure to various formulations of pesticides	N	
Study		

Reliability criteria of exposure studies

Publication: Connolly *et al.,* 2018	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study design clearly described	Y	
Population investigated sufficiently described	Y	
Exposure circumstances sufficiently described	Y	
Sampling scheme sufficiently documented	Y	
Analytical method described in detail	Y	
Validation of analytical method reported	Y?	Not complete.
Monitoring results reported	Y	
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Reliability not assignable		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because more detail could have been provided on the validation of the analytical method. Also the number of participants per exposure scenario was rather limited.		

1. Information on the study

Data point:	CA 5.9/002
Report author	Connolly, A. *et al.*
Report year	2019
Report title	Exploring the half-life of glyphosate in human urine samples
Document No	doi.org/10.1016/j.ijheh.2018.09.004 E-ISSN: 1618-131X
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary of the study according to OECD format

This study aimed to estimate the human half-life of glyphosate from human urine samples collected from amenity horticulture workers using glyphosate based pesticide products.

Materials and methods

Site description and study population - An occupational urinary biomonitoring study for glyphosate was carried out from September 2016 to September 2017. Sample collection took place at the Irish

Commissioner for Public Works (OPW) field sites. The tasks completed by the workers sampled were subdivided into three similar exposure groups (SEGs) based on the application method used by the workers to apply glyphosate based pesticide products: manual knapsacks, pressurized applicators and controlled droplet applicators, all of which involved the use of a handheld lance.

Urine collection - Full void urinary spot samples were collected from the participants for 29 work tasks and collected and analysed separately. A minimum of 3 urine samples were collected from each participant: one pre-task sample, one post-task sample and the first morning void sample obtained the day after completing the work task (following first morning void). Participants had an option to provide individual spot urine samples for all urinary voids from the start of the pesticide task to the following first morning void. A pre-labelled sample container was given to every participant for each void and they were asked to write the time and date on the container label. The volume of each urine sample was recorded and the sample was well mixed before taking a 20 mL aliquot to be transferred into a Sterilin™ pot, labelled with a unique identifier number, date and time. Care was taken to avoid any contamination. All samples were stored frozen at -18°C within 24 hours of collection pending analysis.

Urine sample analysis - Glyphosate was extracted from diluted urine samples (200 µl added to 800 µL deionized water) using anion exchange solid phase extraction (SPE) using 10% formic acid in methanol as an eluent. Quantitative analysis was performed by liquid chromatography tandem mass spectrometry (LC-MS/MS). Chromatographic separation was achieved on a Zorbax XDB-C8, 150×4.6 mm, 5 µm column with 0.1% formic acid and acetonitrile with a gradient elution as the mobile phase. The analytical method was linear over the range 0–20 µg L^{-1} and intra- and inter-assay coefficient of variations (CVs) of 3.54% (n = 10) and 9.96% (n = 40, over 4 runs) were achieved. The analytical limit of quantification (LOQ) was 0.5 µg L^{-1}. Creatinine analysis was performed on all urine samples with a Pentra 400 clinical analyser using the alkaline picrate method.

Estimation of the half-life time of glyphosate - To explore the elimination rate and to estimate the potential human biological half-life of glyphosate, only work tasks with at least two spot urine samples collected after the peak exposure were included for excretion profile analysis. The peak urinary exposure value was defined as the highest urinary glyphosate concentration measured in a spot sample after application per task. The elimination time and the estimation of the half-life of glyphosate was assessed using three different measurement metrics: unadjusted glyphosate concentrations (µg/L), creatinine corrected concentrations (µmol/mol creatinine) and Urinary Excretion Rates (UER). The UER (µg/hr) was calculated by taking the glyphosate concentration of the spot urine sample and multiplying it by the volume of the void and dividing it by the time that elapsed between this urine void and the last urine void.

Statistical analysis - All statistical analyses were performed on Microsoft Excel and Stata Software. Glyphosate concentrations were log transformed as the data showed a log normal distribution. The period of peak sample collection was taken as the start time (t = 0) and the time period from the sample collection time (t = 0) to each proceeding sample was calculated. The slope of the glyphosate urine concentration by the time duration (time passed from the start time) was calculated for each task. Linear interpolation using regression analysis was also performed for each of the included tasks. The mean values, as well as the 95% confidence interval of the half-lives were calculated to estimate the half-life range for each measurement metric.

Results

Urine samples from 7 participants performing 8 work tasks involving glyphosate based products were analysed. Data from 6 males and one female worker is included in this study. One male participated twice on two consecutive days. The age range was from 32 to 60 years, with an arithmetic mean (AM) of 48 years. Workers carried out work tasks that involved the application of glyphosate based pesticide products within one of the SEGs, which lasted between approximately 1 and 6 hours daily. The total sampling time duration of the selected 8 work tasks included in the data analysis, ranged from approximately 19 to 26 hours. In total, 28 individual spot urine samples were analysed for the 8 work tasks included in this study

(3 to 4 spot urine samples per sample set). Each sample set was analysed to evaluate the relationships between the measured urinary glyphosate concentrations (µg/L, µmol/mol creatinine or UER) and the duration. The duration started from the peak concentration sample (start time) to each of the subsequent samples. Correlations and linear regression analysis were performed for each sample set. Four sample sets were excluded from the analysis: two creatinine corrected samples sets (µmol/mol creatinine) and two UER calculated sample sets. One creatinine corrected sample set was excluded due to low creatinine levels (< 3 mmol/L) in individual spot urine samples and another because there was no association between concentrations and duration of sampling. This lack of association could relate to a number of factors like gender, diet and hydration.

Each of the sample sets showed a moderate to strong relationship between concentration and duration for all samples (R^2 = 0.42–1.00), with an estimated half-life ranging approximately from 1.5 to 10 hours for unadjusted values or from 4.75 to 20 hours for creatinine corrected values. When the results were restricted to sample sets which showed very strong relationships (R^2 > 0.90), the estimated half-life average (range) was 4.5 (1.5 - 7) hours and 7.5 (4.75 – 9.25) hours for unadjusted and creatinine corrected values, respectively. UER calculated samples showed moderate to strong relationship (R^2 = 0.60–0.95), with an estimated half-life average (range) of 7.25 (3 and 9.50) hours. The average glyphosate half-life including all measuring metrics was approximately 5.5 to 10 hours. The average and range of the half-life on sample sets (numbers 2, 12, 19 and 30) that had all three measuring metrics included was calculated. Sensitivity analysis on the four sample sets, common across all measuring metrics, had an estimated half-life average (range) of approximately 6.5 (4–10), 11.75 (7.25 - 20), and 6.5 (3–7.75) hours for the unadjusted glyphosate concentrations, creatinine corrected concentrations and urinary excretion rate. The limitations of this study are the lack of standardization (pesticide products used, quantity of pesticides applied per task, different application methods and different sampling times). The small sample size prevented the use of more elaborate statistical tests to identify differences due to sex or age. The pharmacokinetic analysis revealed first order kinetics but due to the collection of urine samples over a limited period of time (19-26 hours) multi-phasic kinetics may not have been identified.

Conclusion

The results from this study provide new information on the elimination rate and estimated human biological half-life of glyphosate based on the analysis of urine samples collected during an exposure assessment study. This information can be helpful in the design of sampling strategies, as well as assisting in the interpretation of results for human biomonitoring studies involving glyphosate. The data could also contribute to the development or refinement of Physiologically Based PharmacoKinetic (PBPK) models for glyphosate.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Analytical data for glyphosate obtained from spot urine samples collected during a glyphosate exposure study (Connolly et al., International journal of Hygiene and Environmental Health (2018), Vol. 221, 1012-1022) were used to estimate the human biological half-life of glyphosate. To that end only work tasks with at least two spot urine samples collected after the peak exposure were included for excretion profile analysis. Glyphosate concentrations were log transformed and the slope of the glyphosate urine concentration by the time duration (time passed from the start time) was calculated for each task. When the results were restricted to sample sets which showed very strong relationships (R^2 > 0.90), the estimated half-life average (range) was 4.5 (1.5 - 7) hours and 7.5 (4.75 - 9.25) hours for unadjusted and creatinine corrected values, respectively. UER calculated samples showed moderate to strong relationship (R^2 = 0.60–0.95), with an estimated half-life average (range) of 7.25 (3 and 9.50) hours. This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions in view of the limitations of the study such as the lack of standardization (pesticide products used, quantity of pesticides applied per task, different application methods and different sampling times). The

small sample size prevented the use of more elaborate statistical tests to identify differences due to sex or age. The pharmacokinetic analysis revealed first order kinetics but due to the collection of urine samples over a limited period of time (19-26 hours) multi-phasic kinetics may not have been identified.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions in view of the limitations of the study such as the lack of standardization (pesticide products used, quantity of pesticides applied per task, different application methods and different sampling times). The small sample size prevented the use of more elaborate statistical tests to identify differences due to sex or age. The pharmacokinetic analysis revealed first order kinetics but due to the collection of urine samples over a limited period of time (19-26 hours) multi-phasic kinetics may not have been identified.

Assessment and conclusion by RMS:

Reliability criteria of exposure studies

Publication: Connolly *et al*., 2019	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines/practices.	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y?	Urine monitoring data from a glyphosate exposure study were used to study the urinary excretion kinetics of glyphosate. There are limitations in the study approach.
Test substance		
Exposure to formulations with only glyphosate as a.s.	Y	
Exposure to formulations with glyphosate combined with other a.s.	N	
Exposure to various formulations of pesticides	N	
Study		
Study design clearly described	N	Based on the glyphosate exposure study.
Population investigated sufficiently described	N	Based on the glyphosate exposure study.
Exposure circumstances sufficiently described	N	Based on the glyphosate exposure study.
Sampling scheme sufficiently documented	Y	Based on the glyphosate exposure study.
Analytical method described in detail	Y	
Validation of analytical method reported	Y?	Could be more elaborate.
Monitoring results reported	Y	
Statistical analysis	Y	
Pharmacokinetic analysis	Y	To some extent, supposing first order one-compartment pharmacokinetics.
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	

Publication: Connolly *et al*., 2019	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Reliability not assignable		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions in view of the limitations of the study such as the lack of standardization (pesticide products used, quantity of pesticides applied per task, different application methods and different sampling times). The small sample size prevented the use of more elaborate statistical tests to identify differences due to sex or age. The pharmacokinetic analysis revealed first order kinetics but due to the collection of urine samples over a limited period of time (19-26 hours) multi-phasic kinetics may not have been identified.		

1. Information on the study

Data point:	CA 5.9/003
Report author	Connolly, A. *et al.*
Report year	2017
Report title	Exposure assessment using human biomonitoring for glyphosate and fluroxypyr users in amenity horticulture
Document No	doi.org/10.1016/j.ijheh.2017.06.008 ISSN: 1618-131X
Guidelines followed in study	None
Deviations from current test guideline	NA
Previous evaluation	Non-GLP
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Reliable without restrictions

2. Full summary of the study according to OECD format

This study aims to measure occupational exposures to amenity horticuturalists using pesticides containing the active substances, e.g. glyphosate by urinary biomonitoring. A total of 40 work tasks involving glyphosate were surveyed over the period of June – October 2015. Pesticide concentrations were measured in urine samples collected pre and post work tasks using liquid chromatography tandem mass spectrometry. Pesticide urinary concentrations were higher than those reported for environmental exposures and comparable to those reported in some agricultural studies. Log-transformed pesticide concentrations were statistically significantly higher in post-work samples compared to those in pre-work samples. Urinary pesticide concentrations in post-work samples had a geometric mean / geometric standard deviation of 0.66 / 1.11 $\mu g\ L^{-1}$. Linear regression revealed a statistically significant positive association to exist between the time-interval between samples and the log-transformed adjusted (i.e. post-minus pre-task) pesticide urinary concentrations ($\beta = 0.0039$; $p < 0.0001$).

Materials and Methods

Site description and study population - This study was conducted over the period of June – October 2015

in the Republic of Ireland, at field sites managed by the Office of Public Works (OPW). A walk through survey was performed by the researcher at the selected OPW sites including national parks, ornamental gardens and historic monuments, to collect information on the frequency of use of pesticides containing glyphosate and spraying methods used. In this study 3 similar exposure groups were considered for glyphosate applications: glyphosate with a manual knapsack (8 participants using Roundup Biactive XL, 360 g/L; Pistol, 250 g/L; Destrol Amenity, 250 g/L; Glyfos, 360 g/L; Rambo 360, 360 g/L), glyphosate with a controlled droplet applicator (4 participants using Nomix Dual, 120 g/L), and glyphosate with a pressurized handheld lance (5 participants using Roundup Biactive XL, 360 g/L; Pistol, 250 g/L; Glyfo, 360 g/L; Rambo 360, 360 g/L). Amenity horticulturists (age ranging from 33–66 years) who used glyphosate and worked with the OPW at the designated sites, were invited to participate in the study. Participation in the study was voluntary and recruitment was done in coordination with the OPW Health and Safety Unit. Prior to the commencement of the study, the workers were informed of the sampling protocols and methods and completed a self-administered questionnaire to collect information on the participants including their work activities, out-of-work use of pesticides, dietary habits and smoking status.

Biological Monitoring

Urine collection - On the day workers participated in the study, they were asked to provide a pre-work and a post-work sample of up to 50 mL each. The post-work sample was taken within one hour after completion of the task. The samples were stored at -18°C until laboratory analysis. The sampling time in this study was defined as the time interval between the pre- and post-sample collection. Following information was collected for each task: sampling time, application method, pesticide used, personal protective equipment (PPE) used and climatic conditions. Any changes in PPE, change in weather conditions, breaks taken and problems were also recorded.

Urine sample analysis - Glyphosate standard and glyphosate internal standard (2-$^{13}C^{15}N$-glyphosate) were purchased commercially. 500 µL of urine sample was diluted with 500 µL of deionised water for the analysis of glyphosate. The prepared samples were spiked with 10 µL of internal standard (500 µg/L). Glyphosate was extracted from urine by solid phase extraction (SPE) with subsequent analysis by liquid chromatography tandem mass spectrometry (LC–MS/MS) using a Zorbax SB-C3 150 × 4.6 mm, 5 µm column equipped with a C18 guard column. Mass spectrometry analysis was performed in negative MRM mode. The method was linear over the calibration range 0–20 µg/L. The intra and inter assay coefficients of variation were 3.54% (n = 10) and 9.96% (n = 40, over 4 runs) for glyphosate. The limit of detection (LOD) for glyphosate was set at 0.5 µg/L. Creatinine analysis was performed on all urine samples. Statistical analysis for this study was performed using STATA software. Urinary glyphosate concentrations are expressed as geometric means (GM) and geometric standard deviations (GSD) along with arithmetic mean (AM), minimum and maximum levels.

Results

Demographic and working characteristics - The study population consisted of 18 horticulture amenity gardeners (17 men and one woman), who applied pesticides as part of their work. One participant stated that he was a smoker. In 58% of the tasks sampled, a pesticide task had also been completed on the previous day. Of the 40 tasks analysed in this study, 93% of the workers applied glyphosate by spraying with 53% being involved with mixing and loading. The vast majority of the workers wore PPE for each task: 94% wore gloves, 83% respiratory protective equipment (RPE), and 67% Tyvek suits. 89% of the workers reused their PPE and 78% of the workers did not take breaks during the task. The average duration of exposure to glyphosate (from pre-work to post-work sampling time) was 110 minutes for application with a manual knapsack, 235 minutes for application with a large droplet applicator, and 219 minutes for application with a pressurized lance applicator.

Urinary glyphosate concentrations - A total of 80 samples were collected in this study, 40 pre-work and 40 post-work samples. 58% (23 samples) of the pre-work samples and 43% (17 samples) of the post-work samples had glyphosate concentrations below the LOD. For the combined exposure groups the geometric

mean was 0.42 µg/L for the pre-work samples and 0.66 µg/L for the post-work samples. The geometric means of the glyphosate urinary concentrations of the knapsack and pressurized lance applicators were comparable (0.62 µg/L and 0.57 µg/L, respectively) whereas the geometric mean of the post-work samples of the controlled droplet applicators was higher (1.00 µg/L) although not statistically significantly different from the other exposure groups. The urinary glyphosate concentrations (both simple and corrected for creatinine values) of the pre- and post-work samples were statistically significantly different. When the effects of the time between the collection of the pre- and post-work samples on the urinary concentrations were considered, a positive statistically significant association was observed. Similar results were found with the creatinine corrected values Similar but somewhat less pronounced associations were observed for the controlled droplet applicator and pressurized lance groups, but not for measurements performed during spraying with the manual knapsack. Pesticide urinary concentrations from workers who took breaks during the task were significantly higher by a factor 1.7 on average, compared to those from workers who did not take breaks. Trends were similar when analysis was repeated using the creatinine corrected values. Similarly, median exposure durations were statistically significantly longer for the measurements where workers took breaks compared to those who did not (273 versus 105 minutes), as well as those samples that had pesticide concentrations above the LOD compared to those with concentrations below the LOD (170 versus 98 mins).

Discussion

Studies quantifying occupational exposure to pesticides in amenity horticulture are very sparse. The sensitivity of the analytical method used for glyphosate (LOD of 0.5 µg/L) was comparable to those previously reported. The study results suggest that amenity workers have elevated urinary pesticide levels for glyphosate, above what would be expected from dietary exposures. In 43% of the post-work samples, pesticide concentrations were lower than the LOD. Although very low, the pesticide concentrations in almost all of the post-work samples were higher than those in the pre-work samples. Although no human biomonitoring data are available for the Irish population, the arithmetic mean of the urinary concentrations in the post-work samples (1.35 µg/L) is higher than the mean of 0.21 µg/L reported in an European environmental exposure study. Similarly, both mean and maximum values reported in the current study (1.35 µg/L and 10.66 µg/L, respectively) are also higher than the maximum urinary glyphosate concentrations (0.41 µg/L) reported for German adults with non-occupational exposures to glyphosate. Compared to previous studies among working populations, glyphosate urinary concentrations are comparable to those reported on agricultural exposures and slightly lower but within the range of urinary concentrations reported in studies among farm families in Iowa and farmers in Minnesota and South Carolina. The mean urinary glyphosate concentrations were comparable across all exposure groups although the exposure data from the controlled droplet applicator group appeared to be somewhat more variable than those from the other groups. The observed variation in exposure within this group may reflect inconsistent use of PPE and complacent work practices. Protective coveralls were only worn for 29% of the tasks in this group compared to at least 50% in the other groups.

Previous studies have associated higher pesticide exposures with the mixing and loading of pesticide concentrate. In this study, the majority of the participants performed mixing and loading of the pesticide concentrate as part of the overall task assessed with the exception of the controlled droplet applicators who used a pre-mixed solution. It was therefore not possible to evaluate pesticide exposure during the mixing and loading of the pesticide concentrate. A strong association was found between urinary glyphosate concentrations and exposure duration. The duration of exposure for work involving spraying with the manual knapsack and controlled droplet applicator groups ranged from 5 to 115 min and 33–195 min, respectively. No association between urinary pesticide levels and exposure duration was found for manual knapsack applicators. These study results show that there is a potential for exposure during tasks in horticulture and amenity gardening that typically involve small volumes of pesticides, ranging from 100 mL to 2 L, which warrants further investigation. 89% of the workers reused PPE such as coveralls, gloves, disposable face masks and this may have contributed to the level of exposure. Higher levels of urinary pesticide levels were found among workers who took breaks during the task. Workers who encountered problems during pesticide application such as adjusting the nozzle, leaks or spills, change in climatic

conditions or issues with PPE, had higher average urinary concentrations of glyphosate. The sampling strategy adopted in this study (spot sampling pre- and post-work), most likely underestimates the exposure potential. A larger study incorporating 24–72 h urine samples would provide more reliable estimates of exposure and allow comparison with ESFA's designated ADIs and AEOLs.

Conclusions

The results from this study provide evidence of occupational exposure to glyphosate among amenity horticultural workers. The measured levels of urinary concentrations are comparable to those reported for agricultural workers. Urinary concentrations appeared to be dependent on the duration of exposure and the levels measured were higher among workers who took breaks or performed longer tasks, such as the use of controlled droplet applicators. Further research is currently underway to investigate 24 h exposures, evaluate dermal and inadvertent ingestion exposure and their contribution to total body burden of the pesticides.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Biological monitoring has previously been used in studies evaluating occupational exposures to pesticides in both the agricultural and horticultural sectors. The aim of this study was to characterise the occupational exposures in amenity horticultural workers using a biomonitoring method for glyphosate in urine. The geometric mean of the urinary glyphosate concentrations in the post-work samples of all exposure groups combined was found to be 0.66 µg/L. When the relationship between urinary concentrations of glyphosate and systemic dose as established by Acquavella et al. (Acquavella et al. (2004) Environmental Health Perspectives, 112(3), 321-326) is taken into consideration, the daily systemic dose for the workers in this study is estimated to be 0.000021 mg/kg bw/day. The corresponding daily oral external dose is about 0.0001 mg/kg bw/day when an oral bioavailability of 20% is taken into account. This is 5,000 times lower than the ADI of 0.5 mg/kg bw/day.

This publication is considered relevant for glyphosate risk assessment and reliable without restrictions because it complies with all the reliability criteria of an exposure study.

Assessment and conclusion by RMS:

Reliability criteria for exposure studies

Publication: Connolly *et al*., 2017	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines/practices.		
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Exposure to formulations with only glyphosate as a.s.		
Exposure to formulations with glyphosate combined with other a.s.		

Exposure to various formulations of pesticides	Y	
Study		
Study design clearly described	Y	
Population investigated sufficiently described	Y	
Exposure circumstances sufficiently described	Y	
Sampling scheme sufficiently documented	Y	
Analytical method described in detail	Y	
Validation of analytical method reported	Y	
Monitoring results reported	Y	
Overall assessment		
Reliable without restrictions	Y	
Reliable with restrictions		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate and reliable without restrictions because it complies with all the reliability criteria of an exposure study.		

1. Information on the study

Data point:	CA 5.9/004
Report author	Connoly, A. *et al.*
Report year	2018
Report title	Glyphosate in Irish adults – A pilot study in 2017
Document No	doi.org/10.1016/j.envres.2018.04.025 E-ISSN: 1096-0953
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	None
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Yes/Reliable without restrictions

2. Full summary of the study according to OECD format

The objective of this study was to conduct an exploratory glyphosate exposure assessment study among Irish adults, who were non-occupational users of glyphosate. A biomonitoring survey involving the collection and analysis of 20 ml spot urine samples from 50 Irish adults was conducted in June 2017. Participants completed a short questionnaire to collect information on demographics, dietary habits and lifestyle. Glyphosate was extracted using solid phase extraction (SPE) and analysed by liquid chromatography tandem mass spectrometry (LC-MC/MS). Of the 50 samples analysed, 10 (20%) contained detectable levels of glyphosate (0.80 – 1.35 µg/L). Exposure concentrations were higher than those reported in comparable studies of European and American adults.

Materials and methods

Using a convenient sampling method, 50 Irish adults (> 18 years) were recruited from 16 counties across the Republic of Ireland to participate in the study over June – August 2017. Participants with specific dietary habits (vegetarian/vegan) and those whose occupation involved the use of pesticides were excluded from the study, to prevent dietary exposure bias, due to a proportionally increased intake of wheat products, fruits

and vegetables or from occupational use of glyphosate-based pesticide products. Participants completed a project questionnaire and provided one, 20 mL first morning void urine sample. A questionnaire was designed to collect information on participant demographics, dietary habits and lifestyle.

Glyphosate was extracted using solid phase extraction (SPE) and analysed by liquid chromatography tandem mass spectrometry (LC-MC/MS). The method was linear over the calibration range 0 – 20 µg/L, the limit of detection (LOD) was set at 0.5 µg/L (signal to noise ratio of ≥ 3:1). Creatinine analysis was completed on all samples using an automated alkaline picrate method.

Results

Of the 50 study participants, 60% (n = 30) were female, the mean age was 42 (range 18 − 82), 60% (n = 30) were pet owners and only 10% (n = 5) were smokers. Participants reported that 70% (n = 35) of them consumed few or no portions of organic food. A large proportion of participants, 80% (n = 40), reported consuming 2 portions or less of bread per day.

Home use of pesticides was reported by 40% (n = 20) of participants and 16 of the 20 reported using glyphosate-based products such as Roundup, Gallup, and Weedol, they also reported wearing personal protective equipment when using pesticides (gloves, facemasks or both).

Of the 50 samples analysed, 10 samples (20%) contained detectable levels of glyphosate (0.79 – 1.32 µg/L). Based on results from urinary creatinine analysis, 47 samples were valid (creatinine values < 3.0 or > 30 mmol/L). The three invalid samples had no detectable level of glyphosate. Six of the detectable samples were from females, similar to the gender spread in the full data set. Of the detectable results, 3 out of the 10 participants used glyphosate-based products at home but none of them had used pesticides for at least a month previous (Table 1).

Table 1. Summary of the urinary glyphosate concentrations expressed as µg/L and as µmol/mol creatinine among 50 Irish adults sampled in 2017

Variable		
Samples	**Glyphosate**	
No. of samples analysed *n* (%)	50 (100)	
No. of valid samples *n* (%)	47 (94)	
No. of samples > LOD *n* (%)	10 (20)	
*Sample analysis of 10 samples > 0.5 µg L^{-1} (LOD)	µg L^{-1}	µmol/mol creatinine
Median	0.87	0.41
Maximum	1.35	0.89
Minimum	0.80	0.21

* 20% (10) of the samples analysed had detectable levels.

Conclusion

The proportion of detectable urinary glyphosate concentrations for samples collected from 50 Irish adults is low (20%), which could be due to less localised use of pesticides. A European study that had an LOD of 0.1 µg/L, reported low detection rates in Germany (32%), whereas in the USA, 93% of samples were above 0.5 µg/L (despite an LOD of 0.1 µg/L). The detection rate could possibly have underestimated the true population exposure proportion due to the small sample size and the higher limit of detection of the analytical method used in this study.

3. Assessment and conclusion

Assessment and conclusion by applicant:

This study is newly submitted for purpose of review. A biomonitoring survey involving the collection and analysis of 20 ml spot urine samples from 50 Irish adults on non-occupational setting was conducted. The LC-MC/MS analyses of urinary samples revealed that 10 out of 50 samples analysed (i.e. 20%) contained detectable levels of glyphosate (0.80 – 1.35 µg/L). The low proportion of detectable glyphosate levels could be due to lower localised use of pesticides, having a small sample size or the higher analytical detection limit used in this study (0.5 µg/L).

This publication is considered relevant for the risk assessment of glyphosate and reliable without restrictions because it complies with the quality criteria of a good exposure study.

Assessment and conclusion by RMS:

Reliability criteria of exposure studies

Publication: Connolly *et al*., 2018.	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines/practices.	?	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Exposure to formulations with only glyphosate as a.s.	Y	
Exposure to formulations with glyphosate combined with other a.s.	Y	
Exposure to various formulations of pesticides	Y	
Study		
Study design clearly described	Y	
Population investigated sufficiently described	Y	
Exposure circumstances sufficiently described	Y	
Sampling scheme sufficiently documented	Y	First morning urine void sample.
Analytical method described in detail	Y	To some extent, ref. to other paper.
Validation of analytical method reported	Y	To some extent, ref. to other paper.
Monitoring results reported	Y	
Overall assessment		
Reliable without restrictions	Y	
Reliable with restrictions		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate and reliable without restrictions because it complies with the quality criteria of a good exposure study.		

1. Information on the study

Data point:	CA 5.9/005
Report author	Connolly, A. *et al.*
Report year	2019
Report title	Evaluating glyphosate exposure routes and their contribution to total body burden: a study among amenity horticulturalists
Document No	doi: 10.1093/annweh/wxy104 E-ISSN: 2398-7316
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	None
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Yes/Reliable without restrictions

2. Full summary of the study according to OECD format

The purpose of this study was to evaluate determinants of dermal and inadvertent ingestion exposure and assess their contribution to total body burden among amenity horticultural users using glyphosate-based pesticide products. A dermal and inadvertent ingestion exposure assessment was completed alongside a biomonitoring study among amenity horticultural workers. Linear mixed effect regression models were elaborated to evaluate determinants of exposure and their contribution to total body burden.

A total of 343 wipe and glove samples were collected from 20 workers across 29 work tasks. Geometric mean (GM) glyphosate concentrations of 0.01, 0.04 and 0.05 μg/cm^2 were obtained on wipes from the workers' perioral region and left and right hands, respectively. For disposable and reusable gloves, respectively, GM glyphosate concentrations of 0.43 and 7.99 μg/cm^2 were detected. The combined hand and perioral region glyphosate concentrations explained 40% of the variance in the urinary (μg/L) biomonitoring data. Data show the dermal exposure is the prominent route of exposure in comparison to inadvertent ingestion but inadvertent ingestion may contribute to overall body burden.

Materials and methods

Site description and study population

Exposure assessments were conducted at sites managed by the Irish Commissioner for Public Works (OPW) from September 2016 to September 2017. Workers were grouped into three similar exposure groups (SEGs): manual knapsack, pressurised lance and controlled droplet applicator, based on the applicator used to apply glyphosate-based pesticide products. The manual knapsack applicator SEG (typical capacity of 10 – 15 L), was carried as a knapsack with the pesticide product being applied with a handheld lance. The pressurised lance SEG applied the pesticide product using a handheld lance connected to a motorised knapsack. The controlled droplet applicator SEG, similar to the manual knapsack, but with a capacity 5 L, was purchased with a pre-mixed solution (eliminating the mixing and loading task) and had an adaptable applicator that could increase the droplet size, thus reducing the spray drift.

Study participants were recruited via oral presentation and circulated project information leaflets. Participation was voluntary and all participants gave informed consent. Ethics approval for this project was received from the National University of Ireland, Galway Research Ethics Committee (Ref: 16 July 2019) on the 5th September 2016.

Biomonitoring samples
A biomonitoring study involving the collection of individual full urinary void spot samples was completed and previously been reported (Connolly *et al*., 2018). A minimum of three urine samples were collected from each participant: a sample before the task began, within 1 h of task completion and the following first morning void. For 59% (n = 17) of tasks, participants gave samples for each void over the exposure assessment period (pre-task to the following first morning void). Urine samples were analysed separately for glyphosate, so the sample with the highest glyphosate concentration could be identified for each task. The urine sample with the highest glyphosate concentration measured during the sampling period was selected and referred to as the peak urinary sample for that participant.

Dermal and inadvertent ingestion sampling strategy
Wipe samples of the hands, perioral region and of potentially contaminated work surfaces (pesticide product container, worker mobile phones and steering wheels of work vehicles) were collected. Wipe sampling was conducted using Ghost Wipes™, pre-packaged polyvinyl alcohol wipes wetted with deionised water by the manufacturer. Dermal and perioral wipe samples were collected from the workers before and after the sampling task. Pre-work task wipe samples were required to evaluate whether detectable data collected post-task was as a result of the observed pesticide application task. Workers' glove and surface wipe samples were collected after the pesticide application tasks. The researcher wore disposable nitrile gloves for collecting samples and changed these gloves with each sample obtained. An appropriate number of Ghost Wipes™ and glove field blanks were also collected.

Following sampling, wipes were placed in 100 mL plastic pots and appropriately labelled. Samples were extracted and aliquots were frozen to −18°C within 24 h of collection, shipped and chemical analysis completed at the Health and Safety Executive's Laboratory, Buxton, UK.

Dermal sampling
Hand wipes were collected, using two wipes per hand. The front of the hand was wiped with five strokes from the base of the hand to the top of the palm and then five strokes across the palm, starting from the base of the palm of the small finger. The wipe was folded in half and the same sequence repeated on the back of the hand. The wipes were then folded once more and each individual finger was wiped, starting at the small finger to the thumb, going between the fingers and including the finger web areas. Followed by the tips of each finger wiped in a circular motion. The same procedure was completed again with a second wipe and repeated for both hands. Dermal wipe samples were collected from the hands when workers removed their gloves, either during the task (i.e. lunch break) or after the pesticide application task.

Glove contamination samples
PPE use varied from disposable to reusable chemical resistant nitrile gloves as per company policy. After the work task, disposable glove samples were collected for glyphosate analysis, while only some participants provided their reusable gloves for analysis. Worker gloves were collected after the pesticide application tasks or within the task if gloves were changed during the pesticide task. At the end of the pesticide application task, dermal wipe samples were collected of each hand after the gloves were removed.

Inadvertent ingestion sampling
Perioral wipes were collected starting from the upper lip area and wiped in a clockwise motion around the upper lip and philtrum area and down around to the mentolabial fold to the edge of the mouth of the lower lip area. The wipe was folded in half and similarly wiped in an anti-clockwise direction, starting at the lower lip area and finishing at the upper lip area. One wipe was used for the perioral region.

An inadvertent ingestion observational study was also conducted. The frequency of worker contacts per task, (which in the current study included all surfaces contacted by the worker), frequency of worker hand to mouth contacts, contacts with the body and surrounding area (i.e. potentially contaminated surfaces) were recorded. The frequency of contacts was recorded during only the pesticide task. Worker contacts pre- and post-work task or during work breaks were not recorded.

Potentially contaminated work surface sampling
Potentially contaminated work surfaces were wiped using one Ghost Wipe™, according to an object-specific sampling protocol developed within the study. Specifically, mobile phones were first wiped on the front of the phone, from the top to the bottom of the screen in one stroke. The wipe was folded in half and wiped on the back from top to bottom in one stroke. Finally, the wipe was folded once more and the edge of the phone was wiped, starting at the top right hand corner and completing the full edge in a clockwise motion.

Similarly, work vehicle steering wheel wipe samples were wiped from the top of the steering wheel in a clockwise direction, then folded it in half and repeated in an anti-clockwise direction. The wipe was folded once more and the centre of the wheel was wiped in a clockwise direction and the spokes were wiped from the edge to the base of the steering wheel.

Pesticide product containers were wiped from top to bottom for the full width of the container. The bottom of the container was wiped from right to left in one stroke. The wipe was folded and the top of the container was wiped in a clockwise motion. The container handle and surrounding area were wiped from top to bottom afterwards. The wipe was folded once more and the area around the lid was wiped, and then the lid itself, in a clockwise motion.

Chemical analysis
Wipe samples from the hands and perioral region, as well as the disposable glove samples, were placed into a 100 mL plastic pot and extracted by adding 50 mL of deionised water, shaken vigorously for 30 seconds, then placed on the Denley Spriamax 5 roller mixer for an hour. A 20 mL aliquot was transferred to a labelled Sterilin™ pot for storage and transport before analysis. Glyphosate was extracted from large reusable gloves at the laboratory. One glove was placed into a grip seal bag with 100 mL deionised water. Bags were placed on a gyratory rocker for 1 h with bags being turned over at 30 min. The liquid contents were then transferred to Sterilin™ pots for storage before analysis. The solubility of glyphosate in water (11.6 g/L at 25°C) made it an appropriate extraction solvent.

All samples were prepared and analysed for glyphosate. Glyphosate was extracted from dermal wipe, surface wipe and glove extracted water samples (100 µL diluted with 900 µL deionised water) using strong anion exchange solid phase extraction eluting into 10% formic acid in methanol. The eluent was evaporated under a stream of nitrogen and reconstituted in 200 µL of 0.1% formic acid. Quantitative analysis was performed by liquid chromatography tandem mass spectrometry. Chromatographic separation was achieved on a Zorbax XDB-C8, 150 x 4.6 mm, 5 µm (Agilent, Stockport, UK) column with mobile phases of 0.1% formic acid and acetonitrile with a gradient elution. The method was linear over the range 1–1000 µg/L and the LOQ was 1 µg/L and the LOD was 0.5 µg/L. Where results exceeded the top of the linear range (1000 µg/L) the samples were repeated with dilutions. The method was reproducible with an intra assay CV of 2.9% (n = 12) and an inter assay CV of 4.2% (n = 42, over four runs).

Data processing and analysis
All 23 wipe field blanks had non-detectable glyphosate concentrations. Seventeen glove field blanks were collected from the sites (as some workers used reusable gloves), six having non-detectable glyphosate concentrations, whereas detectable levels were found in the remainder. For each task with detectable glyphosate levels found on the blank glove, each glove sample within that task was field blank corrected. All the samples were corrected for the sample volume and for the surface area wiped. Although samples were not corrected for recovery efficiencies, the mean recovery percentage for plastic containers, disposable chemical resistant nitrile gloves and mobile phones, spike at 20 µg, was 122, 104 and 125%, respectively. Ghost wipes™ have a mean recovery for three samples spiked at 200 µg of 106%.

The average hand surface area measurements were assigned according to published US EPA guidance (US EPA, 2011). The glove adjustments were assigned in the same manner as the surface area for hands. This assumes a 1070 cm^{-2} for both male hands, or 535 cm^{-2} surface per hand and 890 cm^{-2} for both female

hands, or 445 cm^{-2} per hand. Average surface area measurements for the perioral region were assigned as 40 cm^{-2}.

Surface area calculations for the steering wheel were assigned as 1100 cm^{-2} surface area. An average mobile phone surface area of 202 cm^{-2} was calculated using the physical phone dimension measurements, based on the phone type sampled. Similarly, for the product containers, an average surface area value of 2300 cm^{-2} was calculated.

Statistical analysis
Before the conducting statistical analysis, all concentration levels below the LOQ were imputed, in SAS v 9.4 (SAS Institute, North Carolina, USA). A single random imputation method based on maximum likelihood estimation was used. The remainder of the statistical analysis was performed using Stata Statistical Software 15 (StataCorp, 2015).

The data were log normally distributed and thus all statistical analysis was performed with log transformed exposure concentrations. Summary and descriptive analysis was performed on the work demographics and glyphosate concentrations levels for the combined dataset and by SEG. The results for the potentially contaminated surfaces are only shown for the combined dataset.

Pearson's correlation coefficients were estimated to evaluate relationships between glyphosate concentrations on the right and left hand, the dominant hand and both hands combined. Similar tests were performed on the glove data.

A linear mixed effect regression model was elaborated based on exposure determinants for inadvertent ingestion previously identified and evaluated in regression analysis against measurements of metals (Gorman Ng *et al.*, 2017). In this model, the hand contamination and the frequency of contacts per task were entered as fixed effects whereas the worker's id was entered as a random effect to account for correlations between repeated measurements from the same worker. This model had some differences to Gorman Ng *et al.*, 2017 model, including that respiratory protective equipment (RPE) was not considered as it was used by all workers participating in the study and that we used frequency of contacts per task, not just hand to perioral/oral contacts per task. Further models using a forward model-built approach were elaborated to examine the robustness of the derived model as well as to identify determinants for inadvertent ingestion and dermal exposure and their relative contribution to overall body burden. In these models, parameters were entered sequentially based on their level of significance and kept within the model if they had a statistical significance of ($P < 0.1$).

Results

Demographic and working characteristics
Twenty amenity horticulturists who applied glyphosate-based pesticide products as part of their daily duties participated in the study (18 males and 2 females) were grouped into 3 SEGs. The pesticide task duration ranged from approximately a half hour to 6 hours. Work tasks involving the manual knapsack, controlled droplet applicator and the pressurised applicator were, on average, ~3, 3½ and 6 h, respectively.
Good worker compliance with PPE use was observed, with workers using PPE for most of the work tasks sampled; gloves, Tyvek suits and RPE were used for 29 (100%), 26 (90%) and 28 (97%) of the observed tasks, respectively.

Levels of glyphosate concentrations on wipes, gloves and contaminated surfaces
A total of 343 wipe and glove samples across 29 work tasks were collected and analysed for glyphosate. A minimum of seven sets of wipe samples were collected for each task sampled. A sample set consists of a blank wipe, perioral sample and each hand sample (two wipes per hand), before and after each work task.

Glyphosate concentration data for perioral and hand wipes (µg/cm), collected pre and post the work tasks are presented in Table 1 for overall samples and per SEG. Table 2 details the glyphosate concentration data

for the disposable and reusable gloves samples. Seventeen pairs of disposable gloves and seven pairs of reusable gloves were analysed in this study. For three of the work tasks analysed, workers wore disposable gloves over a pair of reusable gloves and gave both sets of gloves for analysis. For eight of the tasks, the workers refused to give their gloves. Glyphosate was detected in all the pre- and post-work task hand wipe samples, as well as on the post-work task glove samples. Only 11 (38%) of the pre-work task and 6 (21%) post-work task perioral wipes had non-detectable glyphosate concentrations. For a third of work tasks sampled (n = 10), workers had started the pesticide task prior to the collection of pre-work task samples. Detectable glyphosate concentrations were also found on 11 (65%) of the blank gloves samples, collected from PPE field stores. Field observations suggest that cross contamination may occur when storing new gloves in close proximity of the pesticide chemical storage area or when handling unused gloves with contaminated hands.

In the current study, arithmetic mean (range) glyphosate concentrations of 2708 (3.0–21 845) µg wipe^{-1} and 2797 (5.0–27 354) µg wipe^{-1}, (right and left hand, respectively) were found on worker hand wipe samples collected after the pesticide application task. Glyphosate concentration levels of 41 (< LOQ–321) µg per wipe were detected on wipes collected from the perioral region. Values reported for hand wipes in this study were higher than those found for agricultural pesticide users 647 (83–2081) µg (Christopher, 2008). However, the perioral glyphosate concentrations were within a range of that reported in Christopher (2008), with an arithmetic mean and range of 39.5 (2.6 – 91) µg. Christopher (2008) also detected glyphosate in worker saliva samples with an arithmetic mean and range of 140 (56–440) ng, which could suggest comparable inadvertent ingestion levels for the current study workers.

Strong positive associations were found between left and right hands, and between the dominant hand, the individual hands and the combination of both hands. Between SEGs, similar glyphosate concentrations were detected on perioral wipes, with the highest geometric mean (GM) and maximum value found in the pressurised lance SEG. Within SEGs, similar glyphosate wipe concentrations were found on both the right and left hands in the manual knapsack group and the pressurised lance applicator. Glyphosate concentrations on reusable gloves were two orders of magnitudes higher than those on disposable gloves.

On some occasions, additional dermal and gloves samples were collected for workers who took a break during the pesticide application task. For three of the work tasks, dermal wipe samples were collected from three participants before taking a break and the glyphosate concentrations for the perioral and both hands (combined) ranged from 0.01 to 0.15 and 0.002 to 0.41 µg/cm, respectively. Similarly, three participants provided their disposable gloves during the break for five of the work tasks, which included two of these participants giving samples on two consecutive days and found glyphosate concentrations that ranged from 1.27 to 20.89 µg/cm. As concentrations were similar to the post-work task samples and due to the limited sample numbers, this data were not included in further analysis. The additional samples collected could only have had a negligible effect on the post-work task samples due to similar detection levels. The assumption would be that workers would wash hands and dispose of contaminated work gloves before their break, removing the contaminant, thus no accumulation of glyphosate concentration occurs on the post-work task samples.

Wipe samples data from pesticide product containers (n = 21), work vehicle steering wheels (n = 10) and participant's personal mobile phones (n = 18) are presented in Table 3. Of all the potentially contaminated work surfaces sampled in this study (n = 49), the highest glyphosate concentrations were detected on the pesticide product containers. A pesticide product container is considered to be handled up to 50 times by the worker before disposal.

Glyphosate was also detectable on all wipes from work vehicle steering wheels (n = 21), with a mean and range of 1928 (478–5984) µg wipe^{-1} (unadjusted values). These included small tractors and vehicles (e.g. vans, cars) used to transport equipment to and around field sites. Participating workers drove the work vehicle, on some occasions to travel to multiple sites within a day, and performed the required pesticide application tasks. Only two (11%) of the mobile phone samples had non-detectable glyphosate concentrations. Most mobile phones were personal use phones.

Table 1. Glyphosate wipe concentration data (µg/cm) for pre- and post-work task perioral and hand (left and right) measurements. Results are presented as for the overall sample and per similar exposure group concerned

Variable	*k*	*N*	<LOQ *N* (%)	GM	GSD	Min	Max	<LOQ *N* (%)	GM	GSD	Min	Max
Combined SEGs				Pre-work task samples					Post-work task samples			
Perioral	20	29	11 (38%)	2.1×10^{-03}	12.93	8.2×10^{-06}	0.15	6 (21%)	0.01	9.05	1.7×10^{-04}	0.40
Hand left	20	29	0	6.1×10^{-03}	8.77	9.4×10^{-05}	0.31	0	0.04	9.21	4.7×10^{-04}	2.56
Hand right	20	29	0	6.5×10^{-03}	9.09	9.4×10^{-05}	0.22	0	0.05	8.73	2.8×10^{-04}	2.04
Manual knapsack				Pre-work task samples					Post-work task samples			
Perioral	10	12	6 (50 %)	1.1×10^{-03}	21.30	8.2×10^{-06}	0.15	4 (33%)	0.01	11.30	1.7×10^{-04}	0.23
Hand left	10	12	0	2.2×10^{-03}	8.47	9.4×10^{-05}	0.07	0	0.01	8.64	4.7×10^{-04}	0.08
Hand right	10	12	0	2.7×10^{-03}	11.00	9.4×10^{-05}	0.14	0	0.01	7.26	2.8×10^{-04}	0.09
Pressurised lance				Pre-work task samples					Post-work task samples			
Perioral	6	10	2 (20 %)	3.9×10^{-03}	8.56	5.8×10^{-05}	0.05	0	0.04	4.68	2.5×10^{-03}	0.40
Hand left	6	10	0	2.2×10^{-02}	10.01	3.7×10^{-04}	0.31	0	0.08	9.17	2.6×10^{-03}	2.56
Hand right	6	10	0	1.8×10^{-02}	10.62	4.7×10^{-04}	0.22	0	0.21	7.41	3.7×10^{-03}	2.04
Controlled droplet				Pre-work task samples					Post-work task samples			
Perioral	5	7	3 (43 %)	2.6×10^{-03}	9.44	1.1×10^{-04}	0.03	2 (29%)	0.01	8.34	6.0×10^{-04}	0.10
Hand left	5	7	0	5.9×10^{-03}	2.39	2.4×10^{-03}	0.03	0	0.04	5.89	3.5×10^{-03}	0.50
Hand right	5	7	0	6.8×10^{-03}	2.09	3.0×10^{-03}	0.02	0	0.06	5.73	7.9×10^{-03}	0.85

k is the number of participants in the group. *N* is the number of samples (one sample per task) in the group. Number of samples below the limit of quantification (LOQ) (1 µg l^{-1}) by number (*N*) and percentage (%). GM is the geometric mean, GSD the geometric standard deviation and the range (min–max).

Table 2. Glyphosate wipe concentration data (µg/cm) for the post-work task glove samples. Results are presented for the overall sample and per similar exposure group concerned

Variable	*k*	*N*	GM	GSD	Min	Max	*k*	*N*	GM	GSD	Min	Max
Combined SEGs			Post-work task disposable gloves						Post-work task reusable gloves			
Glove left	12	17	0.18	6.14	3.9×10^{-03}	2.78	6	6	4.49	4.68	0.81	58.87
Glove right	12	17	0.20	6.96	2.4×10^{-03}	10.62	6	6	4.52	4.43	0.73	66.27
Glove both	12	17	0.43	5.67	0.02	13.41	6	7	7.99	4.14	1.53	125.1
Manual knapsack			Post-work task disposable gloves						Post-work task reusable gloves			
Glove left	7	8	0.06	5.39	3.9×10^{-03}	0.63	1	1	—	—	6.11	6.11
Glove right	7	8	0.07	6.56	2.4×10^{-03}	0.49	1	1	—	—	6.43	6.43
Glove both	7	8	0.15	4.41	0.02	1.12	1	1	—	—	12.54	12.54
Pressurised lance			Post-work task disposable gloves						Post-work task reusable gloves			
Glove left	3	4	0.39	2.42	0.19	1.23	2	2	—	—	6.64	58.87
Glove right	3	4	0.36	1.44	0.26	0.58	2	2	—	—	6.11	66.27
Glove both	3	4	0.80	1.91	0.46	1.76	2	2	—	—	12.74	125.1
Controlled droplet			Post-work task disposable gloves						Post-work task reusable gloves			
Glove left	3	5	0.05	3.83	0.04	2.78	2	3	1.51	1.75	0.81	2.39
Glove right	3	5	0.73	7.29	0.05	10.62	2	3	1.49	1.86	0.73	2.17
Glove both	3	5	1.03	6.76	0.09	13.41	3	4	3.19	1.64	1.53	4.56

k is the number of participants in the group. *N* is the number of samples (one sample per task) in the group. There was no samples in this table that were below the limit of quantification (<LOQ) (1 µg l^{-1}). GM is the geometric mean, GSD the geometric standard deviation and the range (min–max).

Table 3. Glyphosate concentration data (µg/cm) for wipe samples collected from work surfaces. Sampling from those was performed post-work task completion and results are presented as geometric mean (GM), geometric standard deviation (GSD) and range (min–max) for the overall sample

Variables	*k*	*N*	<LOQ*N* (%)	GM	GSD	Min	Max
Combined SEGs							
Product container	15	21	0	2.06	7.48	0.01	27.7
Steering wheel	7	10	0	0.06	2.44	0.02	0.27
Mobile	15	18	2 (11%)	0.004	6.50	1.0×10^{-4}	0.12

k is the number of participants in the group. *N* is the number of samples (one sample per task) in the group. Samples that are below the limit of quantification (<LOQ) (1 µg l^{-1}) by number (*N*) and percentage (%). GM is the geometric mean, GSD the geometric standard deviation and the range (min–max).

Differences in exposure levels across SEGs, sampling and working parameters
Disposable worker gloves had the highest glyphosate concentrations, followed by worker hands (post-work task). Glyphosate concentrations were lowest on perioral wipes (Fig. 1a). The highest glyphosate concentrations were detected on wipe samples collected from the pesticide product containers, followed by much lower levels on the work vehicle steering wheel. The lowest concentrations were detected on the worker mobile phones (Fig. 1b). Three of the participants in this study had their mobile phone wiped on two separate occasions, and on both occasions, glyphosate concentrations were detected.

A strong positive relationship was found between urinary glyphosate concentrations (µg/L) and glyphosate wipe concentrations (µg/cm) on the worker perioral wipe samples (Fig. 2a). Similarly, perioral region and worker hands glyphosate concentrations (µg/cm) correlated positively and strongly (Fig. 2b). Some of the participants started the work task before monitoring began. Although this has no influence on the dermal exposure assessments measurements as samples were taken before and after the work task but it could potentially impact on the urinary concentrations which may not accurately reflect the full day's exposure. The precision of peak urinary concentration estimates used within our analysis may be dependent on the number of samples available for participants. However, no statistically significant differences were found in peak urinary concentrations between participants.

Figure 1. Boxplot showing the post-work task glyphosate concentrations for (a) disposable gloves, the workers hands (under the glove) and the perioral region and (b) for potentially contaminated work surfaces, pesticide product containers, the steering wheel of work vehicles and participants mobile phone (µg/cm). The box is the 25th to the 75th percentile, the line within the box is the median and the whiskers the lower and the upper adjacent values. Single points indicate outliers. *n is the number of samples. Reusable gloves were not included in the boxplot (a) due to the low number of samples. Mean measured concentrations were statistically different between all types of samples (*t*-tests; P < 0.001).

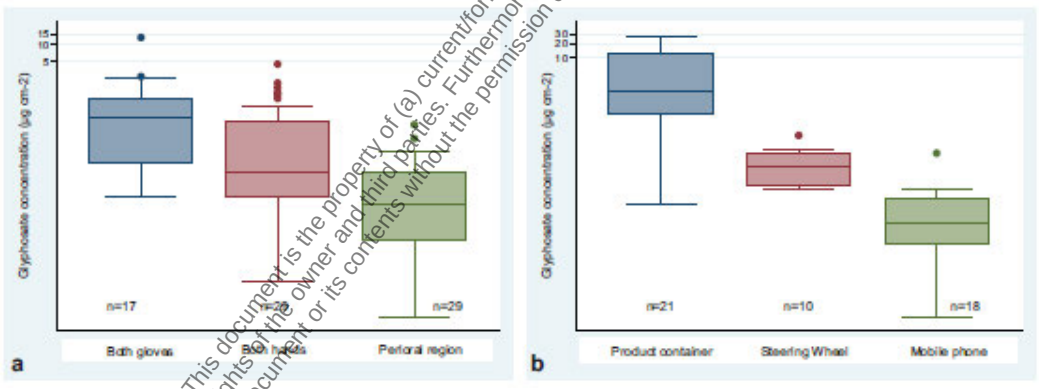
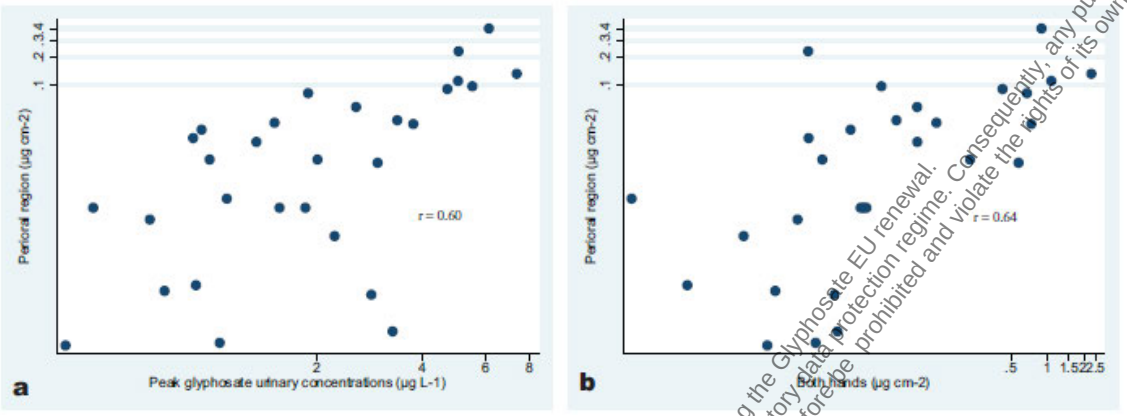


Figure 2. Scatter graph showing moderate relationships between glyphosate perioral glyphosate wipe contamination levels (µg/cm) and (a) peak urinary glyphosate concentrations (µg/L) and (b) both hands glyphosate surface loading contamination levels after a pesticide application task (µg/cm).



Elaborated linear mixed effect regression models are presented in Table 4. The model evaluating previously documented determinants of inadvertent ingestion exposure Gorman Ng *et al.* (2017), explained 45% of the variability for the glyphosate concentrations found on the perioral region. In this model (Model 1), an increase in the frequency of contacts per task and post-task hand contamination was significantly associated with an increased in perioral glyphosate concentrations.

Forward building of the same model on the basis of improvement of the fit parameters resulted with the same parameters being included alongside sampling time. The effects for hand contamination and frequency of contacts per task were comparable to those of the Model 1, whereas perioral concentrations also increased with increasing sampling time ($\beta = 0.01$; $P < 0.08$). This model explained ~50% of the total variability in perioral glyphosate concentrations.

The forward built model examining determinants of participants' hand contamination (Model 2) comprised of the task sampling time, the age of the participant and the SEG and explained 62% of the total variability of glyphosate concentrations measured on the hands.

All the workers who participated in this study wore gloves when applying pesticides. Gloves were used throughout the task however it was observed that on a number of occasions workers would remove their gloves for various reasons (e.g. checking mobile phones, to drive work vehicles, when going on a break, etc.). All had detectable glyphosate concentrations on their hands. Field observations suggest that poor glove doffing procedures or removing gloves during the work task (e.g. answering the phone, adjusting facemasks) may be responsible for hand contamination.

Table 4. Mixed effect models with participants' identification number included in the models as a random effect. Model 1 results are describing the effects of hand contamination and the frequency of contacts per task on glyphosate concentrations measured on the perioral region. Model 2 results describe the effects of sampling time, the age of the workers and work task characteristic on glyphosate surface loading concentrations measured on the hands. Measurements are given as on the log-transformed glyphosate concentrations per surface area (µg cm−2) and were taken from 20 workers over 29 pesticide application tasks.

Covariate	Model 1			Model 2		
	β	SE	*P*	β	SE	*P*
Intercept	−3.91	0.70	0.00	−10.05	1.67	0.00
Glyphosate conc. of both hands, post-work task (µg cm^{-2})	0.56	0.16	0.00			
Sampling time of the work task (mins)				0.01	0.003	0.01
Total frequency of contacts per task	0.02	0.01	0.02			
Age of the participant (years)				0.12	0.04	0.00
Similar exposure groups						
Pressurised lance				1.41	0.67	0.04
Controlled droplet applicator				0.69	0.67	0.30
Manual knapsack				Ref	—	—
Between variance (naive model)	1.18 (2.32)			0.00 (3.94)		
Within variance (naive model)	1.55 (2.62)			1.85 (0.97)		
Total variance explained	45%			62%		

β, regression coefficient for log-transformed exposure data; SE, standard error. The naive model is the results from the model without the inclusion of any fixed effects.

To identify the relative contribution of the routes of exposure to the total uptake of glyphosate, a separate model was forward built using the log-transformed results of the biomonitoring exposure measurements as the dependent covariate. The final elaborated model comprised of only the combined hand and perioral region glyphosate concentration (Table 5). Overall, the model explained 40% of the total variance in urinary concentrations. The hands and perioral data were not entered as separate covariates in the model, as they were highly correlated ($r = 0.64$; $P < 0.001$). Hand and perioral glyphosate concentrations are important determinants of total glyphosate body burden (glyphosate urinary concentrations), explaining 40% of variance in the urine data. Discriminating the individual contribution for each route to the total body burden was not possible. However, hand glyphosate concentrations alone explained approximately one third of the variance in the glyphosate urine concentrations, which would indicate that dermal exposure was the predominant route but that inadvertent ingestion may contribute to overall body burden since the presence of glyphosate contamination in the perioral region may result in ingestion and/or dermal absorption.

Table 5. Mixed effect model describing the effects of glyphosate concentration of the combined hands and the perioral region on the log-transformed glyphosate concentrations (µg/L) measured of 20 workers over 29 pesticide application tasks. Mixed models build with participants' identification number as a random effect.

Covariate	β	SE	*P*
Intercept	1.20	0.19	0.00
Ln concentrations in hand and perioral region surfaces	0.26	0.06	0.00
Between variance (naive model)		0.15 (0.36)	
Within variance (naive model)		0.22 (0.27)	
Total variance explained		40%	

β, regression coefficient for log-transformed exposure data; SE, standard error. The naive model is the results from the model without the inclusion of any fixed effects.

Conclusion

The analysis of a total of 343 wipe and glove samples were collected from 20 workers across 29 work tasks revealed the GM glyphosate concentrations of 0.01, 0.04 and 0.05 µg/cm^2 on wipes from the workers' perioral region and left and right hands, respectively. For disposable and reusable gloves, respectively, GM glyphosate concentrations of 0.43 and 7.99 µg/cm^2 were detected. The combined hand and perioral region glyphosate concentrations explained 40% of the variance in the urinary (µg/L) biomonitoring data. The results of the study show dermal to be a prominent route of exposure but support inadvertent ingestion potential contribution to the total body burden among this worker group. The study also identified a potential for the spread of contamination among non-pesticide users in the workforce and para-occupational exposures. Study results also showed that PPE practice is an important determinant of both inadvertent ingestion and dermal exposure. An implementation of PPE management and work practices policies for pesticide use could potentially reduce both occupational exposures and para-occupational exposures.

3. Assessment and conclusion

Assessment and conclusion by applicant:

This study is newly submitted for purpose of review. The total uptake of glyphosate was assessed in parallel with dermal and inadvertent exposure routes, using urine, wipes and glove samples collected from 20 workers across 29 work tasks. The average hand surface area measurements were assigned according to published US EPA guidance. Geometric mean (GM) glyphosate concentrations of 0.01, 0.04 and 0.05 µg/cm^2 were obtained on wipes from the workers' perioral region and left and right hands, respectively. For disposable and reusable gloves, respectively, GM glyphosate concentrations of 0.43 and 7.99 µg/cm^2 were detected. The combined hand and perioral region glyphosate concentrations explained 40% of the variance in the urinary (µg/L) biomonitoring data. Data show the dermal exposure is the prominent route of exposure in comparison to inadvertent ingestion, but inadvertent ingestion may contribute to overall body burden. The study also identified potential exposure to non-pesticide users in the workplace and para-occupational exposures.

This publication is considered relevant for the risk assessment of glyphosate and reliable without restrictions because it complies with the quality criteria of a good exposure study.

Assessment and conclusion by RMS:

Reliability criteria of exposure studies

Publication: Connolly *et al.*, 2019.	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines/practices.	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Exposure to formulations with only glyphosate as a.s.	Y	
Exposure to formulations with glyphosate combined with other a.s.	Y	
Exposure to various formulations of pesticides	Y	
Study		
Study design clearly described	Y	
Population investigated sufficiently described	Y	
Exposure circumstances sufficiently described	Y	
Sampling scheme sufficiently documented	Y	
Analytical method described in detail	Y	
Validation of analytical method reported	Y	
Monitoring results reported	Y	
Overall assessment		
Reliable without restrictions	Y	
Reliable with restrictions		
Reliability not assignable		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate and reliable without restrictions because it complies with the quality criteria of a good exposure study.		

1. Information on the study

Data point:	CA 5.9/006
Report author	Conrad, U. *et al.*
Report year	2017
Report title	Glyphosate in German adults – Time trend (2001 to 2015) of human exposure to a widely used herbicide
Document No	doi.org/10.1016/j.ijheh.2016.09.016100898843 E-ISSN: 1618-131X
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	None
GLP/Officially recognised testing facilities	Not applicable

Acceptability/Reliability:	Yes/Reliable without restrictions

2. Full summary of the study according to OECD format

The purpose of this study was to elucidate the internal exposure of the general German population to glyphosate and aminomethylphosphonic acid (AMPA) and its change over time.

The broadband herbicide glyphosate (N-[phosphonomethyl]-glycine) and its main metabolite AMPA were analysed by GC-MS-MS in 24 h-urine samples cryo-archived by the German Environmental Specimen Bank (ESB). Samples collected in 2001, 2003, 2005, 2007, 2009, 2011, 2012, 2013, 2014, and 2015 were chosen for this retrospective analysis. All urine samples had been provided by 20 to 29 years old individuals living in Greifswald, a city in north-eastern Germany.

Out of the 399 analysed urine samples, 127 (= 31.8%) contained glyphosate concentrations at or above the limit of quantification (LOQ) of 0.1 g/L. For AMPA this was the case for 160 (= 40.1%) samples. The fraction of glyphosate levels at or above LOQ peaked in 2012 (57.5%) and 2013 (56.4%) after having discontinuously increased from 10.0% in 2001. Quantification rates were lower again in 2014 and 2015 with32.5% and 40.0%, respectively. The overall trend for quantifiable AMPA levels was similar. Glyphosate and AMPA concentrations in urine were statistically significantly correlated (spearman rank correlation coefficient = 0.506, $p \leq 0.001$). Urinary glyphosate and AMPA levels tended to be higher in males. The possible reduction in exposure since 2013 may be due to changes in glyphosate application in agricultural practice.

Materials and Methods

Sampling and study group; This retrospective monitoring study was based on 24 h-urine specimen collected in the annual sampling of the German ESB. To reduce the risk of contamination, all containers needed for sampling and aliquoting were carefully cleaned before use according to standard operating procedures. All samples have been provided by young adults (predominantly students) aged 20 to 29 years. To follow the time trend of human exposure to glyphosate and AMPA, cryo-preserved urine samples collected in 2001, 2003, 2005, 2007, 2009, 2011, 2012, 2013, 2014, and 2015 were analysed. All urine samples were collected from individuals living in Greifswald, a city in north-eastern Germany. Annual ESB sampling in Greifswald was regularly carried out in the period of March and April. From each of the ten study years, 24 h-urine samples donated by 20 male and 20 female participants were randomly selected for analyses. The only inclusion criterion for this main study sample was that no specifically restricted diet – mainly vegetarian or vegan – had been reported by the sample provider in the self-administered ESB questionnaire. In 2001 the questionnaire item on dietary restrictions had not yet been implemented. Therefore, some samples from 2001 may have been provided by vegetarians or vegans. The fraction of vegetarian or vegan ESB participants, however, remained roughly between 2 and 14% from 2002 to 2014 followed by fractions up to 18% in 2015. Therefore, it can be assumed that, if any, only very few participants with restricted diets might have erroneously been included in the 2001 sample. One 2013 measurement had to be excluded from the main study sample, as the participant was later identified not to fulfil the inclusion criterion.

Hence, the main sample of this study consisted of 399 participants living in the ESB sampling location Greifswald with virtually equal sample sizes and sex ratios in each study year (cf. Table 1).

Table 1. Description of sample composition (ESB participants from Greifswald analysed for glyphosate and AMPA concentrations in 24 h-urine (no self-reported specific dietary restrictions)

Year	Sample size (male/female)	Age [years] AM (range)	24 h-urine volume [mL] AM (range)	Creatinine in urine [g/L] AM (range)	BMI [kg/m²] AM (range)
2001	40 (20/20)	23.2 (20–28)	1757 (490–3000)	0.98 (0.38–3.73)	22.3 (17.8–27.4)
2003	40 (20/20)	24.6 (20–28)	1793 (770–2850)	1.06 (0.42–2.11)	23.4 (17.3–31.2)
2005	40 (20/20)	23.1 (20–29)	1910 (895–2841)	0.90 (0.29–2.38)	22.6 (18.1–33.2)
2007	40 (20/20)	23.8 (20–28)	1937 (771–3047)	0.96 (0.36–2.92)	23.5 (18.0–34.6)
2009	40 (20/20)	24.3 (20–29)	1959 (701–3438)	0.85 (0.37–2.39)	23.7 (17.8–34.6)
2011	40 (20/20)	24.3 (20–29)	1893 (783–3045)	0.87 (0.26–2.17)	23.6 (18.0–39.1)
2012	40 (20/20)	24.4 (20–29)	1802 (768–3076)	0.97 (0.32–2.27)	22.8 (17.5–29.8)
2013	39 (20/19)	24.7 (20–29)	1973 (924–3081)	0.75 (0.20–1.60)	23.9 (19.3–40.9)
2014	40 (20/20)	24.1 (20–28)	1958 (760–3069)	0.82 (0.28–2.07)	23.2 (17.9–44.8)
2015	40 (20/20)	24.3 (20–28)	1759 (588–2956)	0.93 (0.18–2.02)	23.0 (17.9–36.8)
Total	399	24.1 (20–29)	1874 (490–3438)	0.91 (0.18–3.73)	23.1 (17.3–44.8)
Male	200	24.4 (20–29)	1881 (490–3076)	1.09 (0.20–3.73)	24.1 (17.4–36.8)
Female	199	23.8 (20–29)	1866 (600–3438)	0.73 (0.18–2.02)	22.0 (17.3–44.8)

Notes: AM = arithmetic mean, BMI = body mass index.

Male ESB participants tended to have higher BMI values and urinary creatinine levels than females. To investigate possible regional/seasonal differences of glyphosate and AMPA levels 40 urine samples collected in January 2005 and 2015 at the ESB sampling location Muenster (a city in north-western Germany) have additionally been analysed. Moreover, 20 urine samples from vegetarian or vegan participants have been analysed as well, in order to investigate differences due to diet. These samples were collected in Greifswald in the years 2007 (10 females) and 2015 (5 males and 5 females) and represent all available samples from vegetarian or vegan participants. A description of the two additionally analysed comparative ESB sub-populations is provided in Table 2. Participants in Muenster tended to have slightly lower BMI values. The other sub-population of self-reported vegetarians/vegans also exhibited lower average BMI values as well as higher 24 h-urine sample volumes and lower urinary creatinine concentrations.

Table 2. Description of two sub-populations from Muenster (no self-reported specific dietary restrictions) and Greifswald (self-reported vegetarians/vegans) analysed for comparison with the main study sample

Year	Sample size (male/female)	Age [years] AM (range)	24 h-urine volume [mL] AM (range)	Creatinine in urine [g/L] AM (range)	BMI [kg/m²] AM (range)
ESB sampling location Muenster (no self-reported specific dietary restrictions)					
2005	40 (20/20)	23.6 (20–28)	1790 (691–2962)	1.03 (0.19–2.41)	22.2 (17.4–29.3)
2015	40 (20/20)	23.6 (20–28)	1991 (271–4601)	0.75 (0.35–1.73)	22.1 (18.3–28.6)
Total	80 (40/40)	23.6 (20–28)	1891 (271–4601)	0.89 (0.19–2.41)	22.1 (17.4–29.3)
Male	40	24.0 (20–28)	1934 (797–2952)	0.98 (0.19–2.41)	23.1 (19.6–28.6)
Female	40	23.3 (20–28)	1847 (271–4601)	0.80 (0.35–1.65)	21.1 (17.4–29.3)
Self-reported vegetarians/vegans (ESB sampling location Greifswald)					
2007	10 (0/10)	24.5 (23–28)	2293 (457–3011)	0.67 (0.21–2.51)	23.0 (19.9–28.1)
2015	10 (5/5)	24.3 (20–28)	1831 (773–2993)	0.72 (0.24–1.40)	22.1 (17.7–25.1)
Total	20 (5/15)	24.4 (20–28)	2062 (457–3011)	0.69 (0.21–2.51)	22.5 (17.7–28.1)
Male	5	25.4 (24–27)	1915 (1135–2813)	0.80 (0.31–1.36)	22.6 (21.3–23.9)
Female	15	24.1 (20–28)	2111 (457–3011)	0.66 (0.21–2.51)	22.5 (17.7–28.1)

Notes: AM = arithmetic mean, BMI = body mass index.

Analytical procedure; The chemical analysis was based on the method by Alferness and Iwata (1994) initially developed for trace analysis of Glyphosate and AMPA in food which uses gas chromatography (GC) coupled with a single quadrupole mass selective detector (MSD). The newly developed method applied in the present study used GC with tandem mass spectrometry (GC-MS-MS) to reach a low limit of quantification (LOQ) in human urine along with high selectivity. Isotope labelled internal standards have been used for further increasing the method's performance.

Standards and reagents; All chemicals were of analytical grade unless stated otherwise. Reference compounds (glyphosate and AMPA) and internal standards (1,2-13C2-15N-glyphosate and 13C-15N-AMPA) were obtained from Dr. Ehrenstorfer (Augsburg, Germany) as solutions in water (10 g/mL each). 2,2,2-trifluoroethanol (99%), trifluoroacetic anhydride (99%) and acetonitrile were purchased from Sigma-

Aldrich (Seelze, Germany). Methanol was obtained from Merck (Darmstadt, Germany). Water was purified by an ultra-water purification system from ELGA (Ransbach-Baumbach, Germany).

Sample preparation; 50 L of urine sample and 25 L of the internal standard (IS) solution (containing 4 ng/mL of each IS) were transferred to 10 mL screw-capped glass tubes containing 1 mL of acetonitrile. After evaporation to dryness in a vacuum centrifuge, 0.5 mL of 2,2,2-trifluoroethanol and 1 mL of freezing cold (-40 °C) trifluoroacetic anhydride were added cautiously to the residue. The derivatization of the analytes was started by heating the closed tubes to 85 ◦C for 1 h in a heating block. After cooling down to room temperature the mixture was cautiously evaporated to dryness. The oily residue was then dissolved in 100 L of methanol and transferred into a microvial. This final solution was used for GC-MS-MS analysis. Mixed glyphosate and AMPA calibration solutions were prepared by serial dilution of a stock solution (each 5 ng/mL) in solutions of 50 L water in 1 mL acetonitrile containing 25 L of the IS-solution. These solutions were processed in the same way as described for human urine samples and represent sample concentrations from 0.1 to 10 g/L.

GC-MS-MS analysis; The derivatised analytes were separated by gas chromatography using a GC system7890 equipped with a split/split less injector (Agilent) and a MPS2 autosampler (Gerstel). The GC column was a HP INNOWAX, 30 m length, 0.25 mm internal diameter and 0.25 m film thickness (Agilent). The mass spectrometric parameter and ion transitions used are summarised in Table 3. While the primary transitions are well suitable for quantification of glyphosate and AMPA at low environmental internal exposure levels, the secondary transitions of glyphosate and AMPA only worked well at urine concentrations beyond approx. 20 g/L to confirm the identity of analytes. As the method was clearly focused on reaching the lowest quantification limits in human urine, the secondary transitions were not considered. The high specificity of the primary ion transition was evaluated during the validation of the analytical method.

Table 3. Mass spectrometric parameter and ion transitions used in glyphosate and AMPA analyses

Mass spectrometric parameters	
Instrument	Agilent 7000
Ion source temperature	150 °C
Ionization type	Negative chemical ionization (NCI)
Chemical ionization gas	Methane 4.5
Collision gas	Argon 5.0
Electron multiplier	Relative +200 V

Mass transfers of analytes and internal standards

Transition	Precursor ion [m/z]	Product ion [m/z]	Collision energy [V]	Use
Glyphosate 1st transition	370	245	10	Analyte quantifier
Glyphosate 2nd transition	351	268	5	Of limited suitability
AMPA 1st transition	351	268	5	Analyte quantifier
AMPA 2nd transition	271	188	5	Of limited suitability
1,2-$^{13}C_2$-^{15}N-glyphosate	371	246	10	Internal standard
^{13}C-^{15}N-AMPA	353	270	5	Internal standard

Notes: The secondary transitions of glyphosate and AMPA are listed for sake of completeness only. As they provide reliable confirming information only at concentrations beyond 20 µg/L, they have not been used in this study.

Validation and quality assurance measures of analytical method; For evaluation of the method performance the requirement of SANCO guideline 825 (European Commission, 2010) were considered which are mandatory for analytical methods in the context of pesticide registration and monitoring. Specificity, linearity, working range, accuracy, precision and LOQ were investigated for method evaluation. It can be concluded that the primary transition was very selective for a reliable quantification of glyphosate and AMPA.

The specificity of the analytical method was checked by the chromatography of unfortified human urine samples which showed no other interfering peaks besides the analytes. Further, the sample solutions of 44 unfortified human urine samples containing residues of glyphosate were analysed in parallel using separation columns with phases of different selectivity. Analysed concentrations of glyphosate (n = 44 > LOQ) and AMPA (n = 25 > LOQ) ranged from 0.2 to 5 µg/L on both columns and correlated well: The respective slopes of the linear regression lines were close to unity (1.03 for glyphosate and 1.12 for AMPA)

and the coefficients of determination (R2) reached satisfactory values (0.9968 for glyphosate and 0.9893 for AMPA). Therefore, it could be concluded that the primary transition was very selective for a reliable quantification of glyphosate and AMPA.

Basic calibration was performed by the measurement of 8 calibration solutions with concentrations ranging from 0.1 to 10 µg/L. A linear relationship between concentration and the ratio of the peak area of glyphosate and AMPA and its internal standards was observed. All calibration curve points were within 15% of their respective theoretical value. The linear correlation coefficients were typically > 0.99. Calibration curves for glyphosate and AMPA based on water and pooled human urine were both linear (each R2 > 0.99) and ran parallel. The slopes differed only by approximately 2%. This indicates that possible matrix effects are well compensated by the internal standards and matrix matched calibration solutions are not required for accurate determination of glyphosate and AMPA. The LOQ for glyphosate and AMPA was determined by fortification of human urine samples. The lower level at 0.1 µg/L demonstrated sufficient recovery (86 to 115%) and precision (8.9to 9.1%) for both analytes. This concentration was set as the LOQ of the GC-MS-MS method. The urine samples were analysed in a randomised order. Blank values (urine substituted by water) were measured during the analysis of urine samples regularly every 15th sample. All blank values were below the LOQ of 0.1 µg/L. Evaluation of the accuracy and precision of the method was performed through recovery experiments. Pooled human urine samples with no detectable amount of glyphosate and AMPA (each <0.1 µg/L) were fortified at 0.5, 1.0, 2.5, and 5 µg/L on 8 replicates each level. The recovery values ranged from 81 to 106% with are lative standard deviation (RSD) below 8.3%. Further, we performed recovery experiments using individual human urine samples to check for possible matrix effects caused by variations in the composition of the samples. Ten individual urine samples free of glyphosate and AMPA (each < 0.1 µg/L) were spiked at 0.5, 2.5 and 5 µg/L and were analysed in triplicate. The recoveries ranged from 87 to 110% proving that possible matrix effects were compensated by the internal standards $^{13}C_2$-^{15}N-glyphosate and ^{13}C-^{15}N-AMPA. In addition, the performance of the method was checked by measuring of control samples spiked at 0.5 and 2.5 µg/L during the analysis of the samples from this study (about every 33rd sample). A summary of the results of the control samples is provided in Table 4.

Table 4. Results of control samples concurrently analysed with the study samples

Analyte	Spiking level [µg/L]	Mean recovery [%]	Range [%]	RSD [%]	Number of samples
Glyphosate	0.5	103.0	84.4–113.3	8.6	15
	2.5	102.9	94.2–111.3	5.1	15
AMPA	0.5	102.1	82.4–112.4	9.4	15
	2.5	101.3	91.2–111.0	5.7	15

Statistical analysis; Glyphosate and AMPA concentrations below the LOQ were set to LOQ/2 prior to statistical evaluation. All data analyses were carried out in SPSS Statistics Version 20 (IBM Corporation, 2011). Differences between frequencies were tested with Pearson's Chi^2 test of independence after cross tabulation. Correlations between variables were quantified by Spearman's rank correlation coefficients, as concentration and other data mostly contained few extreme values. Box-plots were created in R Version 3.2.3 (R Core Team, 2015) displaying the 25th, median and 75th percentile as a box. The whiskers were set to extend to the minimum and maximum value, due to considerable skewness and obvious non-normality of the data. All p-values of 0.05 or lower were considered to indicate statistical significance.

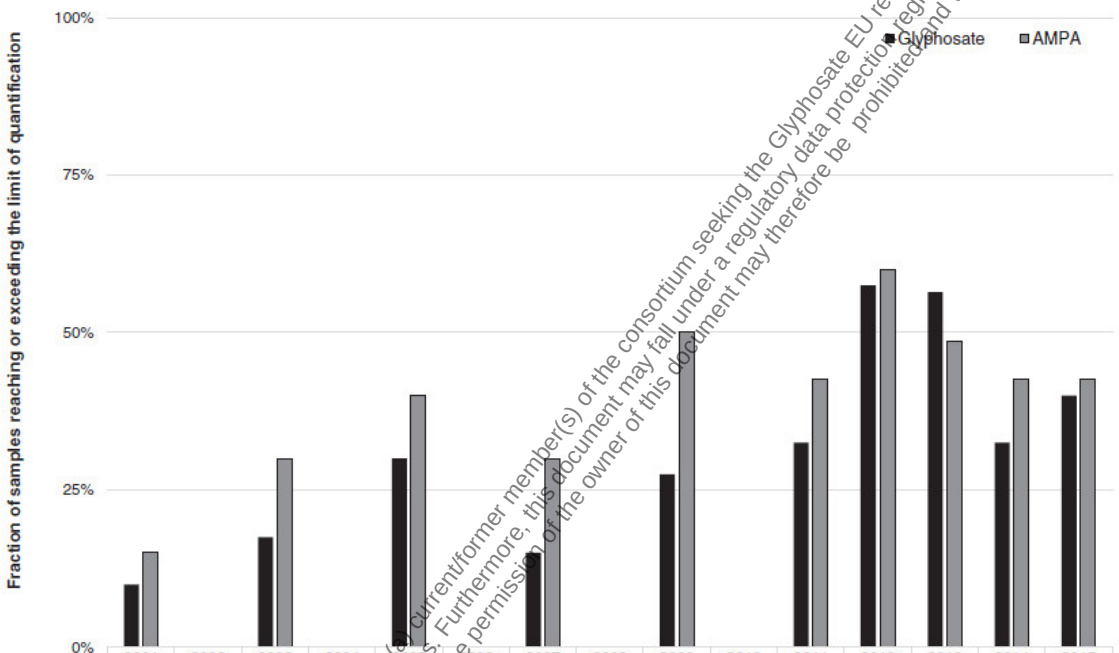
Results

Urinary concentrations of glyphosate and AMPA in the main study sample

Frequency of quantifiable concentrations; Out of the 399 analysed urine samples, 127 (= 31.8%) contained glyphosate concentrations that reached or exceeded the LOQ of 0.1 g/L. For AMPA this was the case for 160 (= 40.1%) of all samples. The fraction of samples at or above LOQ varied significantly over the years investigated, both for glyphosate ($p \leq 0.001$) and AMPA ($p = 0.005$). As displayed in Table 5 and Fig. 1, years with the highest quantification rates were 2012 (57.5%) and 2013 (56.4%) after rates having

discontinuously increased from 10.0% in 2001. Fractions of at least 0.1 g/L were lower again in 2014 and 2015 with 32.5% and 40.0%, respectively. The overall trend for quantifiable AMPA levels was quite similar. The highest fraction of samples reaching or exceeding the LOQ was observed for samples taken in 2012 (60.0%). The fractions of quantifiable levels of glyphosate and AMPA per year were generally higher in males. Especially for glyphosate, the principally increasing trend in urine concentrations was mainly due to samples provided by males (cf. Table 5).

Fig. 1. Temporal trend of glyphosate and AMPA in human 24 h-urine (fraction of samples at or above limit of quantification of 0.1 µg/L, ESB sampling location Greifswald , no self-reported specific dietary restriction)



	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Glyphosate	10.0%		17.5%		30.0%		15.0%		27.5%		32.5%	57.5%	56.4%	32.5%	40.0%
AMPA	15.0%		30.0%		40.0%		30.0%		50.0%		42.5%	60.0%	48.7%	42.5%	42.5%

Fractions of quantifiable glyphosate levels in samples from females were particularly high only in 2012 (55.0%) and 2013 (47.4%). The same difference between males and females was also apparent for AMPA, although the difference was less pronounced. Glyphosate sales in Germany have increased substantially from approximately 3300 t in 2000 to approximately 5400 t in 2014. The interim peak of approximately 7600 t in 2008 might be interrelated with the abolishment of EU set-aside requirements announced in 2007. Against the background of these data, the increase in quantifiable glyphosate and AMPA concentrations in analysed ESB urine samples were in agreement with expectations. Although the internal exposure to glyphosate and AMPA seems to have decreased again since 2013, there was a clear increase in comparison to 2001. The possible reduction in exposure since 2013 indicated by ESB data may be due to changes in application of glyphosate in agriculture: Austria, for example, banned the pre-harvest use of glyphosate in 2013. Also in Germany, intended glyphosate uses as pre-harvest treatment have been restricted (e.g. to partial applications instead of whole field treatments) from 2014 onwards. Currently, no German sales data are available for the year 2015.

Distribution of concentrations; The 50th, 75th, and 95th percentiles and maximal values for glyphosate and AMPA levels by sex and study year are provided in Table 5. Only in 2012 and 2013 the median concentration of glyphosate was slightly above the LOQ of 0.1 g/L. The 75th percentile exceeded the LOQ in all study years after 2007, reaching highest values in 2012 and 2013. The 95th percentiles of glyphosate

concentrations in 24 h-urine were substantially higher in 2013 (1.25 g/L) and 2014 (0.80 g/L) compared to all other years. Also the maximum concentrations of glyphosate peaked in these two years (2013: 2.80 g/L, 2014: 1.78 g/L). The median urinary AMPA concentration only slightly exceeded the LOQ in 2012. With the exception of the first year of the study, 2001, all 75th percentiles exceeded the LOQ with the highest level observed in 2013. The 95th percentiles of AMPA levels peaked in 2013. The two highest AMPA concentrations were observed in samples from 2013 (1.88 g/L and 1.54 g/L). The observed urinary glyphosate and AMPA concentrations were in good agreement with findings from other studies. In view of these results, ESB data was considered to provide a reliable indication of the background exposure to glyphosate and AMPA in Germany and its change from 2001 to 2015. Comparability with other studies was limited, partly due to differences in the study population and in the type of urine samples.

Table 5. Summary statistics for glyphosate and AMPA concentrations in 24 h-urine samples (µg/L) by sex and year of sampling at ESB sampling location Greifswald (no self-reported specific dietary restrictions)

			Glyphosate					*AMPA*				
		N	% ≥ LOQ	P 50	P 75	P 95	Max.	% ≥ LOQ	P 50	P 75	P 95	Max.
2001	Male	20	15.0	<LOQ	<LOQ	0.26	0.40	15.0	<LOQ	<LOQ	0.25	0.29
	Female	20	5.0	<LOQ	<LOQ	<LOQ	0.11	15.0	<LOQ	<LOQ	0.21	0.22
	Total	40	10.0	<LOQ	<LOQ	0.12	0.40	15.0	<LOQ	<LOQ	0.22	0.29
2003	Male	20	20.0	<LOQ	<LOQ	0.25	0.37	40.0	<LOQ	0.14	0.18	0.18
	Female	20	15.0	<LOQ	<LOQ	0.16	0.20	20.0	<LOQ	<LOQ	0.19	0.20
	Total	40	17.5	<LOQ	<LOQ	0.16	0.37	30.0	<LOQ	0.13	0.18	0.20
2005	Male	20	40.0	<LOQ	0.14	0.26	0.26	45.0	<LOQ	0.19	0.24	0.24
	Female	20	20.0	<LOQ	<LOQ	0.19	0.24	35.0	<LOQ	0.13	0.26	0.29
	Total	40	30.0	<LOQ	0.11	0.25	0.26	40.0	<LOQ	0.17	0.24	0.29
2007	Male	20	10.0	<LOQ	<LOQ	0.20	0.26	35.0	<LOQ	0.14	0.23	0.23
	Female	20	20.0	<LOQ	<LOQ	0.14	0.14	25.0	<LOQ	<LOQ	0.19	0.20
	Total	40	15.0	<LOQ	<LOQ	0.14	0.26	30.0	<LOQ	0.13	0.21	0.23
2009	Male	20	40.0	<LOQ	0.11	0.22	0.30	55.0	0.11	0.18	0.55	0.81
	Female	20	15.0	<LOQ	<LOQ	0.12	0.12	45.0	<LOQ	0.16	0.20	0.20
	Total	40	27.5	<LOQ	0.10	0.13	0.30	50.0	<LOQ	0.17	0.26	0.81
2011	Male	20	50.0	<LOQ	0.14	0.38	0.51	60.0	0.13	0.22	0.48	0.65
	Female	20	15.0	<LOQ	<LOQ	0.11	0.11	25.0	<LOQ	0.11	0.32	0.37
	Total	40	32.5	<LOQ	0.11	0.25	0.51	42.5	<LOQ	0.18	0.34	0.65
2012	Male	20	60.0	0.12	0.23	0.48	0.57	65.0	0.15	0.21	0.61	0.66
	Female	20	55.0	0.11	0.16	0.44	0.63	55.0	0.11	0.19	0.46	0.50
	Total	40	57.5	0.11	0.20	0.48	0.63	60.0	0.12	0.21	0.56	0.66
2013	Male	20	65.0	0.18	0.29	0.55	0.63	60.0	0.18	0.35	1.03	1.54
	Female	19	47.4	<LOQ	0.16	2.80	2.80	36.8	<LOQ	0.16	1.88	1.88
	Total	39	56.4	0.11	0.27	1.25	2.80	48.7	<LOQ	0.29	1.54	1.88
2014	Male	20	55.0	0.11	0.20	1.12	1.78	60.0	0.13	0.19	0.25	0.26
	Female	20	10.0	<LOQ	<LOQ	0.63	1.15	25.0	<LOQ	<LOQ	0.60	0.97
	Total	40	32.5	<LOQ	0.11	0.80	1.78	42.5	<LOQ	0.16	0.25	0.97
2015	Male	20	70.0	0.16	0.22	0.45	0.49	50.0	<LOQ	0.18	0.38	0.41
	Female	20	10.0	<LOQ	<LOQ	0.37	0.57	35.0	<LOQ	0.13	0.38	0.39
	Total	40	40.0	<LOQ	0.16	0.45	0.57	42.5	<LOQ	0.16	0.38	0.41

Notes: N = sample size, LOQ = limit of quantification, P = percentiles, Max. = maximum value.

As displayed in Figs. 2 and 3 glyphosate and AMPA concentrations were generally higher in samples from male ESB participants compared to samples from female participants. From 2011 onwards, median levels and 75th percentiles for glyphosate were higher in males. Box-plots for AMPA concentrations showed the same pattern. The maximum values for glyphosate and AMPA concentrations in urine, however, were observed in samples from female ESB participants. The differences in urinary glyphosate might be due to differences in exposure patterns between males and females or to sex-related differences in physiological determinants of glyphosate and AMPA in urine.

Fig. 2. Box-plots of glyphosate concentrations in 24 h-urine samples by study year and sex (ESB sampling location Greifswald, no self-reported specific dietary restriction, concentrations below LOQ of 0.1 µg/L set to LOQ/2 = horizontal solid line, box displays 25th, median and 75th percentile, whiskers extend to minimum and maximum value)

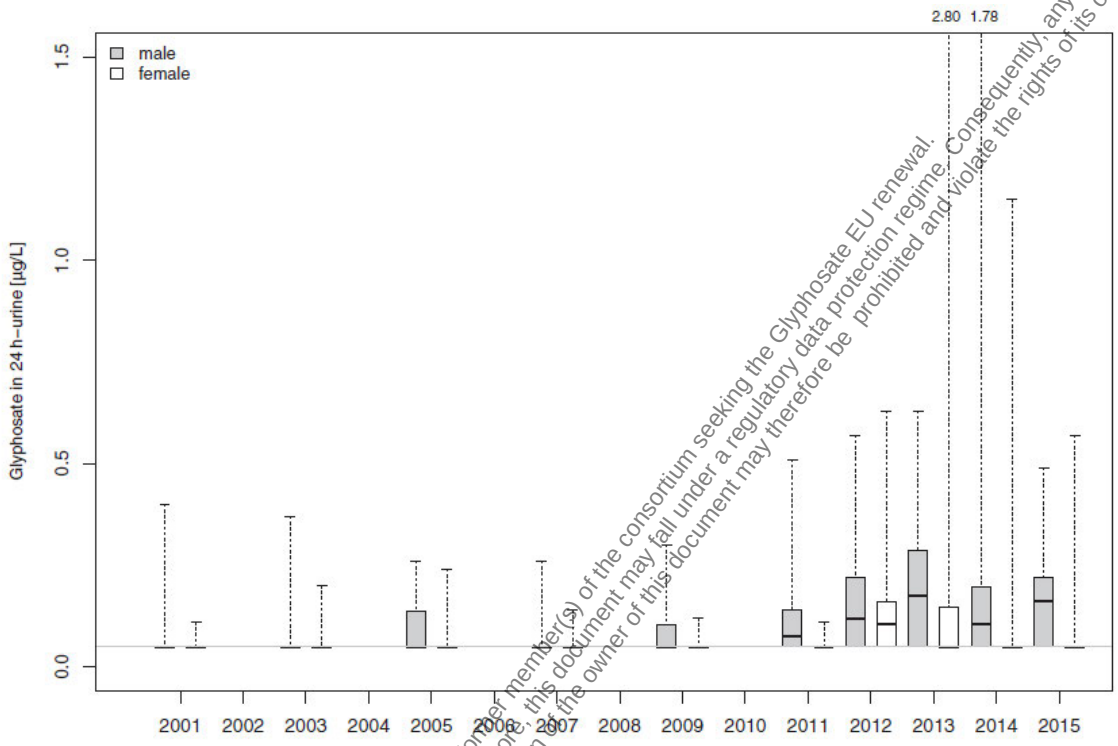
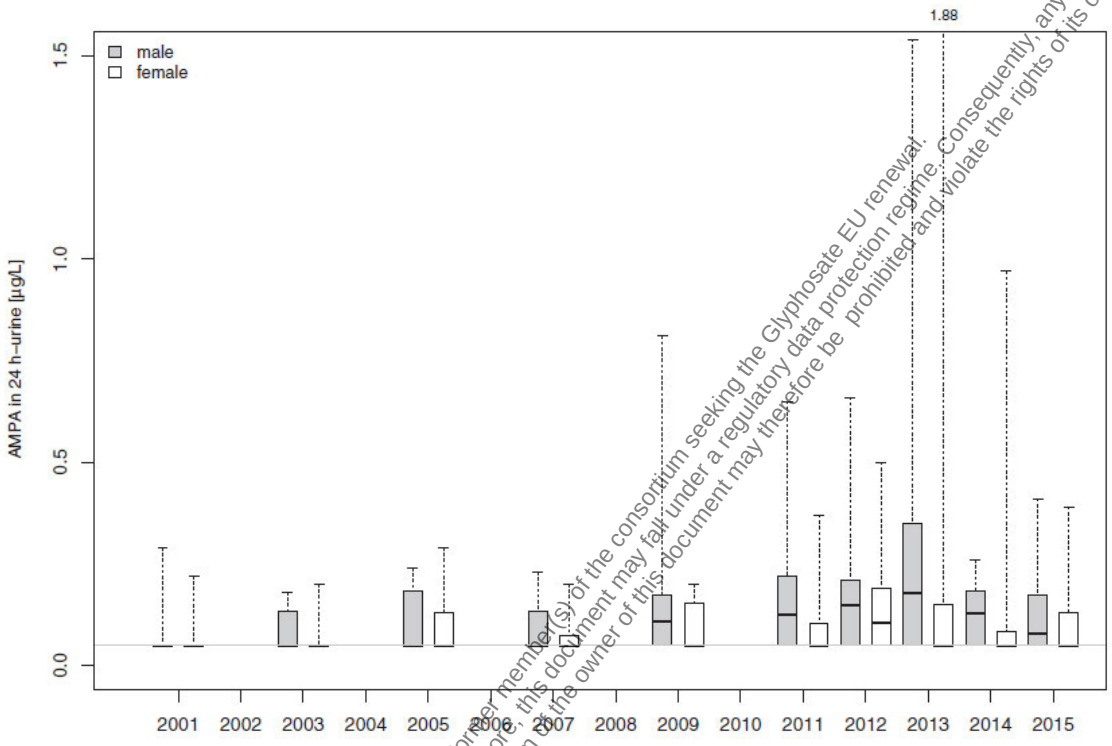


Fig. 3. Box-plots of AMPA concentrations in 24 h-urine samples by study year and sex (ESB sampling location Greifswald, no self-reported specific dietary restriction, concentrations below LOQ of 0.1 µg/L set to LOQ/2 = horizontal solid line, box displays 25th, median and 75th percentile, whiskers extend to minimum and maximum value



Correlations between glyphosate, AMPA and physiological parameters; Spearman rank correlations between glyphosate and AMPA levels in urine and physiological parameters observed in the main study sample are summarised in Table 6.

Table 6. Spearman rank correlation coefficients between glyphosate and AMPA concentrations in 24 h-urine and physiological parameters

		AMPA in 24 h-urine [µg/L]	Body mass index [kg/m²]	Volume of 24 h-urine sample [mL]	Creatinine in 24 h-urine [g/L]
Glyphosate in 24 h-urine [µg/L]	*Corr. coeff.*	**0.506**	**0.161**	**−0.278**	**0.347**
	p-value	≤0.001	0.001	≤0.001	≤0.001
	N	399	399	398	398
AMPA in 24 h-urine [µg/L]	*Corr. coeff.*		0.079	**−0.327**	**0.373**
	p-value		0.114	≤0.001	≤0.001
	N		399	398	398
Body mass index [kg/m²]	*Corr. coeff.*			0.020	**0.252**
	p-value			0.692	≤0.001
	N			398	398
Volume of 24 h-urine sample [mL]	*Corr. coeff.*				**−0.760**
	p-value				≤0.001
	N				397

Notes: N = sample size, statistically significant correlation coefficients ($p \leq 0.05$) highlighted in bold

Glyphosate and AMPA concentrations in urine were statistically correlated (spearman rank correlations coefficient $r_S = 0.506$, $p \leq 0.001$). When calculating coefficients of rank correlation separately for each study year, glyphosate and AMPA levels correlated statistically significantly in all years except for the first two, 2001 and 2003. For the following eight years of the study, r_S ranged between 0.360 and 0.616 (all p-values ≤ 0.05). A statistically significant association between glyphosate and AMPA concentrations in urine was also observed when cross tabulating all quantifiable and non-quantifiable levels for both analytes as

well as when calculating the Pearson product-moment correlation coefficient (data not shown). There were, however, urine samples with comparatively high glyphosate and quite low AMPA concentrations, and vice versa. The coefficients of correlation of glyphosate and AMPA with BMI were comparatively low and statistically significant only for glyphosate. Correlations between BMI and glyphosate concentrations in urine were only statistically significant at the 5% level in 2011 (r_S = 0.344) and 2015 (r_S = 0.365). For AMPA, only the correlation of the concentrations in urine with the participants' BMI in 2015 reached statistical significance (r_S = 0.346). Glyphosate and AMPA concentrations in urine were consistently negatively correlated with the urine sample volume (r_S = -0.278 and -0.327) and positively correlated with urinary creatinine levels (r_S = 0.347 and 0.373). All these coefficients of correlation were statistically significant ($p \leq 0.001$). The BMI was positively correlated with the creatinine concentration in 24 h-urine samples (r_S = 0.252, $p \leq 0.001$). Glyphosate and AMPA concentrations in urine were positively associated with urinary creatinine in all study years. The coefficients of correlation were statistically significant at the 5% level in almost all study years. Examined for the individual years of the study, the r_S of urine sample volume and glyphosate as well as AMPA levels were consistently negative. The correlation, however, was often not statistically significant. These results warrant a further discussion on options for a combined consideration of glyphosate and AMPA in exposure assessment. The quite low, but statistically significant correlation between BMI and glyphosate deserves attention when further investigating glyphosate exposure via food consumption. The negative association of glyphosate and AMPA concentrations with 24 h-urine sample volumes and positive association with urinary creatinine concentrations were in line with expectations, as both parameters reflect the individual urinary dilueness. 24 h creatinine excretion was usually higher in males.

Comparison with other ESB sub-populations

To get a first insight into differences in exposures due to the place of residence and season of sampling, 40 urine samples collected in 2005 and 2015 at the ESB sampling location Muenster were also analysed for glyphosate and AMPA. In contrast to samples being taken in April/May in Greifswald, the annual Muenster sampling is carried out in January. The summary statistics for glyphosate and AMPA in this sub-population are given in Table 7.

Table 7. Summary statistics for glyphosate and AMPA concentrations in 24 h-urine samples (µg/L) by sex and year of sampling in two sub-populations from Muenster (no self-reported specific dietary restrictions) and Greifswald (self-reported vegetarians/vegans) analysed for comparison with the main study sample

			Glyphosate					*AMPA*				
		N	% ≥ LOQ	P 50	P 75	P 95	Max.	% ≥ LOQ	P 50	P 75	P 95	Max.
ESB sampling location Muenster (no self-reported specific dietary restrictions)												
2005	Male	20	0.0	<LOQ	<LOQ	<LOQ	<LOQ	30.0	<LOQ	0.12	0.20	0.22
	Female	20	10.0	<LOQ	<LOQ	0.34	0.54	25.0	<LOQ	<LOQ	0.28	0.30
	Total	40	5.0	<LOQ	<LOQ	<LOQ	0.54	27.5	<LOQ	0.11	0.24	0.30
2015	Male	20	15.0	<LOQ	<LOQ	0.23	0.31	30.0	<LOQ	0.11	0.34	0.45
	Female	20	15.0	<LOQ	<LOQ	0.17	0.17	40.0	<LOQ	0.17	0.28	0.31
	Total	40	15.0	<LOQ	<LOQ	0.17	0.31	35.0	<LOQ	0.15	0.28	0.45
Self-reported vegetarians/vegans (ESB sampling location Greifswald)												
2007	Male	0										
	Female	10	10.0	<LOQ			0.14	0.0	<LOQ			<LOQ
	Total	10	10.0	<LOQ			0.14	0.0	<LOQ			<LOQ
2015	Male	5	60.0	0.26			0.61	40.0	<LOQ			0.33
	Female	5	20.0	<LOQ			0.53	20.0	<LOQ			0.43
	Total	10	40.0	<LOQ			0.61	30.0	<LOQ			0.43

Notes: N = sample size, LOQ = limit of quantification, P = percentiles, Max. = maximum value.

In 2005 and 2015 the percentage of quantifiable glyphosate levels was significantly higher in the main study sample (Greifswald) than in Muenster (2005: 30.0% vs. 5.0%, p = 0.003 and 2015: 40.0% vs. 15.0%, p = 0.012). For AMPA no statistically significant differences between Greifswald and Muenster samples were observed in 2005 (40.0% vs. 27.5%, p = 0.24) and 2015 (42.5% vs. 35.0%, p = 0.49). Also the 75th and 95th percentile of urinary glyphosate concentrations in the main study sample were higher than in samples collected in Muenster. For AMPA these percentiles were quite similar for both populations. A second comparative subsample analysed for glyphosate and AMPA consists of 10 samples provided in

2007 and 2015 by self-reported vegetarians/vegans taking part in Greifswald (cf. Table 7). There was virtually no difference between self-reported vegetarians/vegans and the main study sample concerning quantifiable percentages of glyphosate in 2007 and 2015. For AMPA the fractions of samples with levels of at least 0.1 g/L tended to be lower for vegetarians/vegans (2007: 0.0% vs. 30.0%, p = 0.047 and 2015: 30.0% vs. 42.5%, p = 0.47), being statistically significant only in 2007. In that year, all self-reported vegetarians/vegans who participated in Greifswald were female. When limiting the comparison to samples collected from women, the difference observed in 2007 was less pronounced and no longer statistically significant (0.0% vs. 25.0%, p = 0.083). Glyphosate concentrations in urine seem slightly higher in the main study sample in comparison to the Muenster sub-population. Although there were virtually no differences in urinary AMPA, this result indicated possible regional or seasonal differences in exposure. Against expectations, the results of this study did not considered to advocate urinary glyphosate and AMPA levels being higher in vegetarian/vegan participants. No equal sex distribution could be achieved for the sub-population of self-reported vegetarians/vegans, due to a low participation rate of male vegetarians/vegans. This might have reduced comparability of this sub-population, as males showed a tendency to exhibit higher glyphosate and AMPA concentrations in urine. Another limitation of this comparison was that vegetarian/vegan participants exhibit on average higher 24 h-urine sample volumes than in the main study sample without self-reported specifically restricted diet. In general, the sample sizes of the two sub-populations analysed for comparison were possibly too small to draw general conclusions on seasonal or regional effects and on effects of dietary preferences.

Health-relevance of observed internal exposure; The acceptable daily intake (ADI) for glyphosate derived by the European Food Safety Authority (EFSA) is 0.5 mg/kg/d (EFSA, 2015). Assuming a bodyweight of 60 kg, an oral absorption of 20% with fast elimination via urine, and a daily urine excretion of 1500 to 2000 mL, the concentration in 24 h-urine associated with this ADI resulted in 3000 to 4000 g/L. This concentration was higher than the maximum concentration observed in this study (2.8 g/L) by a factor of 1000. Considering EFSA's risk assessment, none of glyphosate concentration measured in ESB samples was considered problematic for human health. The International Agency for Research on Cancer (IARC), however, classified glyphosate in Group 2A ("probably carcinogenic to humans"; IARC,). Taking this assessment into account, especially the increasing trend in internal glyphosate exposure documented by ESB samples needs an attention with regard to human health.

Conclusion

Retrospective GC-MS-MS analyses of the general German population urinary samples collected during a period covering 2001 – 2015 revealed that 31.8% of analysed samples contained detectable level of glyphosate. For AMPA this was the case for 40.1% samples analysed. A peak of detectable glyphosate level was observed in 2012 (57.5%) and 2013 (56.4%), followed by a decrease in 2014 (32.5%) and 2015 (40.0%), which may be due to changes in glyphosate application in agricultural practice. Urinary glyphosate levels tended to be higher in males. Overall, the urinary level of AMPA showed a similar trend as glyphosate, with a statistically significantly correlation.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The internal exposure levels of glyphosate and its main metabolite AMPA were analysed using the general German population urinary samples collected during a period covering 2001 – 2015 with similar sample sizes and sex distributions. Retrospective GC-MS-MS analyses revealed that 31.8% of analysed samples contained detectable level of glyphosate. For AMPA this was the case for 40.1% samples analysed. A peak of detectable glyphosate level was observed in 2012 (57.5%) and 2013 (56.4%), followed by a decrease in 2014 (32.5%) and 2015 (40.0%), which may be due to changes in glyphosate application in agricultural practice. Urinary glyphosate levels tended to be higher in males. Overall, the urinary level of AMPA showed a similar trend as glyphosate, with a statistically significantly correlation.

This publication is considered relevant for the risk assessment of glyphosate and reliable without restrictions because it complies with the quality criteria of a good monitoring study.

Assessment and conclusion by rMS:

Reliability criteria of exposure studies

Publication: Conrad *et al.* 2017	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines/practices.	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	Retrospective population monitoring study of glyphosate and AMPA in urine.
Test substance		
Exposure to formulations with only glyphosate as a.s.	NA	Exposure to glyphosate and AMPA mainly through the diet.
Exposure to formulations with glyphosate combined with other a.s.	NA	
Exposure to various formulations of pesticides	NA	
Study		
Study design clearly described	Y	
Population investigated sufficiently described	Y	
Exposure circumstances sufficiently described	Y	General population.
Sampling scheme sufficiently documented	Y	
Analytical method described in detail	Y	
Validation of analytical method reported	Y	
Monitoring results reported	Y	
Overall assessment		
Reliable without restrictions	Y	
Reliable with restrictions		
Reliability not assignable		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate and reliable without restrictions because it complies with the quality criteria of a good monitoring study.		

1. Information on the study

Data point:	CA 5.9/007
Report author	Kongtip, P. *et al.*
Report year	2017
Report title	Glyphosate and Paraquat in Maternal and Fetal Serums in Thai Women
Document No	doi.org/10.1080/1059924X.2017.1319315 E-ISSN: 1545-0813
Guidelines followed in study	None
Deviations from current	Not applicable

test guideline	
Previous evaluation	None
GLP/Officially recognised testing facilities	Non-GLP
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary of the study according to OECD format

This longitudinal study measured the glyphosate concentrations found in maternal and umbilical cord serum in 82 pregnant women who gave birth in three provinces of Thailand. Through questionnaires and biological samples collected at childbirth, factors such as personal characteristics, family members occupation, agricultural activities, and herbicide use in agricultural work were evaluated as predictors of glyphosate levels in the pregnant women. Statistical analysis used univariate and binary multiple logistic regression, where the outcome was the probability of exposure to glyphosate above the limit of detection associated with occupation and household factors. The glyphosate concentrations in the pregnant women's serum at childbirth (median: 17.5, range: 0.2–189.1 ng/mL) were significantly higher ($P < .007$) than those in the umbilical cord serum (median: 0.2, range: 0.2–94.9 ng/mL). Women with glyphosate levels >limit of Detection (LOD) in serum at childbirth were 11.9 times more likely to report work as an agriculturist ($P < .001$), 3.7 times more likely to live near agricultural areas ($P = .006$), and 5.9 times more likely to have a family member who worked in agriculture ($P < .001$).

Materials and Methods

Subjects for a pilot birth cohort were recruited from pregnant women who came for prenatal care at three hospitals in Thailand: Amnatchareon Hospital in Amnatchareon Province in the northeast, Sawanpracharak Hospital in Nakhorn Sawan Province in the lower north, and Paholpolpayuhasena Hospital in Karnjanaburi Province in the west of Thailand, from May to December 2011. To be recruited, the women had to be in their 7th month of pregnancy, 19–35 years of age, not have diabetes or hypertension, and plan to give birth and have follow-up infant care at the recruiting hospital. The 82 subjects for this study included 81 full-term normal birth neonate and 1 full-term cesarean birth neonate.

During their 7th month of pregnancy, the women were interviewed about their general health, diet, and work exposures, including agricultural work, as well as about use of pesticides at home and work. Several questionnaires, each with several sections, constituted the data collection. The questionnaire was based on the type of information collected in previous studies of agricultural workers and modified for the conditions of agriculture and types of pesticide exposures experienced in Thailand. The questionnaires were reviewed by staff at each hospital, then piloted and revised based on comments. One questionnaire collected data on demographics and the mother's general health, whereas another collected birth data. The pesticide exposure questionnaire consisted of six sections; the first section contained 12 items related to pesticide use in the home or outside the home, as well as sources of drinking water. The second section contained 9 items about the woman's work history outside the home. The third section, with 10 items, covered agricultural activities if conducted by the woman. The fourth section, with 3 items, covered agricultural activities conducted by the woman during pregnancy. The fifth section, with 6 items, asked about the agricultural work of family members. The sixth section, with 30 items, collected detailed information only from those who were agricultural workers or had family members who were agricultural workers and included detailed information on the mixing and spraying of pesticides. To summarize, the pesticide exposure questionnaire had 40 items for all subjects and 30 items only for those who were self-identified as agricultural workers or who had family members who were agricultural workers. For comparisons with agriculturists, women with other occupations were used as the control group. The nurses at the prenatal clinics in the three study hospitals were trained to recruit and interview subjects. The maternal and umbilical cord serum was collected during delivery by the delivery nurses and was frozen at -45°C until analysis. This study was reviewed and approved by the Ethics Committee on Human Rights Related to Human Experimentation,

Mahidol University, and the University of Massachusetts Lowell Institutional Review Board.

Glyphosate was obtained from Sigma-Aldrich Inc., Singapore. Acetonitrile, trimethylamine, methylene chloride, dichloromethane (all high-performance liquid chromatography [HPLC] grade), and sodium dodecyl sulfate (SDS) (gas chromatography [GC] grade) were purchased from Apex Chemical, Bangkok, Thailand. Other chemicals were of analytical grade. Analysis was performed with an HPLC system (Agilent 1200 Series) with a fluorescence detector for glyphosate. The serum sample was analysed on Luna 5 µm C18 (150 × 4.6 mm) column (Phenomenex, Torrance, CA, USA) with guard column at 45°C. A calibration curve was developed using hospital serum samples of non-subjects tested for the non-presence of glyphosate and then spiked with glyphosate to yield final concentrations of 12.5, 25, 50, 100, 150, and 200 ng/mL (n = 3 replicates). Samples of glyphosate were prepared using 250-µL of serum and 30 µL of derivatized glyphosate and injected into the HPLC. Evaluation of the detection limit was performed following the National Institute for Occupational Safety and Health method. For determination of accuracy and precision, concentrations of hospital serum samples of non-subjects tested for the non-presence of pesticide were used to prepare concentrations of 50, 100, and 150 ng of glyphosate/mL. Three replicates of each concentration were analysed on three separate days. The calibration curve for glyphosate was linear over the concentration range of 12.5–200 ng glyphosate/mL serum with the correlation coefficient of 0.998. The detection limit for the analysis of glyphosate in serum was0.4 ng/mL. Values below the detection limit for serum glyphosate were used as the detection limit divided by 2 since the data were highly skewed. The recovery of the method ranged from 94.33% to 99.03% with a relative standard deviation (RSD) of <3% for glyphosate concentrations of 50–150 ng/mL.

The descriptive statistics were calculated using SPSS (SPSS version 18; PASW Statistics Base 18, Bangkok, Thailand). Since exposures were highly skewed due to the large percentage of values below the limit of detection (LOD), concentrations were reported as the median and range. For comparison of the paired mother and cord blood serum concentrations, only pairs with both measurements above the LOD levels were used with the Wilcoxon signed-rank test. Binary logistic regression was used to evaluate whether various factors were significantly associated with the probability of having glyphosate concentrations over the LOD. Several a priori factors were evaluated for inclusion as potentially significant covariates, such as maternal location/province and educational level of the mother, but these were found not to be significantly associated with the probability of having glyphosate concentrations over the LOD. Thus, occupational factors were evaluated in single-factor models and reported as unadjusted odds ratios (ORs). In models examining secondary exposure factors, maternal occupation was included as a covariate in the model to control for the influence of maternal agricultural work on serum levels.

Results

The average age of the 82 women who gave birth during the study was 26 years old (range: 19–34 years), with most having completed secondary school (41%), although 26% only completed primary school. Of the 82 women, 39% of the women described their occupation as agriculturist/farmer, whereas 21% listed themselves as a housewife, and about 13% were employees or owned their own business (often these are small retail stands with food or other items for sale). Of the 82 pregnant women who gave birth during the study, cord blood was only collected from 75 newborns, all born full term (37–41 weeks).

The percentage of maternal samples of glyphosate that were at or below the LOD was 46.3%, whereas for cord serum 50.7% were ≤LOD. Comparison of the glyphosate concentrations in paired serum samples of mother and cord blood that were both >LOD (n = 36) found that they were significantly different (P < .001), with the mother's serum levels the higher of the two. With regard to occupational factors predicting glyphosate exposures, the odds of having a detectable level of glyphosate in serum were 11.9 higher (95% confidence interval [CI] 3.6-39.5) than the LOD among women who worked in the fields compared with those who did not. Pregnant women who worked in agricultural fields during the first, second, or third trimester of pregnancy also had significantly elevated ORs of 13.5 (95% CI 2.8-64.3), 7.7 (95% CI 2.0-29.8), and 12.4 (95% CI 1.5-102.7), respectively, of having serum levels >LOD compared with those women who never worked in the fields. Likewise, pregnant women who reported picking crops during

pregnancy had a significantly elevated OR (5.4; 95% CI 1.4-20.8)), whereas those who reported the agricultural activity of digging in farm soil or controlling weeds during pregnancy did not have elevated ORs.

With regard to secondary factors, pregnant women who lived near agricultural fields (<0.5 km) were significantly more likely to have serum glyphosate levels >LOD than those women living far from agricultural fields (OR=3.7, 95% CI 1.5-9.2). Note that there was no significant relationship between home location and agricultural occupation; thus, the adjusted OR did not reflect collinearity or confounding. Those pregnant women who reported a family member who was an agriculturist/farmer living in the same house were significantly more likely to have elevated serum glyphosate than those who did not (OR=5.9; 95% CI 2.2-16.1); however, when adjusted for occupation, the OR became non-significant because of the high correlation between having a family member who was a farmer in the same house and maternal occupation as a farmer.

The most striking finding of this study was that glyphosate concentrations over the LOD were significantly more likely to be found in the serum of pregnant women collected at childbirth if their occupation was an agriculturist/farmer or conducted various agricultural tasks or if they lived near agricultural fields sprayed with pesticides (unspecified), when compared with women who had no agricultural exposures. Researchers measured glyphosate levels in urine among farmers in the United States and found that farmers who had skin contact with pesticides or did not use rubber gloves had significantly higher glyphosate concentrations in their urine. Among those who did not wear rubber gloves, appreciable differences were found in the urinary glyphosate levels between those who repaired equipment during applications or spilled during mixing/loading or application. This supports the notion that dermal contact is an important route of exposure for this herbicide. Researchers also reported urinary glyphosate in one of the children who lived 1.5 km away from the farm where their father sprayed glyphosate in France. However, in a US study, very few of the spouses (4%) and children (12%) who lived in a home within 1 mile of the sprayed farm fields had measurable urinary glyphosate on the day of spraying, and an even lower percentage had measurable levels on days 1–3 post application. Among the applicators, 60% had measurable glyphosate on the spraying day, with a decrease to 27% on the third day post application. This raises the question of the half-life of glyphosate and how the Thai agricultural women giving birth had measurable levels of glyphosate in their serum. Although these women had regular, repeated exposures to glyphosate, even during their final trimester of pregnancy, it is unlikely that they were exposed in the field on the day prior to giving birth. It is possible that these serum levels were caused by the drift of glyphosate spray, skin contact with the pesticide contaminated family member working in the fields, contamination of the home through clothing (take home exposure), or through poor storage of pesticides near the home. Alternatively, little is known about the metabolism and storage of pesticides during pregnancy and the impact on pesticide half-life. The median (range) glyphosate concentrations of maternal and umbilical cord serums in this current study were 17.5 (0.2–189.1) and 0.2 (0.2–94.9) ng/mL, respectively. The maternal serums of glyphosate at birth were significantly higher than those in cord blood serums. It is not known why maternal serum levels were higher than cord serum samples. At this time, only one study has looked at serum levels of pesticides in pregnant and non-pregnant Canadian women who were not agriculturists or living with a spouse who worked with pesticides. They found no detectable levels of glyphosate in the pregnant women before birth or in their cord blood samples. However, 5% (2/39) of the non-pregnant women in that study had measurable glyphosate serum levels (including one with a level of 93.6 ng/mL). Potential exposures in that study were assumed to be through consumption of pesticides associated with genetically modified foods. In our study, we found measurable glyphosate levels (>LOD) in 14.7% of the maternal serum samples of women who were not agriculturists/farmers by occupation. This is concerning, because researchers studied the effect of glyphosate exposures on the risk of miscarriage among women living on farms in Ontario, Canada, and found that women who were exposed to glyphosate before conception (from 3 months before up to conception) had a higher risk of spontaneous abortion.

Conclusion

This study suggests that agricultural activities do increase maternal serum levels of glyphosate even in

samples taken on the day of birth. In the case of glyphosate, living near farmland where pesticides are sprayed can also significantly increase the risk of serum levels >LOD at birth. These results suggest that a study evaluating the long-term health of children exposed to herbicides during gestation should be considered. Given that herbicides make up the largest volume of pesticide imports in Thailand, and that imports continue to increase, further regulation of the sale and use of pesticides may help safeguard the health of Thai children.

3. Assessment and conclusion

Assessment and conclusion by applicant:

This study suggests that agricultural activities increase maternal serum levels of glyphosate, even in samples taken on the day of birth. Living near farmland where pesticides are sprayed can also significantly increase the risk of serum levels >LOD at birth. Limitations of this study include a small sample size (N=82) and large percentages of maternal samples cord serum that were at or below the LOD for glyphosate (46.3% and 50.7%, respectively).

This publication is considered relevant to the risk assessment of glyphosate but reliable with restrictions because the analytical method used for glyphosate could have been described in more detail.

Reliability criteria of exposure studies

Publication: Kongtip *et al*., 2017	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines/practices.	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Exposure to formulations with only glyphosate as a.s.	NA	Subjects of a pilot birth cohort being monitored for glyphosate and paraquat.
Exposure to formulations with glyphosate combined with other a.s.	NA	
Exposure to various formulations of pesticides	NA	
Study		
Study design clearly described	Y	
Population investigated sufficiently described	Y	
Exposure circumstances sufficiently described	Y	
Sampling scheme sufficiently documented	Y	
Analytical method described in detail	Y	Analytical method for glyphosate could be described in more detail (derivatization).
Validation of analytical method reported	Y	
Monitoring results reported	Y	
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Reliability not assignable		

Not reliable		
This publication is considered relevant to the risk assessment of glyphosate but reliable with restrictions because the analytical method used for glyphosate could have been described in more detail.		

1. Information on the study

Data point:	CA 5.9/008
Report author	McGuire M. K. *et al.*
Report year	2016
Report title	Glyphosate and aminomethylphosphonic acid are not detectable in human milk
Document No	doi: 10.3945/ajcn.115.126854 ISSN: 1938-3207
Guidelines followed in study	None
Deviations from current test guideline	NA
GLP/Officially recognised testing facilities	Non-GLP
Acceptability/Reliability:	Yes/Reliable without restrictions

2. Full summary of the study according to OECD format

It was sought to determine whether glyphosate and its metabolite aminomethylphosphonic acid (AMPA) could be detected in milk and urine produced by lactating women and, if so, to quantify typical consumption by breastfed infants. Milk (n = 41) and urine (n = 40) samples from healthy lactating women living in and around Moscow, Idaho and Pullman, Washington was collected. Milk and urine samples were analysed for glyphosate and AMPA with the use of highly sensitive liquid chromatography–tandem mass spectrometry methods validated for and optimized to each sample matrix. The milk assay, which was sensitive down to 1 µg/L for both analytes, detected neither glyphosate nor AMPA in any milk sample. Mean ± SD glyphosate and AMPA concentrations in urine were 0.28 ± 0.38 and 0.30 ± 0.33 mg/L, respectively. Because of the complex nature of milk matrixes, these samples required more dilution before analysis than did urine, thus decreasing the sensitivity of the assay in milk compared with urine. No difference was found in urine glyphosate and AMPA concentrations between subjects consuming organic compared with conventionally grown foods or between women living on or near a farm/ranch and those living in an urban or suburban non-farming area.

Materials and Methods

Human subjects – A total of 41 healthy lactating women living in and around Pullman, Washington, and Moscow, Idaho, were included in the study. To be eligible for participation, women had to be 1–3 months postpartum, breastfeeding and/or pumping milk 5 times/day and more, and aged 18 years and older. All but 1 subject also completed a 5-question survey documenting potential glyphosate exposure from the environment and diet.

Milk and urine collection and preservation - After cleaning the breast, approx. 30 mL milk was collected with an hospital-grade electric breast pump into a single-use sterile collection container. A midstream urine sample was also collected into a single-use sterile collection container. The sample containers were immediately placed in ice, separated into aliquots and frozen at -20^{0}C pending analysis. One subject failed to provide a urine sample.

Glyphosate and AMPA analyses - Milk and urine samples were analysed for glyphosate and AMPA using liquid chromatography-tandem mass spectrometry in the multiple reaction monitoring mode. Two precursor product ion transitions for each analyte and a stable isotope labelled internal standard for each analyte were used to ensure the selectivity of the analytical method. Although 2 quantitative precursor-product ion transitions were monitored, the results were reported using the most sensitive transition for each analyte. The assay was validated separately for milk and urine. Limits of detection (LOD) and quantification (LOQ) for glyphosate in milk were 1.0 and 10.0 µg/L, respectively. The LOD and LOQ for glyphosate in urine were 0.02 and 0.10 µg/L, respectively. The LOD and LOQ for AMPA in milk were 1.0 and 10.0 µg/L, respectively and those for AMPA in urine were 0.03 and 0.10 µg/L, respectively. Glyphosate and AMPA concentrations in milk were independently confirmed by another laboratory using the same liquid chromatography-tandem mass spectrometry method with minor modifications. Because of differences in instrumentation, the LODs with the more sensitive quantitative ion transitions were 6.0 and 9.0 µg/L for glyphosate and AMPA in human milk, respectively. The LOQ for human milk was 25.0 µg/L for both analytes. Duplicate aliquots from each milk sample were sent to each laboratory separately.

Statistical analyses - For glyphosate and AMPA concentrations in urine a generalized linear mixed model was used assuming a Poisson distribution with a logarithmic link function. For concentrations less than the respective LOD values, one-half LOD (0.01 and 0.015 µg/L for glyphosate and AMPA, respectively) nominal values were used for assessment. For concentrations between the LOD and LOQ, one-half LOQ (0.05 µg/L for both glyphosate and AMPA) nominal values were used for assessment. All values are presented as means ± SDs.

Results

Description of study population and glyphosate exposure - Women were aged 29 ± 5 years, 67 ± 17 days postpartum, and had a BMI (kg/m^2) of 26.8 ± 8.6. 75% of them lived in an urban or suburban non-farming region of the Palouse, and 58% of them reported that they made no effort to eat foods characterized as organic, although they sometimes included them in their diets for convenience. 15% reported ever having personally mixed or used any type of weed killer. All but one of the women having reported ever doing so had mixed or used a weed killer containing glyphosate.

Glyphosate and AMPA concentrations in milk - Regardless of where the samples were analysed, none of the milk samples contained detectable amounts of either glyphosate or AMPA.

Glyphosate and AMPA concentrations in urine - Glyphosate was detectable in nearly all (n =37) of the urine samples and was quantifiable in 29 of them. Glyphosate values ranged from below the LOD (<0.02 µg/L) to 1.93 µg/L, with a mean concentration of 0.28 ± 0.38 µg/L. AMPA was also detectable in nearly all (n = 38) of the urine samples and quantifiable in 29 of them. Urine AMPA values ranged from below the LOD (<0.03 mg/L) to 1.33 µg/L, with a mean concentration of 0.30 ± 0.33 µg/L. There were no statistically significant effects of consuming organic compared with conventional foods or living on/near a farm compared with living in an urban/suburban region on concentrations of glyphosate in urine, respectively. Neither were there statistically significant effects of consuming organic compared with conventional foods or living on/near a farm compared with living in an urban/suburban region on concentrations of AMPA in urine. Adjusting for potential covariates (age, time postpartum, BMI, parity) did not alter these conclusions. When raw, untransformed values were used in the analysis, there was a statistically significant positive correlation between urinary glyphosate and AMPA concentrations.

Discussion and Conclusion

The results provide evidence that the concentrations of glyphosate and AMPA in milk produced by healthy women are below the detection limits of available validated analytical assays. In urine, glyphosate and AMPA were detectable in many samples, but the concentrations were very low (<0.02 to 1.93 and <0.03 to 1.33 µg/L, respectively) and well below values reported in other healthy adult populations. The RfD for glyphosate is 1.75 mg/kg bw/day. US EPA considers AMPA to be of similar or lesser toxicity than

glyphosate and determined that it should be exempt from regulation regardless of concentrations observed in food or feed. Thus, a woman with a typical weight for the study participants, 75 kg, could consume as much as 131.25 mg glyphosate/day with no expected negative effects. Taking an oral bioavailablity of 20% into account and assuming that all glyphosate absorbed is excreted within 24 hours and all absorbed glyphosate is excreted in urine, the urinary output would be 26,250 µg/day. In the current study, the highest reported glyphosate concentration in urine was 1.93 µg/L. As such, even allowing for a relatively high urine output (3 L/day), the highest glyphosate excretion in our study would be 5.79 µg/day, a value which is more than 4,500 times lower. Applying similar parameters and logic to a 5-kg infant with a mean milk intake of 0.7 L/day and a milk glyphosate concentration of 1 µg/L (the LOD value), then the maximum daily consumption of glyphosate would be 0.7 µg/day which is more than 12,000 times lower than the RfD. It is important to emphasize that the larger international study from which these samples originate was not designed to detect small differences in urine glyphosate and AMPA concentrations based on dietary choices, location of residence (e.g., urban compared with rural), or occupational glyphosate exposure. Detecting small-effect sizes at statistically significant concentrations and adequate statistical power would require 4–5 times as many observations than used in this study.

3. Assessment and conclusion

Assessment and conclusion by applicant:

In this study breast milk and urine samples from lactating women were analysed for glyphosate and AMPA. The results provide evidence that the concentrations of glyphosate and AMPA in milk produced by healthy women are below the detection limits of available validated analytical assays. In urine, glyphosate and AMPA were detectable in many samples, but the concentrations were very low and well below the values reported in other healthy adult populations.

This publication is considered relevant for the risk assessment of glyphosate and reliable without restrictions because it complies with all the reliability criteria of an exposure study.

Assessment and conclusion by RMS:

Reliability criteria for exposure studies

Publication: McGuire *et al*., 2016	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines/practices.		
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Reference material (glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	N	
Exposure to formulations with only glyphosate as a.s.		
Exposure to formulations with glyphosate combined with other a.s.		
Exposure to various formulations of pesticides	Y	Exposure mainly via food
Study		
Study design clearly described	Y	Monitoring of glyphosate in urine

Reliability criteria for exposure studies

Publication: McGuire *et al*., 2016	**Criteria met? Y/N/?**	**Comments**
		and breast milk of lactating women
Population investigated sufficiently described	Y	
Exposure circumstances sufficiently described	Y	
Sampling scheme sufficiently documented	Y	
Analytical method described in detail	Y	
Validation of analytical method reported	Y	
Monitoring results reported	Y	
Overall assessment		
Reliable	Y	
Reliable with restrictions		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate and reliable without restrictions because it complies with all the reliability criteria of an exposure study.		

1. Information on the study

Data point:	CA 5.9/009
Report author	Sierra-Diaz, E. *et al.*
Report year	2019
Report title	Urinary pesticide levels in children and adolescents residing in two agricultural communities in Mexico
Document No	doi:10.3390/ijerph16040562 ISSN: 1660-4601
Guidelines followed in study	None.
Deviations from current test guideline	Not applicable
Previous evaluation	None
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary of the study according to OECD format

The objective of this study was to measure the concentration and prevalence of pesticides in a cross-sectional study involving children and adolescents under 15 years of age in two small farming communities located in in State of Jalisco in Mexico, with both communities having very similar characteristics.

Urine samples (first-morning urine) were taken from children under 15 years of age in Agua Caliente located near the largest lake in Mexico (n = 192) and in Ahuacapán located in the south coast of the state (n = 89). A total of 281 urine samples obtained in both communities and processed for the determination of pesticides with high-performance liquid chromatography together with tandem mass spectrometry.
In 100% of the samples, at least two pesticides of the 17 reported in the total samples were detected. The presence of glyphosate was detected in more than 70% of the cases. The mean urinary level of glyphosate 0.363 ± 0.3210 ng/mL in Agua Caliente and 0.606 ± 0.5435 ng/mL in Ahuacapán, detected in 72.91% and

100% of their respective total samples. Substantial differences were detected regarding the other compounds.

Materials and methods

A cross-sectional study was carried out simultaneously in two communities in State of Jalisco, which is one of the three states in Mexico with the greatest index of people poisoned due to the application of pesticides. The first of these was, Agua Caliente, near Lake Chapala, the largest lake in Mexico, and the second was a community in the region of the south coast of the state (Ahuacapán).

Beginning hundreds of years ago, multiple autochthonous communities of people of Nahua native origin who were dedicated to fishing and agriculture settled on the bank of Lake Chapala. Since 2016, the Department of Public Health of the University of Guadalajara has carried out studies in the zone, specifically in the community of Agua Caliente, Poncitlán Municipality, State of Jalisco. In this community, health problems have been detected such as malnutrition and albuminuria, specifically in children and adolescents under the age of 17 years.

The community is inhabited by 998 persons, whose main activities are farming (37.9%), construction work (29.3%), and laboring 7.2% and who alternate with fishing as a means of subsistence. The most common local crops are corn, seasonal beans, and chayote (*Sechium edule*), which the inhabitants irrigate with lake water. The weekly average family income is approximately 52.63 USD.

The community of Ahuacapán is found toward the coast of the Pacific Ocean, in a 180 km straight line from Agua Caliente. A total of 950 inhabitants live there and their principal economic activity is agriculture, producing sugar cane, corn, tomatoes, citrus, and horticultural products. The water necessary for agriculture derives from springs, deep wells, and the Ayuquila River. The weekly family income in this community is approximately 75 USD. In both localities, there is a total of 550 children and adolescents (58%) aged between less than 1 year and 15 years.

For the urine sampling, communities were invited to participate voluntarily, with the approval of the Department of Public Health of the University of Guadalajara and the local authorities of the two communities. The sample included only children and adolescents aged under 17 years in Agua Caliente and those under 12 years of age in Ahuacapán. Their parents were informed concerning the objective of the study and, after obtaining the parents' signed consent, the minors were asked for a urine sample (first-morning urine). In both communities, anthropometric measurements (weight and height) were performed on the minors.

Urine samples were transported to the laboratory and were processed for the determination of pesticides with the HPLC/MS/MS (high-performance liquid chromatography coupled with tandem mass spectrometry) method with Agilent Technologies® Model 1200 equipment for HPLC and Model 6430B for MS/MS spectrometry. The method for HPLC used a column Zorbax Eclipse XDB C18, Rapid Resolution, 2.1 × 50 mm, 3.5 µm. Mobile phases: A, 0.1% formic acid in water; B, acetonitrile (ACN); gradient of 40% to 100% B; injection volume, 5 µL; flow, 0.5 min; curve range for each pesticide, 0.01 to 1000 µg/mL. The latter was performed at the Laboratory of Applied Pharmacokinetics of the University Center of Exact and Engineering Sciences of the University of Guadalajara. With this method, it was possible to determine the presence of 16 pesticides, as presented in Table 1.

Table 1. Category of pesticides analysed in urine samples from the two communities.

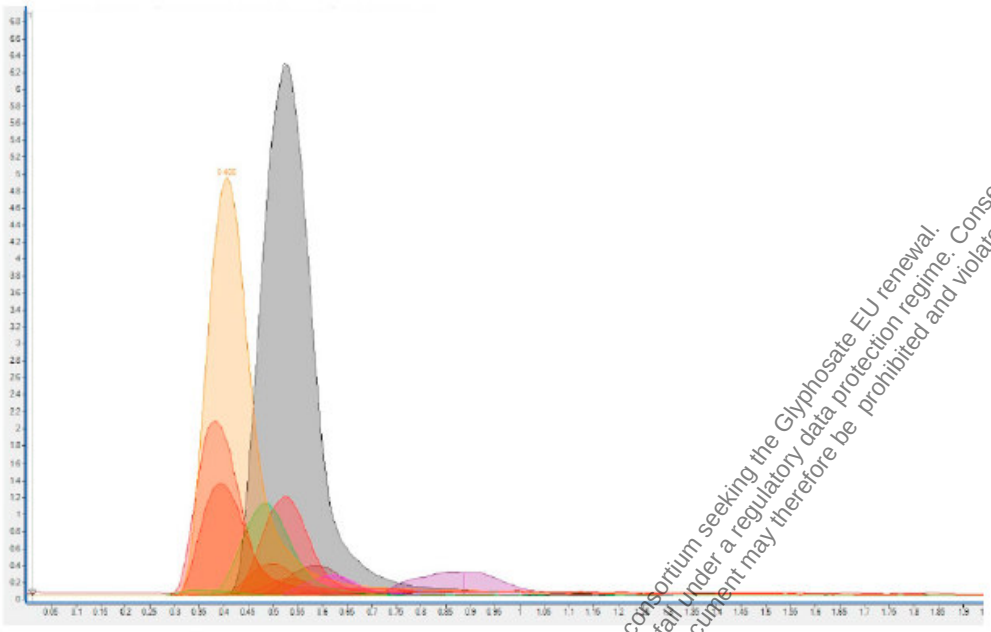
Name	IUPAC ID	PubChem CID	Agrochemical Category
Acetochlor	2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)acetamide	1988	Herbicide
Atrazine	6-chloro-4-N-ethyl-2-N-propan-2-yl-1,3,5-triazine-2,4-diamine	2256	Herbicide
Carbendazim	methyl N-(1H-benzimidazol-2-yl)carbamate	25429	Fungicide
Carbofuran	(2,2-dimethyl-3H-1-benzofuran-7-yl) N-methylcarbamate	2566	Insecticide, Nematicide, Acaricide
Cyhalothrin	[cyano-(3-phenoxyphenyl)methyl] 3-[(Z)-2-chloro-3,3,3-trifluoroprop-1-enyl]-2,2-dimethylcyclopropane-1-carboxylate	5281873	Insecticide
Diazinon	*O,O*-Diethyl O-[4-methyl-6-(propan-2-yl) pyrimidin-2-yl] phosphorothioate	3017	Insecticide, Acaricide
Dimethoate	2-dimethoxyphosphinothioylsulfanyl-N-methylacetamide	3082	Insecticide, Acaricide
Emamectin	4″-Deoxy-4″-epi-methylamino-avermectin B1; Epi-methylamino-4″-deoxy-avermectin	11549937	Insecticide
Enilconazole (imazalil)	1-[2-(2,4-dichlorophenyl)-2-prop-2-enoxyethyl]imidazole	37175	Fungicide
Glyphosate	2-(phosphonomethylamino)acetic acid	3496	Herbicide
Malathion	diethyl 2-dimethoxyphosphinothioylsulfanylbutanedioate	4004	Insecticide, Acaricide
Methomyl	methyl (1E)-N-(methylcarbamoyloxy)ethanimidothioate	5355758	Insecticide
Metoxuron	3-(3-chloro-4-methoxyphenyl)-1,1-dimethylurea	29863	Herbicide
Molinate	S-ethyl azepane-1-carbothioate	16653	Herbicide
Pyraclostrobin	Methyl N-[2-[[1-(4-chlorophenyl) pyrazol-3-yl]oxymethyl]phenyl]-N-methoxycarbamate	6422843	Fungicide, plant growth regulator
Thiabendazole	4-(1H-benzimidazol-2-yl)-1,3-thiazole	5430	Fungicide

Conditions for MS/MS spectrometry are described in Table 2 and Figure 1 shows a chromatogram urine sample.

Table 2. Mass spectrometer conditions for pesticide determination.

Mass spectrometer conditions				
Electrospray Interface Condition				
Gas emperature	350 °C			
Gas flow	12 L/min			
Nebulizer	25 psi			
Capillary	+4000	−4000		
Compound name	**Precursor Ion**	**Product Ion**	**Fragmentor**	**Polarity**
L-Cyhalotrin (225.1)	467.1	225.1	80	Positive
Meclizina (201.1)	391.2	201.1	90	Positive
Pyraclostrobin (163)	388	163	120	Positive
Malation (99)	331	99	80	Positive
Clorpyrifos (200)	325	200	30	Positive
Oxandrolona (289.2)	307.2	289.2	100	Positive
Oxandrolona (271.2)	307.2	271.2	100	Positive
Oxandrolona (229.1)	307.2	229.1	100	Positive
Diazinon (153)	305	153	100	Positive
Imazalil (159)	297	159	100	Positive
Paration (264)	292	264	90	Positive
Paration (236)	292	236	90	Positive
Acetoclor (224.2)	270.1	224.2	60	Positive
Acetoclor (148.4)	270.1	148.4	60	Positive
Picloram (222.9)	240.9	222.9	90	Positive
Picloram (194.9)	240.9	194.9	90	Positive
Dimethoate (171)	230	171	80	Positive
Metoxuron (72.1)	229.1	72.1	93	Positive
Ametryn (186)	228.1	186	120	Positive
Ametryn (96)	228.1	96	120	Positive
Carbofuran (123)	222	123	120	Positive
Atrazine (132)	216	132	120	Positive
Thiabendazole (131)	202	131	120	Positive
Carbendazim (160)	192.1	160	110	Positive
Molinate (55.1)	188.1	55.1	78	Positive
Methomyl (106)	163.1	106	30	Positive
Methomyl (88.1)	163.1	88.1	30	Positive
Methomyl (65)	163.1	65	30	Positive
Emamectina (158.1)	887.1	158.1	60	Positive
Glyphosate (149.9)	168	149.9	80	Negative
Glyphosate (124.2)	168	124.2	80	Negative
2,4-D (161.1)	219	161.1	50	Negative

Figure 1. Shows a chromatogram of a urine sample.



Statistical analysis

For the statistical description, absolute frequencies, percentages, means, and standard deviations (SD) were used. Statistical significance was evaluated by means of the Mann–Whitney U, the Chi-squared, and the Fisher exact tests. To compare the two populations and evaluate the differences in the urine pesticide levels, the Mann–Whitney U test was used. Similarly, to compare the frequency of detection rate, the Fisher test was used. Statistical significance was considered with a p of ≤ 0.05. For data processing, Excel® (Microsoft, Redmond, WA, USA) and Epi Info ver. 7.2 (Centers for Disease Control and Prevention (CDC) Atlanta, GA, USA) statistical software were used.

This research was carried out with the authorization of the ethics committee of the Department of Public Health of the University of Guadalajara (registration number DCSP/CEI/2016/260618/038).

Results

A total of 281 children participated, of whom 192 (68.3%) corresponded to the community of Agua Caliente with an average age of 9.4 years (range, 5–15 years). In the community of Ahuacapán, 89 (31.7%) samples were collected, with an average age of 8.31 years (range, 5–13 years; Table 3).

Table 3. Demographic and anthropometric data of the children in both communities.

Variable	Agua Caliente (n = 192)	Ahuacapán (n = 89)
Gender		
Female	84 (43.8%)	40 (44.9%)
Male	108 (56.3%)	49 (55.1%)
Age (years)	9.40 (SD 2.52)	9.31 (SD 2.05)
Age groups		
5–8 y	78 (40.6%)	49 (55.1%)
9–11 y	67 (34.9%)	34 (38.21%)
12–15 y	47 (24.5%)	6 (6.7%)
Weight (kilograms)	29.39 (SD 10.06)	32.27 (SD 11.73)
Height (centimeters)	131.58 (SD 14.12)	132.94 (SD12.97)
Body mass index (k/m^2)	16.46 (SD 2.44)	17.72 (SD 3.66)

Detection of the pesticides was frequent in both communities. Substantial differences in exposure and detection rate were also identified (Table 4). Glyphosate was detected with a higher frequency in Ahuacapán than in Agua Caliente, which presented in 100% of the minors studied in Ahuacapán vs. 73% in Agua Caliente ($p > 0.001$), with minimal values of 0.0020 ng/mL and maximal values of 2.63 ng/mL. Glyphosate is currently one of the most utilised herbicides in Mexico. The differences between the two localities were considered to be associated with the agro-industrial activity and the practices that have become generalised among the small producers of basic crops.

In general terms, 100% of the study subjects were exposed to at least two of the compounds identified in urine. Positive results are noteworthy of six of the compounds in more than 70% of the subjects studied in both communities: malathion, metoxuron, glyphosate, dimethoate, enilconazole, and acetochlor. The greatest prevalence was for the herbicides (60.49%), in second place, fungicides (39.05%), and lastly, the insecticides (20.92%).

The urinary samples in this study was collected during winter, during which there is a significant diminution in the agricultural use of pesticides. However, the authors emphasised that consumption of a conventional diet with seasonal products could play a very important role regarding the presence of these pesticides in urine.

Table 4. Frequencies, percentages, means, and standard deviations (SD) of pesticides in urine.

Pesticide	Agua Caliente	Ahuacapán	
	n (%)	*n* (%)	*p* (Fisher Test)
	Mean ng/mL (SD)	Mean ng/mL (SD)	*p* (Mann–Whitney)
Acetochlor	161 (83.85) 0.008 (0.0867)	44 (49.43) 0.001 (0.0017)	<0.01 0.04
Atrazine	22 (11.45) 0.016 (0.0486)	22 (24.71) 0.043 (0.0930)	<0.01 0.06
Carbendazim	29 (15.10) 0.141 (0.4192)	52 (41.57) 0.330 (0.5040)	<0.01 <0.01
Carbofuran	1 (0.52) 0.246	0	NA NA
Cyhalothrin	138 (71.87) 0.083 (0.0823)	45 (50.56) 0.080 (0.0855)	<0.01 0.52
Diazinon	29 (15.10) 0.007 (0.0199)	20 (22.47) 0.008 ± 0.0180	0.09 0.41
Dimethoate	179 (93.22) 0.146 (0.1834)	44 (49.43) 0.169 (0.2299)	<0.01 0.03
Emamectin	6 (3.12) 0.006 (0.0339)	9 (10.11) 0.019 (0.0582)	0.02 0.34
Enilconazole	177 (92.18) 1.582 (5.6623)	29 (32.58) 0.069 (0.1023)	<0.01 <0.01
Glyphosate	140 (72.91) 0.363 (0.3210)	89 (100) 0.6060 (0.5435)	<0.01 <0.01
Malathion	191 (99.47) 0.681 (0.6431)	55 (61.79) 0.177 (0.1330)	<0.01 <0.01
Methomyl	46 (23.95) 0.016 (0.0292)	0	<0.01 <0.01
Metoxuron	188 (97.91) 0.038 (0.0403)	50 (56.17) 0.037 (0.0414)	<0.01 0.10
Molinate	79 (41.14) 0.191 (0.3698)	55 (61.79) 0.273 (0.4240)	<0.01 <0.01
Pyraclostrobin	62 (32.29) 0.049 (0.2331)	37 (41.57) 0.042 (0.0509)	0.08 0.18
Thiabendazole	29 (15.10) 0.007 (0.0511)	24 (26.96) 0.002 (0.0046)	<0.01 0.12

Conclusion

In a comparative cross-sectional study in two agricultural communities with very similar characteristics in Mexico (a total of 281 children participated in the study), glyphosate was detected in more than 70% of the cases in both communities, having a higher prevalence rate along with malathion, metoxuron, compared to other pesticides analysed in the study. The mean urinary levels of glyphosate were 0.363 ± 0.3210 ng/mL in Agua Caliente and 0.606 ± 0.5435 ng/mL in Ahuacapán. In general, substantial differences in exposure and detection rate were also identified between the communities.

3. Assessment and conclusion

Assessment and conclusion by applicant:

In a comparative cross-sectional study using the urine of children living in two agricultural communities with very similar characteristics in Mexico (a total of 281 children participated in the study), glyphosate was detected in more than 70% of the cases in both communities. The mean urinary levels of glyphosate were 0.363 ± 0.3210 ng/mL in Agua Caliente and 0.606 ± 0.5435 ng/mL in Ahuacapán.
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions

because no validation data were presented for the analytical method employed.

Assessment and conclusion by RMS:

Reliability criteria of exposure studies

Publication: Sierra-Diaz *et al*., 2019.	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines/practices.	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y?	
Test substance		
Exposure to formulations with only glyphosate as a.s.		
Exposure to formulations with glyphosate combined with other a.s.		
Exposure to various formulations of pesticides	Y	
Study		
Study design clearly described	Y	Survey of glyphosate concentrations in children.
Population investigated sufficiently described	Y	
Exposure circumstances sufficiently described	?	No pesticide exposures reported.
Sampling scheme sufficiently documented	Y	Production of one early morning urine sample.
Analytical method described in detail	Y	
Validation of analytical method reported	N	
Monitoring results reported	Y	
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because no validation data were presented for the analytical method employed.		

1. Information on the study

Data point	CA 5.9/010
Report author	Steinborn, A. *et al.*
Report year	2016
Report title	Determination of Glyphosate Levels in Breast Milk Samples from Germany by LC-MS/MS and GC-MS/MS
Document No.	doi.org/10.1021/acs.jafc.5b05852 E-ISSN: 1520-5118
Guidelines followed in study	Guidance document on analytical quality control and validation procedures for pesticide residues in food and feed, SANCO/12571/2013
Deviations from current	None

test guideline	
GLP/Officially recognised testing facilities	No/Not stated
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary of the study according to OECD format

This study describes the validation and application of two independent analytical methods for the determination of glyphosate in breast milk. They are based on liquid chromatography − tandem mass spectrometry (LC-MS/MS) and gas chromatography − tandem mass spectrometry (GC-MS/MS), respectively. For LC-MS/MS, sample preparation involved an ultra filtration followed by chromatography on an anion exchange column. The analysis by GC-MS/MS involved an extraction step, clean-up on a cation exchange column, and derivatization with heptafluorobutanol and trifluoroacetic acid anhydride. Both methods were newly developed for breast milk and are able to quantify glyphosate residues at concentrations as low as 1 ng/mL. The methods were applied to quantify glyphosate levels in 114 breast milk samples, which had been collected from August to September of 2015 in Germany. The mothers participated at their own request and thus do not form a representative sample. In none of the investigated samples were glyphosate residues above the limit of detection found.

Materials and Methods

Chemicals – Glyphosate standard solution (10 µg/mL), reference glyphosate and internal standard ($^{13}C_2{}^{15}N$ labeled glyphosate) were purchased from LGC Standards, Wesel, Germany and from Dr. Ehrenstorfer, Augsburg, Germany.

Collection of breast milk samples - Breast milk samples were collected in August and September 2015 by the Governmental Institute of Public Health of Lower Saxony, Germany and by the Bavarian Authority for Health and Food Safety Germany for the analysis of glyphosate. All participants signed a declaration of consent concerning the use of their samples for scientific purposes. Participating mothers had not been selected by random sampling and thus do not form a representative sample. There were no restrictions relating to, for example, age and point of sampling during the lactation period for participating in the monitoring program. The milk samples for this study were collected and stored in polypropylene tubes which remained frozen during storage and shipment. In total, 114 milk samples were analysed and the participants completed a self-administered questionnaire. Information on sample collection, biometric data and self-reported pesticide exposure of the participants is given in Table 1.

Table 1: Biometric data of study participants

parameter	samples from Bavaria, Germany	samples from Lower Saxony, Germany
number of samples	17	97
age of mother (years)		
median	32.1	32.0
range	26–39	22–39
body weight of mother (kg)		
median	63.0	67.0
range	54–90	48–102
duration of lactating period (weeks)		
median	11.0	18
range	3–80	4–52
self-reported exposure to pesticides	6 participants	32 participants

The questionnaire also asked for the place of residence and the jobs practiced in the last 10 years. Thirty-eight participants declared the use of chemical insecticides, herbicides or wood preservatives. At least one participant has worked in a residue analytical laboratory and used pesticide standards regularly. Twenty of the 114 breast milk samples were divided each into two subsamples to allow the parallel analysis by LC-MS/MS and GC-MS/MS.

Fortification of breast milk samples for performance tests - A homogeneous sample of breast milk was prepared and spiked with different volumes of a glyphosate standard solution in water at 10 µg/mL. Twenty-eight stored breast milk samples from a previous study of the Governmental Institute of Public Health of Lower Saxony were pooled and 4 aliquots of 100 mL were spiked with glyphosate resulting in concentrations of 0.5, 1, 3, and 5 ng/mL. An additional aliquot of the pooled sample served as the control. All performance samples were divided into two subsamples and analysed in parallel with LC-MS/MS and GC-MS/MS. These samples served as independent quality control samples.

Sample Preparation for LC-MS/MS analysis - Removal of fat by centrifugation and removal of proteins by ultra-filtration in one step through centrifugal filtration using a molecular weight cutoff filter of 30 kDa was found to be suitable. To 3 mL of sample, 30 µL of internal standard solution containing 1000 ng $^{13}C_2{}^{15}N$ glyphosate/mL was added to obtain a concentration of 10 ng/mL. After mixing, the sample was transferred to the top part of the cutoff filter tube. The filter was centrifuged at 3500 rpm for 20 minutes and 500 µL of the filtrate was then transferred to the LC filter vial, the solution was filtered and the vial used for measurement. After this procedure, one mL of final extract contained the glyphosate residue of one mL breast milk.

LC-MS/MS analysis – The LC-MS/MS system used consisted of a Nexera UHPLC system from Shimadzu equipped with a 5500 Qtrap system (Sciex) in triple-quad mode. The LC system consisted of an anion exchange LC column (Dionex Ionpac AS 11 (2 × 250 mm) and a AG-11 guard column (2 × 50 mm) from Thermo Fischer Scientific). Twenty-five µL of standard solution or filtrate were injected into the LC system and glyphosate was eluted from the column using a gradient of water (A) and 1 mM citric acid solution brought to a pH of 11 by addition of a dimethyl amine solution (B). Gradient elution consisted of following steps: 100% A from 0 to 2 minutes, linear to 25% B in 5.5 minutes, and linear to 50% B in 2.5 minutes which was held for 4 minutes. After returning to 100% A in 0.5 minutes the system was re-equilibrated for 7.5 minutes before the next injection. The total run time (including injection) was 22.5 minutes with a flow rate of 0.4 mL/min. A column temperature of 40°C was maintained while the temperature of the samples in the autosampler was 12°C. All transitions were measured using a declustering potential of -75 V, an entrance potential of -10 V and a dwell time of 50 ms. The Turbospray source was used in negative

electrospray mode using the following parameters: Curtain gas 20 arbitrary units, collision gas medium, ion spray - 4000 V, temperature 400 °C, ion spray gas 1: 40 and ion spray gas 2: 50 arbitrary units. The mass transitions for evaluation [m/z] were 168.2 → 62.8 for quantification, 168.2 → 79.0 for confirmation and, 171.2 → 62.8 for $^{13}C_2$ ^{15}N glyphosate.

Sample Preparation for GC-MS/MS analysis - A 2 mL milk sample was extracted with 3.75 mL of 0.6% acetic acid and centrifuged for 5 minutes at 4000 rpm. Two mL of the supernatant liquid was extracted with 2 mL dichloromethane for 2 minutes and the two phases separated by centrifugation at 4000 rpm for 5 minutes. One mL of the supernatant liquid was filtered using a 0.45 µm Nylon filter and a cation exchange clean-up was performed using disposable Bio-Rad Poly-Prep columns filled with 1.72 g (equivalent to 2 mL filling volume) of AG 50W-X8 resin (H^+-form). A 0.55 mL aliquot of the filtered extract (corresponding to 0.2 mL breast milk) and 0.100 mL of internal standard solution (20 ng/mL) were added onto the cation exchange column and were eluted followed by 2.0 mL of CAX solution (800 mL HPLC grade water, 13.5 mL 10 N HCl solution and 200 mL methanol). Both eluates were discarded. Glyphosate residues were eluted from the column with 12.5 mL of CAX solution and evaporated to dryness. The residues were dissolved in 1.0 mL of CAX solution. Derivatization reagent (2,2,3,3,4,4,4-hepta fluoro-1-butanol and trifluoroacetic acid anhydride 1:1) was cooled to a temperature of - 20°C. 0.05 mL of the dissolved eluate (corresponding to 0.01 mL breast milk) was added to 1.5 mL of the chilled reagent and after 5 minutes derivatization is started by heating to 92-97°C for 1 hour. After cooling, the excess of derivatization reagent was removed by evaporation. The dry residue was dissolved in 0.2 mL of ethyl acetate containing 0.2 mL/L citral and then concentrated to a final volume of 20 µL. Citral was used to prevent adsorption of the analytes in the inlet and the GC column. Following this procedure, one mL of final extract contained the glyphosate residue of 0.5 mL breast milk.

GC-MS/MS analysis - The GC-MS/MS system consisted of a Thermo Trace GC Ultra equipped with a TriPlus liquid autosampler, split/splitless injector and MS detector TSQ Quantum with triple quadrupole (Thermo Fisher Scientific). The GC column was a Optima 5HT of 30 m length and 0.25 mm internal diameter coated with a 0.25 µm film (Macherey-Nagel). Four µL of the extracts were injected splitless with the injector temperature at 280 °C. The oven temperature was held at 80°C for 1.5 minutes, ramped up at 10 °C/minute to 180 °C, then ramped up at 30°C/minute to 300 °C and held at 300 °C for 2.8 minutes. The carrier gas was helium and the flow rate 1 mL/minute. The expected retention time for the glyphosate derivative was 9.1 minutes. The temperature of the ion source was 280 °C and the electron impact (EI) energy was 70 eV with an emission current of 50 µA. Mass transition for evaluation [m/z] was 612 → 213 for quantification, 611 → 261 for confirmation, and 615 → 213 for $^{13}C_2$ ^{15}N glyphosate. Calculations were performed using the ratio of the peak areas of the quantifier transition of glyphosate derivative and the internal standard derivative. Calibration solutions were prepared by volumetric dilution of a glyphosate stock solution in a solution containing 20 ng/mL internal standard. The dilutions were made in CAX solution. Aliquots of 0.05 mL of these calibration solutions were derivatized as described for the breast milk extracts. The concentration of the derivatized calibration solutions ranged from 0.01 to 10 ng/mL. The concentration of the internal standard in the final extract was always 5 ng/mL.

Results and Discussion

Since a very low transfer was observed of glyphosate into muscle, milk and fat in farm animal metabolism studies the LOQ should be as low as possible. The LC-MS/MS method was validated for glyphosate in accordance with the requirements of the EU guidance document for quality control and validation procedure. Recovery and precision of glyphosate were determined for 6 or 7 replicates at two fortification levels. The linearity of the system was tested by injecting 8 standards in water in a concentration range from 0 to 50 ng/mL. A linear relationship between concentration and the ratio of the peak area of glyphosate and its internal standard was observed with a coefficient of determination greater than 0.99. All calibration points were within 20% of the theoretical value. Quantification was performed using single-point calibration which is acceptable if the response of the analyte in the samples is close to the response in the standard. The average recovery of the LC-MS/MS method was 99% at 1 ng/mL and 91% at 5 ng/mL with a relative standard deviation of 16% and 7%, respectively. The LOQ of the LC-MS/MS method of 1 ng/mL

demonstrated sufficient recovery and precision. Possible matrix effects were corrected using the stable isotope labeled internal standard $^{13}C_2{}^{15}N$ glyphosate. At a concentration of 0.5 ng/mL, a signal-to-noise ratio of approximately 4 was obtained. This concentration is considered the LOD of the LC-MS/MS method.

For the analysis of glyphosate by GC-MS/MS, extraction with acidified water was combined with clean-up on a cation exchange column to remove interfering substances present in breast milk. To enable analysis by gas chromatography glyphosate was derivatized using heptafluoro-1-butanol and trifluoroacetic acid anhydride. Calibration was performed with freshly prepared derivatives of 8 glyphosate standard solutions in the concentration range from 0.01 to 10 ng/mL. All standard solutions contained the internal standard at 5 ng/mL and the coefficient of determination was always equal to or greater than 0.9980. The average recovery of the GC-MS/MS method was 84% at 1 ng/mL and 83% at 10 ng/mL with a relative standard deviation of 13% and 8%, respectively. All results obtained by GC-MS/MS had to be corrected for (derivatization reagent) blank interferences. A set of at least 4 reagent blanks were analysed within each set of breast milk samples. The average measured blank values ranged from 0.2 to 0.6 ng/mL. The relative standard deviations of blank values in the sample sets ranged from 19% to 33%. Considering the blank values from the derivatization reagent, the LOQ of the GC-MS/MS method was 1 ng/mL. Notwithstanding the interference problem of the GC-MS/MS method, both analytical methods were able to measure glyphosate residues in breast milk with an LOQ of 1 ng/mL.

In total, 114 different breast milk samples were analysed for glyphosate. Seventy-five samples were analysed by LC-MS/MS only. Because of the lower performance, only 19 samples were analysed exclusively by GC-MS/MS. Twenty milk samples were analysed by both methods. In addition to the 114 samples, 5 samples for performance testing were analysed by both LC-MS/MS and GC-MS/MS, 4 breast milk samples which were spiked in advance with glyphosate and one control sample. Glyphosate was identified by LC-MS/MS in all samples spiked with glyphosate. The recoveries for the LC-MS/MS method were 110%, 97% and 102% for the spiking levels of 1, 3, and 5 ng/mL, respectively. In the sample spiked at 0.5 ng/mL, glyphosate could still be detected by the LC-MS/MS method. Due to the interference problem in GC-MS/MS method, no clear detection of glyphosate was possible at this level.
The recoveries for the GC-MS/MS method were 70%, 70%, and 54% for the spiking levels of 1, 3, and 5 ng/mL, respectively. Generally, the GC-MS/MS method tended to result in lower concentrations, probably due to the correction for derivatization reagent blank values. The higher bias of the GC-MS/MS method might be due to dilution steps using very small volumes. The concentration step to yield the final volume might have resulted in a partial loss of the glyphosate derivative. Nevertheless, both methods are able to quantify glyphosate residues in breast milk at or above a concentration of 1 ng/mL. Because of the lack of significant blank values in the LC-MS/MS method, residues of glyphosate higher than 0.5 ng/mL are still detectable by this method. In none of the 114 breast milk samples obtained from German women glyphosate was detected.

Conclusion

A LC-MS/MS and a GC-MS/MS method were developed for the detection and quantification of glyphosate in human breast milk. Both methods have been fully validated and are suitable for the determination of glyphosate with an LOQ of 1 ng/mL. The LC-MS/MS method allows the detection of glyphosate at or above a level of 0.5 ng/mL. The LC-MS/MS method is much faster than the GC-MS/MS method, thus making it suitable for higher sample throughput. The positive findings of glyphosate in breast milk of American women could not be confirmed by the results of this study. In none of the 114 breast milk samples collected from German women in August and September 2015 glyphosate was found within the detection limitations of the analytical methods. Available data from farm animal studies on glyphosate with non-labeled material support these results. They provide no indication of a significant carry-over into fatty tissues or milk even at high dosing levels.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Two analytical methods were developed for the determination of glyphosate in human breast milk. In the first method fat was removed by centrifugation and the proteins by ultra-filtration using a molecular weight cutoff filter of 30 kDa. The final extract was then analysed by LC-MS/MS. In the second method the milk sample was acidified with acetic acid, centrifuged and the supernatant extracted with dichloromethane. The aqueous phase was filtered and cleaned-up using a cation exchange resin. The final extract was then analysed by GC-MS/MS after derivatization with heptafluoro-1-butanol and trifluoroacetic acid anhydride. $^{13}C_2{}^{15}N$ glyphosate was used as the internal standard in both methods. Both analytical methods were validated according to the EU guidance document on analytical quality control and validation procedures for pesticide residues in food and feed (SANCO/12571/2013) and were found suitable for the determination of glyphosate in human breast milk with an LOQ of 1 ng/mL. In August and September 2015, 114 breast milk samples were collected from German women and were analysed for glyphosate. In none of the samples analysed glyphosate concentrations were found at or beyond the LOQ.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the breast milk samples analysed were collected on a voluntary basis and there were no restrictions for participating in the monitoring program. As a consequence the samples cannot be considered representative of the German population. Both analytical methods developed were validated in accordance with the EU guidance on the procedures for the analysis of pesticide residues in food and feed.

Assessment and conclusion by RMS:

Reliability criteria of exposure studies

Publication: Steinborn et al., 2016.	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines/practices.	Y	Guidance document on analytical quality control and validation procedures for pesticide residues in food and feed, SANCO/12571/2013.
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Exposure to formulations with only glyphosate as a.s.		Self-reported exposure to pesticides.
Exposure to formulations with glyphosate combined with other a.s.		
Exposure to various formulations of pesticides		
Study		
Study design clearly described	Y?	Mainly the development of methods for the analysis of glyphosate in human breast milk and applied to a set of 114

Reliability criteria of exposure studies

Publication: Steinborn et al., 2016.	**Criteria met? Y/N/?**	**Comments**
		samples from German women.
Population investigated sufficiently described	Y?	Biometric data of the study population were rather limited. The samples were collected on a voluntary basis and without restrictions.
Exposure circumstances sufficiently described	Y?	No detail was provided on self-reported exposure to pesticides.
Sampling scheme sufficiently documented	Y?	More detail could have been provided.
Analytical method described in detail	Y	
Validation of analytical method reported	Y	
Monitoring results reported	Y	None of the 114 samples analysed was positive for glyphosate.
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Reliability not assignable		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the breast milk samples analysed were collected on a voluntary basis and there were no restrictions for participating in the monitoring program. As a consequence the samples cannot be considered representative of the German population. Both analytical methods developed were validated in accordance with the EU guidance on the procedures for the analysis of pesticide residues in food and feed.		

1. Information on the study

Data point:	CA 5.9/011
Report author	Trasande, L. *et al.*
Report year	2020
Report title	Glyphosate exposures and kidney injury biomarkers in infants and young children
Document No	doi.org/10.1016/j.envpol.2019.113334 E-ISSN: 1873-6424
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	None
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Yes/Reliable without restriction

2. Full summary of the study according to OECD format

The goal of this study was to assess biomarkers of exposure to glyphosate and assess potential associations with renal function in children. While previous studies have indicated that glyphosate may have nephrotoxic effects, few have examined potential effects on kidney function in children. In this study, three cohorts across different phases of child development and measured urinary levels of glyphosate. Associations of glyphosate with three biomarkers of kidney injury was evaluated: albuminuria (ACR), neutrophil gelatinase-associated lipocalin (NGAL), and kidney injury marker 1 (KIM-1). Multivariable regression analyses examined associations of glyphosate with kidney injury biomarkers controlling for covariates. Glyphosate was identified in 11.1% of the total participants. The herbicide was detected more frequently in the neonate population (30%). Multivariable regression models failed to identify significant associations of log-transformed glyphosate with any of the kidney injury biomarkers, controlling for covariates age, sex, and maternal education. While detectability of glyphosate in children's urine at various ages and stages of life was confirmed, there was no evidence for renal injury in children exposed to low levels of glyphosate.

Materials and methods

Study populations; We briefly describe a longitudinal birth cohort of children (Starting Early), in whom urine samples were measured at 10-19 months of age, as well as two cross-sectional studies Preventing Environmental Exposures in Pregnancy (PEEPS), and Bright Start which represent older children (ages 3-8) and newborns (<30 days), respectively. The three cohorts are comprised of chronologically ordered categories of age in children as follows: Bright Start (newborns), Starting Early, and PEEPS. Inclusion in the present study was determined if sufficient urine was available for the experimental testing. All research was performed in accordance with relevant guidelines/regulations. In particular, informed consent was obtained from participants' parents or their legal guardians for use of collected samples for use in future studies such as this one of glyphosate exposure.

Urine collection and storage; Urine samples for each cohort were obtained at study visits using age-appropriate methods (urine bags and cotton balls in untrained babies and infants, freshly voided urine in trained children) and immediately transferred to sterile polyethylene cups. Within 2 h after collection from the infants, urine was transferred to cryovials for storage at -80 °C prior to laboratory analysis. We measured urinary markers of kidney injury NGAL, and KIM1 using Luminex xMAP technology (see below). Urine albumin and creatinine were measured using standard methods of quantitative spectrophotometry at the ARUP National Reference Laboratory (Salt Lake City, Utah) and the results were used to calculate ACR (Albumin-to-Creatinine Ratio [mg:mg]).

Analysis of urine samples for glyphosate; 200 µL of urine sample was transferred into a 15 mL polypropylene (PP) tube and spiked with the labeled internal standard mixture (2-^{13}C, 99%; ^{15}N, 98+% Glyphosate), allowed to stand at room temperature for 30 min and then diluted to 1.0 mL (5-fold dilution) with 1% formic acid in water. The diluted sample was vortexed for 1 min, centrifuged and filtered through a nonsterile regenerated cellulose (RC) membrane filters (0.2 mm; Phenomenex, Inc., Torrance, CA, USA). The filtrate then transferred into an auto sampler vial for LC-MS/MS analysis. An Agilent 1260 Series HPLC system (Agilent Technologies Inc., Santa Clara, CA, USA) coupled with an ABSCIEX 4500 QTRAP mass spectrometer (Applied Biosystems, Foster City, CA, USA) running under negative mode electrospray ionization was used for the analysis. An anion-exchange column, Dionex IonPac AS 21 (2 mm x 250 mm, 7 µm) was employed for the separation of target chemicals under isocratic elution condition with a mobile phase consist of 1% formic acid in water and acetonitrile (95:5) mixture. Isotopic dilution mass spectrometry with MRM mode of analysis was used for selective quantification of the target chemicals. The QA/QC protocols included matrix spike (mean spike recoveries (n=5) for glyphosate was 109.5%) and procedural blanks (non-detected or < LOQ). Midpoint calibration standard and HPLC grade water were injected between every 20 samples analysed to check the instrument detection linearity and carry-over effects, respectively. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated as three and ten times of signal to noise ratio, respectively. LOD for glyphosate was 0.1 ng/mL, and LOQ was 0.33 ng/mL.

Analysis of kidney injury biomarkers; NGAL was measured in human urine using the Luminex performance human kidney Biomarker kit (FCSTM16-01, R&D Systems). Urine samples were thawed and diluted 1:10 in calibrator diluent and protocol followed manufacturer's instructions. Data were captured on a Luminex MAGPIX instrument with xPonent 4.2 software. Standard curve R^2 values were 0.98 and high- and low-quality controls were within expected range. KIM-1 was measured in human urine using the Milliplex MAP Human Kidney Injury Magnetic Bead Panel Kit (HKI1MAG-99K, EMD Millipore). Urine samples were thawed and diluted 1:2 in assay buffer and protocol followed manufacturer's instructions. Data were captured on a Luminex MAGPIX instrument with xPonent 4.2 software. Standard curve R2 values were 0.97 and high- and low-quality controls were within the expected range.

Statistical analysis; We first described the demographics of our study population (N = 108), including age, sex, maternal education, race/ethnicity, BMI category, and intervention arm in the case of the Starting Early group. Body mass index (BMI), calculated as weight in kilograms divided by height in meters squared, was used to measure adiposity in the Starting Early group. We used standardized BMI *z* scores given that BMI varies widely by age and sex, following the Centers for Disease Control and Prevention year 2000 norms. Overweight and obese were categorized as BMI *z*-score of 1.036 or greater (85th percentile for age and sex) and 1.64 or greater (95th percentile), respectively. We calculated median glyphosate, albuminuria, KIM-1, and NGAL levels, as well as interquartile ranges for all biomarkers and percent detected for glyphosate levels by study group. We also described mean and standard deviation in each one of the mentioned biomarkers of interest in the total population. Univariate regressions examined log-transformed glyphosate concentrations and their detectability (using logistic regression) as dependent variables with covariates examined singly. We created dummy variables for maternal education with one category for those children whose mothers did not complete a high school education and another for those whose mothers at least completed high school, and created a dummy variable for missing values (N = 34) that we used as reference. We also created an ordinal variable reflecting the age of the children in increasing order from youngest to oldest type as follows: Healthy Start, Starting Early, and PEEPS (N ¼ 108). We also performed univariate analyses by study arm in the Starting Early sample (N = 66). Multivariable analyses of continuous log-transformed glyphosate exposure examined age, sex, and maternal education as predictors (N ¼ 108). We assessed differences in detectability of glyphosate using multivariable logistic regressions with age, sex, and maternal education as predictors. All regressions examining predictors of log-transformed glyphosate were retransformed from the logarithmic scale. We then examined associations of glyphosate with kidney injury. First, we calculated Pearson correlation coefficients for all log-transformed kidney injury markers KIM-1, NGAL, and ACR, as well as log-transformed glyphosate levels. For glyphosate levels below the limit of detection (LOD), we imputed values equal to LOD/square root of 2. We also performed univariate and multivariable regressions of urinary log-transformed glyphosate as predictor of log-transformed kidney injury markers KIM-1, NGAL, and ACR, controlling for age, sex and maternal education. Sensitivity analyses were also included; we performed univariate and multivariable regressions of creatinine-adjusted urinary log-transformed glyphosate as predictor of log-transformed kidney injury markers KIM-1, NGAL, and ACR. Statistical analysis was performed with Stata/IC Version 14 (StataCorp: College Station, TX).

Results

The population was largely Hispanic, and comprised roughly equal numbers of males and females (Table 1). Only 4% of our participants had a mother with a college degree but the majority of parents in our study population graduated from high school (62.1%). The mean urinary glyphosate concentration was 0.278 ± 0.228 µg/mL, with a range of 0.105-2.125 µg/mL. We identified glyphosate in 11.1% of the participants. The herbicide was detected in 7.6-30% of the three cohorts: 30% of neonates had glyphosate exposure above the LOD, followed by PEEPS cohort (12.5%) and Starting Early (7.6%). We also observed a wide detection range in NGAL biomarkers. We also noted that 40% of children in the Starting Early cohort were overweight or obese. Younger children had higher urinary levels of glyphosate in both univariate and multivariable regressions. Children with mothers without a high school diploma had slightly lower levels of urinary glyphosate relative to those with mothers who attained a high school education in our multivariable regression. However, we found no other significant associations of demographic covariates

with glyphosate in univariate or multivariable models. Similarly, we found no statistically significant associations of demographic factors in relationship to detectability of urinary glyphosate in children. No univariate regressions with urinary glyphosate identified a statistically significant association with increased renal biomarker injury (Fig. 1A-C respectively and Table 2). Multivariable regressions with urinary glyphosate failed to identify statistically significant associations of log-transformed glyphosate with KIM-1, NGAL, or ACR (Table 2). In sensitivity analyses of univariate and multivariable regressions with creatinine-adjusted log-transformed glyphosate, there was also no statistically significant evidence for increased levels of markers for renal injury (Table 2). In additional sensitivity analyses without the newborns similar results were reported with no statistically significant associations of log-transformed glyphosate with kidney injury biomarkers (Table 2).

Table 1: Study population (n=108)[a]

Demographics			N	%
Male (Sex)			56	51.9
Study	Bright Start (Neonates)		10	9.3
	Starting Early		66	61.1
		Intervention Arm	39	59.1
		Control Arm	27	40.9
	PEEPS		32	29.6
Maternal Education	None		7	9.5
	Elementary/Middle		21	28.4
	High School/General Equivalency Diploma		35	47.3
	Some college		7	9.5
	College grad		3	4.1
	Other		1	1.4
Race/Ethnicity	Hispanic		67	90.5
	Non-Hispanic White		5	6.8
	Non-Hispanic Black		1	1.4
	Non-Hispanic Asian		1	1.4
BMI Categories[b]	Underweight		2	3.0
	Normal		40	60.6
	Overweight		12	18.2
	Obese		12	18.2

Distribution of Glyphosate and Kidney Injury Biomarkers	Mean (SD)	Detection Range (Min. & Max. Concentrations)
Glyphosate (ng/mL)	0.278 (0.228)	0.105–2.125
KIM-1 (ng/mL)	80.17 (76.92)	12.48–560.6
NGAL (pg/mL)	12,486 (21,934)	430.7–131,196
ACR[c] (mg/g)	26.23 (25.43)	1.414–119.0

Distribution of Glyphosate and Kidney Injury Biomarkers by Study Groups (N = 108)			
Study	Healthy Start (Neonates)	Starting Early	PEEPS
Glyphosate (ng/mL), Median (IQR)	<LOD (<LOD-1.06)	<LOD (<LOD-LOD)	<LOD (<LOD-LOD)
Percent Detected	30.0%	7.58%	12.5%
KIM-1 (ng/mL), Median (IQR)	88.9 (71.0–114)	47.1 (33.8–71.7)	76.5 (40.3–140.2)
NGAL (pg/mL), Median (IQR)	3770 (2520–7280)	9080 (3880–18,700)	1920 (1240–5770)
ACR[c] (mg/g), Median (IQR)	9.45 (6.00–343)	28.0 (19.1–42.4)	5.66 (3.54–9.00)

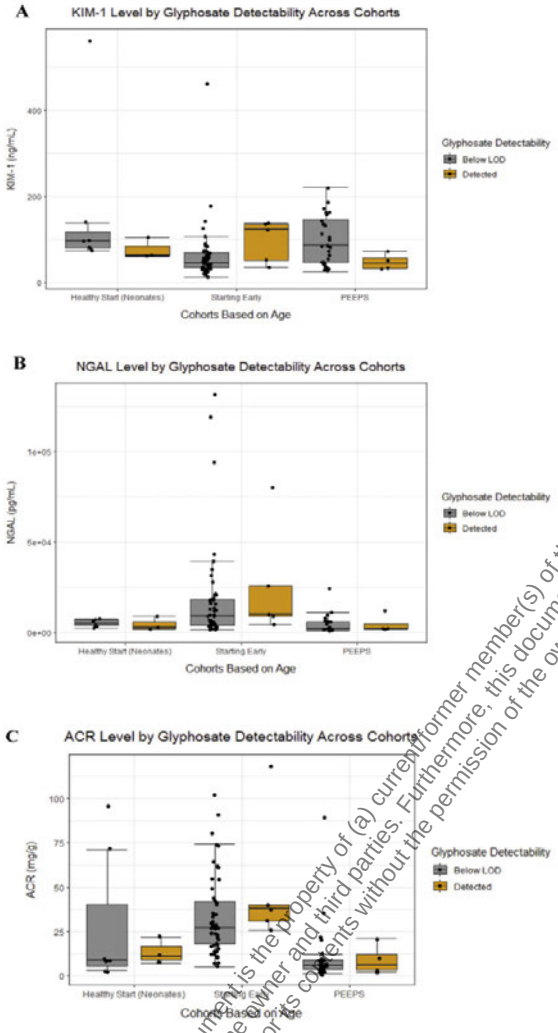
[a] Maternal Education 34 missing, Race/Ethnicity 34 missing, BMI 42 missing, KIM-1 9 missing, NGAL 13 missing, and ACR 12 missing.
[b] Underweight (Below −1.036 SD); Normal (Between −1.036 and + 1.036 SD); Overweight (Between + 1.036 and + 1.64 SD); Obese (Above +1.64 SD).
[c] Albumin-to-creatinine ratio.

Table 2: Multivariable regressions of creatinine-adjusted urinary log-transformed glyphosate as predictor of log-transformed KIM-1, NGAL, ACR biomarkers[a]

All ages N = 108 [a]			
Increment per one log unit increase	KIM-1	NGAL	ACR
Glyphosate Univariate	0.974 (−17.92, 27.78)	−663.8 (−2462, 2641)	−0.287 (−6.773, 10.91)
Glyphosate Multivariable	−1.55 (−14.93, 17.62)	−887.8 (−1800, 662.2)	−5.05 (−14.14, 9.85)
Creatinine-adjusted models[b]			
Glyphosate Univariate	−1.648 (−15.22, 17.47)	−640.5 (−3242, 4079)	7.603 (−9.522, 32.88)
Glyphosate Multivariable	−3.70 (−13.47, 10.26)	−819.8 (−1688, 657.8)	0.858 (−13.80, 22.43)
Non-neonates N = 98[c]			
Increment per one log unit increase	KIM-1	NGAL	ACR
Glyphosate Univariate	−5.351 (−24.13, 27.39)	1489 (−4383, 17944)	3.002 (−10.66, 35.99)
Glyphosate Multivariable	−3.568 (−14.90, 15.96)	395.3 (−5128, 12782)	12.05 (−37.98, 106.6)
Creatinine-adjusted models[d]			
Glyphosate Univariate	−4.89 (−20.66, 22.43)	2083 (−5679, 23234)	8.24 (−16.60, 54.17)
Glyphosate Multivariable	−3.73 (−14.45, 14.88)	132.8 (−5165, 11668)	10.37 (−25.33, 72.33)

[a] KIM-1 N = 99; NGAL N = 95; ACR N = 96. Multivariable regression models controlling for demographic covariates age, sex, and maternal education, where maternal education missing observations were imputed as the majority category "Equal or Greater Than High School".
[b] Creatinine-adjusted regression models KIM-1 N = 95; NGAL N = 92; ACR N = 96.
[c] KIM-1 N = 89; NGAL N = 86; ACR N = 86. Multivariable regression models controlling for demographic covariates age, sex, and maternal education, where maternal education missing observations were imputed as the majority category "Equal or Greater Than High School".
[d] Creatinine-adjusted regression models KIM-1 N = 85; NGAL N = 83; ACR N = 86.

Figure 1: A. The dot plot illustrates the urinary KIM-1 level in participants enrolled in the different studies, 1B. The dot plot illustrates the urinary NGAL level in participants enrolled in the different studies, 1C. The dot plot illustrates the urinary albumin:creatine ratio (ACR) in participants enrolled in the different studies.



Discussion

The main findings of this study are the substantial and frequent prevalence of glyphosate in children across the first decade of life in three independently assembled samples. We identified glyphosate in 11.1% of the total participants, and the herbicide was detected more frequently in the neonate population (30%). However, urinary concentrations detected in the neonates, toddlers, and young school age children was significantly lower than levels documented in adults with occupational or environmental exposure. No association of urinary glyphosate was found with any of the three renal biomarkers (KIM-1, NGAL or ACR). Urinary excretion of albumin and serum creatinine concentration are conventional biomarkers for renal dysfunction. Novel biomarkers such as urinary excretion of KIM-1 and NGAL have been developed and it has been that they have diagnostic utility for earlier detection of acute kidney injury (AKI) prior to significant loss of function. KIM-1 also predicted renal damage from acute exposures in animal studies. This study has a number of limitations.

We used maternal education as a proxy for family educational attainment or socioeconomic status (SES) which are well-known factors for different levels of chemical exposures in children or renal injury outcome. We emphasize that low sample size also limited us to perform analyses in the three cohorts in conjunction, in lieu of stratified analysis. We acknowledge that the strength of any conclusion about the frequency of detecting glyphosate in newborns is limited by the sample size. The source of the glyphosate in the newborns, which was detected more frequently than in the other groups but not at high levels, could be via lactation or infant formula. It is possible that immature or different metabolic pathways for glyphosate in neonates, kidney injury during delivery, or lactation may have affected the findings in this age group. However, the addition of neonates in our study evaluating the association of glyphosate and kidney injury biomarkers may have biased findings towards the null. Our results also suggest that glyphosate levels declined with increasing age. The differences by age may represent differences in dietary behavior and access to foods free of contamination or improved elimination of the herbicide, though further research is needed. Previous larger scale case-control studies have shown in the past changes in renal function, kidney injury, or chronic kidney disease of unknown etiology (CKDu) upon glyphosate exposure, especially of adult occupational exposures. Several strengths in this study are notable. Our study is the first to document the prevalence of glyphosate exposures in young children through age 8 years. Our frequent detection of glyphosate suggests the need for inclusion and biomonitoring of glyphosate exposures in national surveys or screenings in populations of interest, which could provide more information in regards to these exposures and outcomes. Glyphosate biomonitoring could be added into studies such as the National Health and Nutrition Examination Survey (NHANES). Our study is novel in that we examine specific renal biomarkers in relationship to glyphosate in a young population. The lack of evident renal toxicity in association with glyphosate exposure in young children does not exclude a potential adverse impact of longer term exposure to the pesticide. Problems during labor and delivery can compromise renal perfusion leading to increased excretion of tubular injury biomarkers in the neonatal period. This could interfere with the ability to detect an independent effect of glyphosate in this age group. In addition, the regenerative capacity of the renal tubular epithelium in infants and young children may mask an adverse effect of the pesticide on tubule integrity. Urinary biomarkers such as NGAL and KIM-1 have been demonstrated to be useful biomarkers of tubular injury even without reduced kidney function, highlighting their utility in detecting subclinical insults to the kidney. Further research could explore more in detail the association between glyphosate and kidney injury biomarker types across different age groups in larger cohorts, and assess whether more prolonged exposure could have progressive deleterious effects.

Conclusion

In this study, we identify frequent prevalence of levels of glyphosate (11.1%), present across all three age ranges, and particularly present in the youngest population of neonates. There is no evidence for renal injury in young children exposed to low levels of glyphosate. Further studies of larger sample size are indicated to better understand the potential deleterious effects of the herbicide after different levels, routes, and duration of exposure.

3. Assessment and conclusion

Assessment and conclusion by applicant:

This study evaluated three cohorts across different phases of child development and measured urinary levels of glyphosate. They evaluated associations of glyphosate with three biomarkers of kidney injury: ACR, NGAL, and KIM-1. Sample collection and analysis as well as statistical evaluation of data have been conducted using well described methodologies. Multivariable regression models failed to identify significant associations of log-transformed glyphosate with any of the kidney injury biomarkers, controlling for covariates age, sex, and maternal education. The authors confirm detectability of glyphosate in children's urine at various ages and stages of life, there is no evidence in this study for renal injury in children exposed to low levels of glyphosate.

This publication is considered relevant for the risk assessment of glyphosate and reliable without restrictions

because it complies with the quality criteria of a good monitoring study.

Assessment and conclusion by RMS:

Reliability criteria of exposure studies

Publication: Trasande et al., 2020.	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines/practices.	Y	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Exposure to formulations with only glyphosate as a.s.	Y	
Exposure to formulations with glyphosate combined with other a.s.	Y	
Exposure to various formulations of pesticides	Y	Environmental exposure.
Study		
Study design clearly described	Y	Longitudinal birth cohort and 2 cross sectional studies. Study of the association between renal biomarkers and glyphosate in urine.
Population investigated sufficiently described	Y	
Exposure circumstances sufficiently described	Y	Environmental exposure.
Sampling scheme sufficiently documented	Y	
Analytical method described in detail	Y	
Validation of analytical method reported	Y	
Monitoring results reported	Y	
Statistical analysis	Y	
Overall assessment		
Reliable without restrictions	Y	
Reliable with restrictions		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate and reliable without restrictions because it complies with the quality criteria of a good monitoring study.		

Appendix 1: Overview on case reports. No review information or epidemiologic information is listed in this table. The complete references on any kind of medical data are available in the literature search report, CA 9-01.

Reference	Evaluation	Abstract
Bando, H., 2010, The Japanese journal of toxicology, (2010 Sep) Vol. 23, No. 3, pp. 246-9	This case report describes severe hyperkalemia in the setting of suicidal ingestion of potassium salt glyhoate formulations. This is not unexpected and since it describes a suicidal ingestion it should not impact re-registration	CASE REPORT: A 65-year-old female was transferred to our emergency and critical care center after taking two kinds of commercially available glyphosate herbicide products. On admission, her conscious level was depressed to Glasgow Coma Scale E3, V2, and M6. Vital signs were as follows ; blood pressure 83/33mmHg, pulse 59/min, and respiratory rate was 24/min. Arterial blood gas analysis showed metabolic acidosis and an extreme hyperkalemia of 9.22 mEq/L. Electrocardiogram showed absence of P wave and a tall, tapering T wave. On admission, gastric lavage was followed by an intragastric administration of activated charcoal together with cathartic. Immediately after recognition of hyperkalemia, sodium bicarbonate, glucose plus insulin, and calcium gluconate were also administered intravenously. Five hours later, plasma concentration of potassium decreased to 4.31 mEq/L, and the patient discharged on day 10. Later, it was disclosed that the new Roundup Maxload contains high concentration of glyphosate potassium. CONCLUSION: In case of Roundup poisoning, we have to take it consideration that the poisoning may results in a hyperkalemia.
Beswick, E. et al. (2011) Journal of the Intensive Care Society, (January 2011) Vol. 12, No. 1, pp. 37-39	This is a case report of a fatality after a formulated glyphosate suicidal overdose. Multiorgan failure is not uncommon in these overdoses. As this is a case report of a suicidal ingestion it should not impact re-registration decisions.	A 29-year-old man was admitted following deliberate ingestion of approximately 300 mL of 'Roundup Ace,' a herbicide containing glyphosate. On presentation, he was agitated and required intubation and admission to ICU. He developed severe and persistent lactic acidosis, hyperkalaemia, hypotension, torrential watery diarrhoea and abdominal distension in the first 24 hours. The patient was supported with a continuous noradrenaline infusion and continuous veno-venous haemodiafiltration. The clinical course was further complicated by cardiac arrhythmias and an episode of cardiac arrest. By day three, he had bone-marrow, liver and worsening respiratory failure. There was no hope of recovery; therefore, noradrenaline was discontinued, and the patient died 15 minutes later. Glyphosate-surfactant herbicide (GlySH) is a general-purpose herbicide with no anticholinesterase effect and no organophosphate-like CNS effects. GlySH intoxication has a case fatality rate of between 3.2% and 29.3%. There is no antidote and the mainstay of treatment for systemic toxicity is decontamination and aggressive supportive therapy. Early renal replacement therapy may improve prognosis but there is no evidence to support this. There is one published case of a patient who survived severe GlySH poisoning following administration of intravenous fat emulsion. Glyphosate-surfactant herbicide, commercially known as 'Roundup' is a widely available herbicide commonly used in both professional and domestic settings. This is a case of a young man who deliberately ingested GlySH at home and rapidly developed multi-organ failure, culminating in his death.© The Intensive Care Society 2011.
Bosak A. B. et al., 2014 Journal of Medical Toxicology (2014), Vol. 10, No. 1, pp. 72	This is a report about multi-organ failure after suicidal ingestion of formulated glyphosate and should not impact re-registration.	Background: Glyphosate is a commonly used herbicide associated with toxicity and death following large ingestions. Surfactants are implicated as the primary contributor. Hypothesis: Glyphosate herbicide preparations contain various surfactants and salts which may lead to diverse clinical toxicity. Methods: (Case 1) A 50-year-old man presented with N/V, abdominal pain, and somnolence 12 h after ingesting 6.5 oz of concentrated Roundup ® containing 50.2 %

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Reference	Evaluation	Abstract
		glyphosate as isopropylamine salt. Labs revealed metabolic acidosis (AG 40), lactate (9.2 mmol/L), creatinine (2.8 mg/dL), potassium (4.5 mmol/L), WBC (41.5 K/mm3), and lipase (2,528 IU/L). Electrocardiogram QRS was 142 ms and responsive to bicarbonate. Patient became hypoxic and hypotensive requiring intubation, vasopres-sors, and continuous veno-venous hemodialysis (CVVHD) for anuric renal failure. A CT scan showed multiple loops of dilated small bowel and possible pneumatosis with negative exploratory surgery on day 1. On day 5, he had peritonitis prompting the resection of the ischemic terminal ileum and cecum. Over the next 3 weeks, he developed recurrent GI bleeds unrelated to surgery, remained anuric on CVVHD, and ventilator dependent. (Case 2) A 48-year-old landscaper ingested 250- 350 mL of concentrated glyphosate herbicide and presented with AMS and N/V. Labs showed WBC (21.2 K/mm3), potassium (7.1 mmol/L), metabolic acidosis (AG 10), and creatinine of 1.2 mg/dL (0.60 baseline). Electrocardiogram QRS was 192 ms with wide complex rhythm, bradycardia, and responsive to bicarbonate. Persistent hyperkalemia continued despite medical treatment requiring high flux hemodialysis. He required ventilator support for hypoxia and had a negative EGD. Results: Case 1 developed anuric renal failure, QRS prolongation, ischemic bowel, and recurrent GI bleeds. Care was withdrawn on day 25. Case 2 had persistent hyperkalemia, non-anuric renal failure, QRS prolongation, and bradycardia. Discussion: We hypothesize that case 2 ingested a potassium salt preparation due to the persistent hyperkalemia that only resolved with hemodialysis. QRS prolongation was present in both cases responsive to bicarbonate. Conclusion: Glyphosate containing herbicides may have diverse clinical toxicity depending on surfactant and salt preparation.
Brunetti R. et al., 2019 HeartRhythm Case Rep. (2020), Vol. 6, pp. 63	This article claims that dermal exposure to a small amount of glyphosate led to cardiac arrhythmia and claims that the patient developed a Brugada syndrome & long Qt syndrome after exposure. The measured QTC in a wide-complex tracing is uninterpretable. Brugada syndrome is largely due to sodium channel block in cardiac myocytes, LQT syndrome is largely due to potassium channel block in the cardiac myoctyes. Glyphosate	Not available

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Reference	Evaluation	Abstract
	does neither. Moreover, glyphosate is not dermally absorbed and multiple GLP studies have shown that glyphosate is not cardiotoxic.	
Chan C-W. et al., 2016 Critical care medicine (2016), Vol. 44, No. 1, pp. E45	This paper looked at the use of ECMO in a critically ill patient after formulated glyphosate product overdose. ECMO is sometime of utility in treating overdose patients. This paper should not impact re-registration.	OBJECTIVE: To describe the experience of emergency extracorporeal membrane oxygenation in treating life-threatening glyphosate-surfactant intoxication. DESIGN: Case report. SETTING: Emergency department and ICU. PATIENT: A patient with cardiopulmonary failure after glyphosate-surfactant intoxication. INTERVENTION: Extracorporeal membrane oxygenation. CASE REPORT: A 47-year-old man presented with mildly decreased consciousness in our emergency department after ingesting approximately 100 mL of glyphosate-surfactant 1.5 hours previously. Respiratory failure, persistent ventricular tachycardia, profound shock refractory to inotropic agents, and metabolic acidosis developed in the patient within 2 hours. Extracorporeal membrane oxygenation was applied within 4 hours of cardiopulmonary failure. The patient's condition improved considerably. He was transferred to the general ward on the eighth day with stable hemodynamic status and complete neurological recovery. CONCLUSIONS: On the basis of our research, this was the first case in which extracorporeal membrane oxygenation was used to treat severe glyphosate-surfactant intoxication. We recommend early initiation of extracorporeal membrane oxygenation therapy to mitigate cardiopulmonary compromise in patients with glyphosate-surfactant intoxication.
Cho, Y. et al. (2019) AMERICAN JOURNAL OF EMERGENCY MEDICINE, (AUG 2019) Vol. 37, No. 8.	This case report describes the sucessful treatment of hyperkalemia and monitoring of blood glyphosate concentrations after a formulated glyphosate overdose. It is expected that the glyphosate level would be high on admission and then decrease rapidly. This is a case report of a suicidal ingestion and should not impact re-registration.	Introduction: This report describes changes in blood and urine concentrations of glyphosate potassium over Lime and their correlations with clinical symptoms in a patient with acute glyphosate potassium poisoning. Case report: A 67-year-old man visited the emergency center after ingesting 250 mL of a glyphosate potassium based herbicide 5 h before. He was alert but presented with nausea, vomiting, and bradyarrhythmia with atrial fibrillation (tall T waves). Laboratory findings revealed a serum potassium level of 6.52 mEg/L. After treatment with an injection of calcium gluconate, insulin with glucose, bicarbonate, and an enema with polystyrene sulfonate, the patient's serum potassium level normalized and the bradyarrhythmia converted to a normal sinus rhythm. During admission, the blood and urine concentration of glyphosate and urine aminomethylphosphonic acid (AMPA, a glyphosate metabolite) was measured at regular time intervals. The patient's glyphosate blood concentration on admission was 11.43 mg/L, and it had decreased rapidly by 16 h and maintained about 1 mgl/L by 70 h after admission. Urine glyphosate and AMPA levels had also decreased rapidly by 6 h after admission. Discussion: Glyphosate potassium poisoning causes hyperkalemia. Blood concentrations of glyphosate were decreased rapidly by 16 h after

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Reference	Evaluation	Abstract
		admission, and urine concentrations were also decreased by 6 h after admission. (C) 2019 Elsevier Inc. All rights reserved.
Choi B. et al., 2013 Toxicology Letters (2013), Vol. 221, Supp. 1, pp. S66	This is a report about measuring IMA rather than lactate as a marker of shock after suicidal ingestion of formulated glyphosate and should not impact re-registration.	Introduction: To date, plasma lactate level has been thought that the most important monitoring tool to measure systemic tissue hypoxia. However, the time lag and relatively low value in some cases are frequently encountered. Case: Sixty-seven year old female was admitted emergency room after 80 min from acute pesticide poisoning. She drank 500mL of glyphosate with intension of suicide. She was alert but her vital signs were unstable at admission; BP 84/37mm Hg, PR 97/min, SpO2 98%. After the initial resuscitation management including general decontamination treatment, she moved to the Acute Care Unit and continuous resuscitation due to her unstable hemodynamic status. During the hospital course, we daily measured the ischemic modified albumin (IMA) level using albumin-cobalt binding assay that its value could tell us the tissue hypoxia. Among the monitoring values, the trend of IMA and base deficit would be correlated with the clinical progress (Fig. 1). Conclusion: We experienced that IMA has a more sensitive monitoring value than lactate in critically ill patient in our acute pesticide poisoning patient. IMA could be measured in venous blood and may be an alternative monitoring laboratory value as base deficit. Also, further study is warranted.
De Raadt W. M. et al., 2015 Sarcoidosis, vasculitis, and diffuse lung diseases : official journal of WASOG (2015), Vol. 32, No. 2, pp. 172	This article is a case report of a smoker who developed eosinophilic pneumonia after glyphosate exposure. Glyphosate is not a sensitizer as established by multiple GLP regulatory studies. Nozzle application of formulated glyphosate producess aerosols of between 200-350 microns. In humans, it takes droplets of <100 microns to cause inhalational injury. The claim that formulated glyphosate can cause inhalational injury in a setting where it isn't aspirated is not biologically plausible.	We report a case of a female patient who developed acute eosinophilic pneumonia (AEP) after recent onset of smoking and exposure to glyphosate-surfactant.The additional exposure associated with the recent start of smoking may have contributed to the development and/or severity of AEP.A clinical relapse after re-challenge four years later both with smoking and glyphosate-surfactant made the association highly likely.Respiratory distress is a factor of poor outcome and mortality after ingestion of glyphosate-surfactant.This case highlights the importance of a thorough exposure history e.g., possible occupational and environmental exposures together with drug-intake.Genotyping should be considered in cases of severe unexplained pulmonary damage.

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Reference	Evaluation	Abstract
Deo S. P. et al., 2012 Journal of the Nepal Medical Association (2012), Vol. 52, No. 185, pp. 40	Large ingestions of formulated glyphosate can often result in caustic injury secondary to the surfactant's detergent actions on the mucous membranes of in people who ingest them. That said, they shouldn't cause microstomia, which tends to result from much more corrosive and scarring chemicals. This should not impact re-registration.	Glyphosate (GlySH) is a broad spectrum, nonselective herbicide, widely used in agriculture. This case report describes a 25-year-old man presenting with extensive chemical burns and ulceration of the oral cavity as a result of accidental exposure to GlySH. This paper aims to illustrate the typical appearance of GlySH related chemical mucosal burn and to demonstrate the severity of the corrosive effect of GlySH which need team approach to prevent unfavorable sequelae such as microstomia.
Elsner, P. (2018) Journal der Deutschen Dermatologischen Gesellschaft = Journal of the German Society of Dermatology : JDDG, (2018 Jan) Vol. 16, No. 1, pp. 70-71	This paper describes a patient who spilled formulated glyphosate on her arms then washed it off. A week later she developed psoriasis which spread from her arms to her body. She filed a medicolegal claim which was then reported to this journal as a correspondence. The MD wrote that he reviewed the 1986 Maibach paper which evaluated >300 volunteers and showed no evidence of irritation or sensitization and countered this study with 2 case reports alleging sensitizatioin, that the psoriasis on the patient's hands and arms was related to glyphosate exposure, whereas the rest of the psoriasis on her body was not related to the exposure. There is	Not available

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Reference	Evaluation	Abstract
	not a mechanism for glyphosate to cause psoriasis, especially 1 week post exposure.	
Frappart, M. (2011) Vol. 30, No. 11, pp. 852-4.	This case report describes caustic injury to the GI tract and multiorgan failure after formulated glyphosate overdose. The clinical course is consistent with previous reports of overdose and should not impact re-registration.	Not available
Garlich F. M. et al., 2014 Clinical toxicology (2014), Vol. 52, No. 1, pp. 66	This article discusses the sucessful use of haemodialysis in a patient who was critically ill after a formulated glyphosate overdose.	CONTEXT: Ingestion of glyphosate-surfactant herbicides (GlySH) can result in acute kidney injury, electrolyte abnormalities, acidosis, cardiovascular collapse, and death. In severe toxicity, the use of hemodialysis is reported, but largely unsupported by kinetic analysis. We report the dialysis clearance of glyphosate following a suicidal ingestion of a glyphosate-containing herbicide. CASE DETAILS: A 62-year-old man was brought to the emergency department (ED) 8.5 h after drinking a bottle of commercial herbicide containing a 41% solution of glyphosate isopropylamine, in polyoxyethyleneamine (POEA) surfactant and water. He was bradycardic and obtunded with respiratory depression necessitating intubation and mechanical ventilation. Initial laboratory results were significant for the following: pH, 7.11; PCO2, 64 mmHg; PO2, 48 mmHg; potassium, 7.8 mEq/L; Cr 3.3, mg/dL; bicarbonate, 22 mEq/L; anion gap, 18 mEq/L; and lactate, 7.5 mmol/L. Acidosis and hyperkalemia persisted despite ventilation and fluid resuscitation. The patient underwent hemodialysis 16 h post ingestion, after which he demonstrated resolution of acidosis and hyperkalemia, and improvement in clinical status. Serum glyphosate concentrations were drawn prior to, during, and after hemodialysis. The extraction ratio and hemodialysis clearance were calculated to be 91.8% and 97.5 mL/min, respectively. DISCUSSION: We demonstrate the successful clearance of glyphosate using hemodialysis, with corresponding clinical improvement in a patient with several poor prognostic factors (advanced age, large volume ingested, and impaired consciousness). The effects of hemodialysis on the surfactant compound are unknown. Hemodialysis can be considered when severe acidosis and acute kidney injury complicate ingestion of glyphosate-containing products.
Han Sang Kyoon et al. (2010) Vol. 48, No. 6, pp. 566-8.	This is a case report of a suicidal ingestion of formulated glyphosate that was treated with lipid emulsion and	CONTEXT: Circulatory shock is a major cause of mortality in glyphosate-surfactant herbicide (GlySH) poisoning, and this condition responds poorly to conventional therapies. We report a case of GlySH poisoning with shock that was refractory to vasopressors but responsive to intravenous fat emulsion (IFE). CASE DETAILS: A 52-year-old man was brought to the emergency

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Reference	Evaluation	Abstract
	improved. Since this is a descriptionof medical management of a suicidal overdose, this should no impact re-registration	department by ambulance. He was found unconscious in his living room along with an empty bottle of GlySH herbicide, which contained glyphosate, polyoxyethyleneamine (POEA) surfactant, and water. He was drowsy at presentation. His heart rate was 44 beats/min, his blood pressure could not be measured with an arm cuff, but he had a palpable femoral pulse. After about 2.5 h of supportive care after admission, he remained hypotensive, and his systolic blood pressure was 80 mmHg. A 500 mL bottle of 20% IFE product was prepared. As a bolus, 100 mL of IFE was injected, and the remaining 400 mL was then infused. His blood pressure was 100/60 mmHg 1 h after the bolus injection. At 5 h after IFE injection, his blood pressure reached 160/100 mmHg and vasopressors were tapered. CONCLUSION: IFE should be considered in cases of refractory hemodynamic instability caused by GlySH after aggressive fluid and vasopressor support.
Hansen N. B. et al., 2013 Clinical Toxicology (2013), Vol. 51, No. 4, pp. 354	This is a case report of an accidental ingestion of formulated glyphosate resulting in mild corrosive injury to the GI tract in a small child and should not impact re-registration.	Objective: Glyphosate is a broad-spectrum systemic herbicide. Severe toxicity is rare. Case report: A 2.5 year-old boy ingested unknown amounts of concentrated glyphosate (Round-Up), decanted into a soda bottle. Immediately after ingestion the boy was crying, experienced excessive salivation, vomited several times, spontaneous and provoked. Fifteen minutes after ingestion the National Poison Center was contacted, and advised immediate emergency ward contact. The Poison Center alerted the hospital staff about the patient and treatment plan including activated charcoal and examination of oral and gastrointestinal mucosa. On emergency ward arrival the child was awake, crying, reacted adequately, was warm and dry, respiratory frequency (RF) 19/min, oxygen saturation 100%, heart rate 164 beats/min and blood pressure 104/71 mmHg. Activated charcoal 1 g/kg (body weight 14.5 kg) was dosed 1 hour after ingestion. Corrosive injury was suspected because of hypersalivation and red marks in the mouth and throat. Gastroscopy 3 hours after ingestion showed Grade 1b ulceration in the esophagus, pylorus and gastric mucosa. Antibiotic treatment (cefuroxime and metronidazole), with antiemetic (dexamethasone), and analgesics (diclofenac and paracetamol), was initiated. A gastric tube was placed for observation. Hemorrhage was not observed. Coagulation parameter deterioration with elevated international normalized ratio (INR 1.4) and decreased activated partial thromboplastin time (APTT 25 sec), was observed from 3 hours after ingestion and persisted to day 4. All other biochemical parameters were within normal range, and there was no sign of acute kidney injury. Three days after ingestion soft diet was initiated and the following day the patient was well and discharged. Conclusion: Typical symptoms from glyphosate ingestion include nausea, vomiting, abdominal pain, mouth and throat pain. In severe cases gastrointestinal ulceration and bleeding, renal and hepatic deterioration, and circulatory effects might be observed. There have been deaths after rapid onset of respiratory and circulatory collapse. In this case the ingested amount is unknown but is supposed to be a limited minor amount of a highly concentrated product. Immediate development of symptoms and gastric mucosa ulceration confirmed by gastroscopy support the suspicion of concentrated glyphosate

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Reference	Evaluation	Abstract
		ingestion. Unfortunately it was not possible to get a product sample for chemical analysis. The effect on coagulation parameters was unexpected and calls for increased attention in future glyphosate poisonings.
Iwai K. et al., 2014 Journal of Clinical Toxicology (2014), Vol. 4, No. 6, pp. 1	This article discusses the use of endoscopy to treat formulated glyphosate overdose and medical management of suicidal ingestions and therefore should not impact registration decisions.	Introduction: Roundup® is a herbicide widely used in Japan in gardening and agriculture. When ingested, Roundup is highly toxic, but gastrointestinal decontamination, including gastric lavage, is not routinely performed after ingestion. Endoscopy may be useful in managing individuals with liquid herbicide poisoning, by identifying gastric residual contents, assessing mucosal damage and retrieving herbicide directly by aspiration. Case report: A 73 year old, 40 kg female with a history of depression was transported to our emergency room by ambulance 1 h after attempting suicide by ingesting 100 ml Roundup, which contains 48% glyphosate-potassium, and 52% surfactant and water. This volume was below a fatal dose (<5000 mg/kg), but may have caused organ dysfunction and mucosal damage. After confirming respiratory and circulatory stability and after obtaining informed consent from the patient, endoscopy (XQ 260; Olympus, Tokyo, Japan) was performed in the emergency room to retrieve residual herbicide. About 80 ml of herbicide in the stomach were aspirated endoscopically with only mild erosion observed in the mucosa of the stomach. The patient was able to resume oral intake 2 days after endoscopy and was discharged without any complications on day 5. Conclusion: Endoscopy may be useful in cases of liquid poisoning including, Roundup, both to determine the amount of residual toxin and to remove it from the stomach.
Jovic-Stosic J. et al., 2013 Clinical Toxicology (2013), Vol. 51, No. 4, pp. 288.	This is a case series that included one patient with a formulated glyphosate overdose and treatment with ILE. This describes medical management of overdoses and should not impact re-registration.	Objective: To assess the efficacy and complications of intravenous lipid emulsion (ILE) antidotal use in acute human poisoning. Methods: Prospective clinical study on ILE (Intralipid 20%) effects given as fast intravenous infusion in total dose of 500- 1000 mL. The main criteria for administration were cardiocirculatory failure caused by liposoluble agents and poor response to vasopressors. Effects on blood pressure (BP), electrocardiogram (ECG), and central nervous system (CNS) depression were assessed. Pre- and post-lipid administration concentrations of drugs were obtained. Results: A total of nine patients were treated with ILE. Poisonings were caused by glyphosate/polyethyloxylated tallowamine (POEA) herbicide (1 patient), verapamil and benzodiazepines (3 patients), propranolol combined with alcohol or psychoactive drugs (2 patients) and mixed ingestion of various drugs including carbamazepine, lamotrigine, sertraline, risperidone, amitriptyline, clozapine, haloperidol, valproic acid/valproate and chlorpromazine (3 patients). Significant increase of BP leading to vasopressor therapy reduction was noted in all patients after the initial dose of 500 mL, but in some cases this effect was transient and an additional dose of Intralipid was necessary. The most prominent effect was on wide complex tachycardia which developed in two patients (ingested glyphosate/POEA or propranol/alcohol) as sinus rhythm was regained before the end of Intralipid infusion. ECG changes in others included slight widening of QRS or QT prolongation. There was no rapid normalisation that could be attributed to ILE. All patients were comatose (Glasgow Coma Sscale

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		(GCS) 3-5). Improvement in GCS was noted in all except the cases with predominant carbamazepine and valproate. There were no significant changes in drug concentrations in blood after lipid administration. In cases of verapamil toxicity, analysis after lipid removal by ultracentrifugation revealed a decrease in concentration. The only complication which may be connected with ILE treatment was acute respiratory distress syndrome (ARDS) in a case of severe verapamil intoxication. Conclusion: Our results, though limited by the small number of patients, revealed that the most invariable effect of ILE was the increase of BP, and the most impressive was the fast reversal of wide complex tachycardia. Efficacy of ILE in case of poor response to other therapies indicated that the fear of adverse interactions with other conventional drugs for the treatment of cardiotoxicity may not be rational.
Jovic-Stosic J. et al., 2016 Vojnosanitetski pregled (2016), Vol. 73, No. 4, pp. 390	Medical case of intentional ingestion. ILE has been proposed as a possible therapy for formulated glyphosate overdoses. As this was a suicide attempt, this should not impact re-registration.	Introduction: Glyphosate is the first widely used herbicide against weed in genetically modified crops. Though glyphosate itself has a low toxicity, commercial products are more dangerous because of increased toxicity due to surfactants addition. There is no specific antidote for the poisoning with glyphosate-surfactant (Gly-SH). In recent times, the efficacy of intravenous lipid emulsion (ILE) administration for the treatment of acute poisoning caused by Gly- SH has been investigated. Case Report: A 50-year-old man was admitted 3 hours after self-poisoning with herbicide containing glyphosate and polyoxyethyleneamine, as a surfactant. On admission, the patient was in a coma, hypotensive (80/50 mmHg) and without spontaneous breathing. Electrocardiogram showed widecomplex tachycardia, and arterial blood gas (ABG) revealed acidosis (pH 7.07). Conventional treatment included mechanical ventilation, intravenous fluids, bicarbonate and dopamine. As there was no improvement, ILE was started. The patient received 100 mL of 20% Intralipid® bolus followed by infusion of 400 mL over 20 minutes. Prior to expiration of infusion, a gradual rise in blood pressure was noted, and within 2 hours sinus rhythm was restored. Conclusion: This case report suggests that the use of ILE may be an additional option for the treatment of cardiocirculatory disturbances caused by commercial products of glyphosate herbicide.
Jovic-Stosic J. et al., 2016 Clinical Toxicology (2016), Vol. 54, No. 4, pp. 476	This is a report about using ILE to treat overdoses with 1 patient who ingested formulated glyphosate. This paper should not impact re-registration.	Objective: Intravenous lipid emulsion (ILE) may successfully resuscitate patients with cardiotoxicity. However, the indications for its use, as well as its efficacy, are not sufficiently defined. Methods: An observational clinical study on the effects of lipid (Intralipid 20%) given as an intravenous infusion to a total dose of 500-1000 mL. The main criteria for administration of ILE were cardiocirculatory failure caused by liposoluble chemicals (drugs or pesticides) and poor response to conventional treatment which included dopamine, glucagon, bicarbonate and calcium chloride. Effects on blood pressure (BP), electrocardiogram (ECG) and survival of patients were assessed. Results: There were 31 patients (aged 28-83 years) treated with ILE, which comprised approximately 1% of the total number of patients hospitalized in the intensive care unit due to poisoning during 5-year follow up. ILE was most frequently used in calcium channel

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		blockers poisoning (15 patients). In 9 patients who had ingested verapamil, or a combination of verapamil and benzodiazepines or angiotensin converting enzyme (ACE) inhibitors (enalapril, cilazapril), ILE was effective in reversing hypotension and dysrhythmias. There were 5 cases of multi-drug poisoning including amlodipine. Lethal outcome occurred in a patient poisoned by a combination of amlodipine with cilazapril, metformin and gliclazide. Combination of nifedipine and metoprolol was fatal despite treatment including ILE and pace-maker administration. Beta-blockers, including propranolol, metoprolol and bisoprolol were among the ingested drugs in 7 cases. Metoprolol was also lethal in combination with gliclazide, mianserin and benzodiazepines. Administration of ILE rapidly improved conduction delay and increased BP in cases of propafenone (1 patient) and glyphosate herbicide (1 patient) toxicity. In two cases of organophosphate insecticide poisoning with cardiovascular collapse, only transient increase of BP was noted. The remaining cases involved psychoactive drugs. ILE was successful in the treatment of clomipramine, maprotiline, sertraline and risperidone overdoses, but failed to reverse cardiotoxicity in patients who ingested carbamazepine, lamotrigine or valproate. Conclusion: Although all our patients received multiple therapies, the improvement observed in most of them soon after administration ILE can be attributed to its beneficial effects. Our experience revealed that the most invariable result of ILE administration was an increase in BP. The most impressive effect was the fast reversal of wide complex tachycardia in 3 different cases (ingestion of propranolol, glyphosate and propafenone) which may suggest effectiveness of ILE in the treatment of sodium channel blockade. However, ILE was not effective in all cases in which it could be expected on the basis of the toxic agent's liposolubility.
Jyoti W. et al., 2014 Journal of postgraduate medicine (2014), Vol. 60, No. 3, pp. 346	Formulated glyphosate can cause caustic injury to the mucosa membrane after ingestion. The esophagus is especially prone to perforation. Due to the absence of a serosa, the esophagus is notoriously difficult to repair & heal. This is not an unusual feature of caustic injury. As this was a suicide attempt, this should not impact re-registration.	Not available
Kamijo Y. et al., 2012 Clinical toxicology	This article discusses the fact that certain glyphosate-potassium	Not available

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Reference	Evaluation	Abstract
(2012), Vol. 50, No. 2, pp. 159	salt formulations can cause fatal hyperkalemia in overdose. This article discusses a feature of suicidal ingestions and therefore should not impact registration decisions.	
Kato Y., 2015 The Japanese journal of toxicology (2015), Vol. 28, No. 4, pp. 368	This article describes a case series of three patients who presented with extreme hyperkalemia after suicidal ingestion of formulated glyphosate. This is not unexpected in an ingestion involving glyphosate formulated product with potassium salts and should not affect re-registration.	Not available
Knezevic V. et al., 2012 Srpski arhiv za celokupno lekarstvo (2012), Vol. 140, No. 9-10, pp. 648	Glyphosate based formulations can cause renal injury in overdose, and the K+ formulations may result in hyperkalemia. It is therefore reasonable to start hemodialysis or hemofiltration in critically ill patients with kidney failure or hyperkalemia. As this was a suicide attempt, this should not impact re-registration.	INTRODUCTION: Treating severe acute glyphosate-surfactant poisoning requires intensive therapy including dialysis. Cases of hemoperfusion and hemodialysis use in renal failure induced by herbicide ingestion have been reported in the current medical literature. We present a case report of successful patient treatment with continuous venovenous hemodiafiltration in acute glyphosate-surfactant poisoning. CASE OUTLINE: A 36-year-old male patient attempted suicide by drinking approximately 300 ml of glyphosate-surfactant about an hour before coming to our Clinic. On admittance the patient was somnolent, normotensive, acidotic and hyperkalemic. Six hours after poison ingestion there was no positive response to symptomatic and supportive therapy measures. The patient became hypotensive, hypoxic with oliguric acute renal failure, so that post-dilution continuous veno-venous hemodiafiltration was started. During the treatment the patient became hemodinamically stabile, diuresis was established along with electrolyte and acid-base status correction and a gradual decrease of blood urea nitrogen and creatinine levels. After a single 27.5-hour treatment, clinical condition and renal function parameters did not require further dialysis. Complete recovery of renal function was achieved on the fifth day. CONCLUSION: Early introduction of continuous veno-venous hemodiafiltration with other intensive therapy measures led to complete recovery in a hemodinamically instable patient.

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Lee, G. (2018) Journal of Veterinary Clinics (2018), Volume 35, Number 4, pp. 144-145, 10	Multiple GLP studies have been conducted to evaluate whether glyphosate is neurotoxic. There is no mechanism by which glyphosate can cross the Blood Brain Barrier or enter the bundles of neurons in the cauda equina to cause a lower motor neuron lesion. Given the clinical description, this sounds more like a Guillan-Barre syndrome which has been reported in dogs.	A 4-year-old Yorkshire terrier was presented with hindlimb paresis and urinary incontinence after accidental ingestion of an herbicide. Based on neurologic examinations, decreased hindlimb proprioception with flaccid paresis were revealed. Other possible causes of the clinical signs were excluded. The clinical signs gradually improved after administration of anti-inflammatory and antioxidant therapy. This case report is the first to describe the long-term outcome of hindlimb paresis and urinary incontinence induced by glyphosate surfactant herbicide (GPSH) poisoning in a dog.
Lee B. K. et al., 2012 hong Kong Journal of Emergency Medicine (2012), Vol. 19, No. 3, pp. 214	This is a report about multi-organ failure and the use of CVVHD after suicidal ingestion of formulated glyphosate and should not impact re-registration.	Intractable hypotension is a major cause of death after glyphosate-surfactant herbicide poisoning. However, there is no specific treatment besides conservative care. Herein, we report a patient poisoned by glyphosatesurfactant herbicide experiencing cardiac arrest but was successfully resuscitated and treated with continuous venovenous haemodiafiltration (CVVHDF). The 60-year-old patient was brought to our emergency department after ingesting glyphosate-surfactant herbicide. He developed pulmonary oedema, severe metabolic acidosis (pH 6.960), and hyperkalaemia (serum potassium 8.8 mmol/L). Although he experienced cardiac arrest for about 12 minutes, the use of CVVHDF improved the metabolic acidosis and hyperkalaemia, and finally stabilised his vital signs. He regained an alert mental state after therapeutic hypothermia. CVVHDF, which is a better tolerated renal replacement therapy than haemodialysis in haemodynamically unstable patients, should be considered in glyphosate-surfactant poisoned patients of intractable hypotension with severe metabolic acidosis or hyperkalaemia. © 2006 Hong Kong College of Emergency Medicine.
Lee D. H. et al., 2017 Hong Kong Journal of Emergency Medicine (2017), Vol. 24, No. 1, pp. 40	This is a report about multi-organ failure and the use of dialysis after suicidal ingestion of formulated glyphosate and should not impact re-registration.	Glyphosate-surfactant is one of the most commonly used herbicides in the world. Its key component, glyphosate, is a competitive inhibitor of the shikimate pathway, a metabolic pathway found only in plants. However, severe intoxication, including lethal cases by ingestion of, glyphosate-surfactant has been reported. We describe the full recovery of two patients from glyphosate-polyoxyethyleneamine surfactant intoxication and multi-organ system failure following continuous renal replacement therapy. Both patients developed persistent shock, acute kidney injury, lactic acidosis, hyperkalaemia and multi-organ failure despite of resuscitation. We believe that continuous renal replacement therapy should be initiated immediately for removal of glyphosate-polyoxyethyleneamine surfactant in patients with signs of cardiopulmonary compromise, lactic acidosis, and renal failure. We propose the addition of glyphosate-

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Reference	Evaluation	Abstract
		polyoxyethyleneamine surfactant to the list of toxins for which early haemodialysis should be indicated.
Luo W. et al., 2019 Medicine (2019), Vol. 98, No. 30, pp. e16590	This article describes a case report of gastric ulceration and swelling causing pyloric obstruction in a patient who ingested formulated glyphosate. This is not unexpected as formulations contain surfactants which can cause caustic injury to the GI tract with suicidal ingestions. This should not impact re-registration.	RATIONALE: Oral ingestion of glyphosate can induce gastrointestinal symptoms such as vomiting, abdominal pain, and hematochezia. Timely and effective treatment of pyloric stenosis caused by glyphosate poisoning is important. PATIENT CONCERNS: The patient had a poor appetite, accompanied by nausea and vomiting of a small amount of dark brown material that resembled blood clots several times a day. Gastroscopy revealed gastric ulcer, a large pyloric antrum ulcer, and a deformed stomach cavity. DIAGNOSIS: Pyloric stenosis due to glyphosate poisoning in a 36-year-old man. INTERVENTIONS: The patients received distal gastrectomy and subsequently transferred to the ICU for further treatment. A mechanical ventilator was used to assist breathing. OUTCOMES: Follow-up was conducted 3 years after surgery. The patient had no problem with food ingestion and experienced no discomfort, such as vomiting, nausea, coughing, or expectoration. LESSONS: Gastrectomy is necessary to treat pyloric stenosis caused by glyphosate poisoning.
Mahendrakar K. et al., 2014 Indian journal of critical care medicine (2014), Vol. 18, No. 5, pp. 328	ILE has been proposed as a possible therapy for formulated glyphosate overdoses.	Glyphosate is a widely used herbicide in agriculture, forestry, industrial weed control and aquatic environments. Glyphosate potential as herbicide was first reported in 1971. It is a non-selective herbicide. It can cause a wide range of clinical manifestations in human beings like skin and throat irritation to hypotension, oliguria and death. We are reporting a case of a 35-year-old male patient who was admitted to our tertiary care hospital following intentional ingestion of around 200 ml of herbicide containing glyphosate. Initially, gastric lavage done and the patient was managed with intubation and mechanical ventilation, noradrenaline and vasopressin infusion, continuous veno-venous hemodiafiltration and intravenous (IV) lipid emulsion (20% intralipid 100 ml), patient was successfully treated and discharged home. This case report emphasizes on timely systemic supportive measure as a sole method of treatment since this poison has no known specific antidote and the use of IV lipid emulsion for a successful outcome.
Malhotra, R.C. (2010)	This paper describes prolonged encephalopathy in a suicidal glyphosate ingestion. There is no mention of the medication that was used for sedation while the patient was intubated in the ICU. Accumulations of lorazepam and other	Glyphosate-surfactant (GlySH) is a commonly used herbicide that has been used in attempted suicide. Most reports of GlySH toxicity in patients have followed ingestion of the commercial product "Round-up" (Monsanto Ltd; Melbourne, Victoria, Australia), which consists of a mixture of glyphosate (as a isopropylanine salt) and a surfactant (polyoxyethyleneamine). Ingestion of Round-up is reported to cause significant toxicity including nausea, vomiting, oral and abdominal pain. Renal and hepatic impairment and pulmonary oedema may also occur. Impaired consciousness and encephalopathy have been reported as sequelae but there are limited data on the central nervous system (CNS) effects of Round-up toxicity. We report a 71-year-old male who attempted suicide with GlySH and developed a prolonged

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	sedatives may result in prolonged coma. In formualted glyphosate overdose with multiorgan failure it is common to sedate patients until their hemodynamics improve. In the setting of suicidal overdose, this paper should not impact re-registration.	but reversible encephalopathy suggestive of acute CNS toxicity.
Nakae H. et al., 2015 Acute medicine & surgery (2015), Vol. 2, No. 3, pp. 214	This article describes alternative medicine therapies that were used to treat a Japanese woman with a paralytic ileus after glyphosate ingestion. It is not uncommon for patients in a critical care setting to develop an ileus. These tend to resolve on their own without intervention. I cannot be commented on whether this intervention increases GI motility.	Case: A 65-year-old woman ingested glyphosate-surfactant herbicide in an attempt to commit suicide. She experienced glyphosate intoxication associated with multiple organ failure and developed a paralytic ileus. Daijokito, a traditional Japanese Kampo medicine was given to the patient to improve constipation and psychological symptoms. Next, rikkunshito was given to increase her gastric motility. Finally, daikenchuto was given to improve overall digestive peristalsis. Outcome: All abdominal symptoms ultimately improved after treatment with daikenchuto. Conclusion: Kampo medicines may help improve abdominal symptoms associated with glyphosate intoxication in cases where modern medical treatment alone proves inadequate.
Nakayama T. et al., 2019 Clinical and experimental nephrology (2019), Vol. 23, No. 6, pp. 865	This was a suicidal ingestion of formulated glyphosate that resulted in poor renal perfusion & multiorgan failure. Since this was a suicidal ingestion, the outcome is not unexpected and should not impact the re-egistration.	A 70-year-old woman presented with abdominal pain 2 h after ingesting 500 ml of glyphosate–surfactant herbicide (GPSH). As her abdominal pain worsened, contrast-enhanced computed tomography was performed 12 h after the admission, demonstrating renal blood flow shunting through the medulla which is called “reverse rim sign” (Fig. 1). She had been anuric since hospitalization with sufficient mean artery pressure and negative blood culture. Despite intensive care, she died of multiple organ failure on the 6th day of hospitalization. [No abstract was available; this is copied from article. MSB]
Ozaki T. et al., 2017 Therapeutic apheresis and dialysis (2017), Vol. 21, No. 3,	This article discusses the use of haemodialysis and haemofiltration in formulated glyphosate	INTRODUCTION: In our case report we describe the case of a patient who experienced a stroke in her left hippocampus that was found following an attempted suicide via glyphosate overdose. To the best of our knowledge this is the first case report to describe a hippocampal infarction associated with a drug overdose.

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pp. 296	overdoses.This article discusses medical management of suicidal ingestions and therefore should not impact registration decisions.	CASE PRESENTATION: A 64-year-old Japanese woman was brought to our emergency department after ingestion of an unknown dose of glyphosate surfactant herbicide in order to attempt suicide. On admission, she was assumed to be presenting with depression or psychiatric illness, however, sudden-onset memory deficit became apparent. The patient manifested delirium, confusion, and severe anxiety. In addition, short-term memory loss was prominent, with the patient forgetting her attempted suicide. Following an array of standard tests and a brain computed tomography scan (which only showed an old infraction), we performed a magnetic resonance imaging scan and neuropsychological evaluations. The brain magnetic resonance image revealed a small high-intensity lesion in the dorsal part of the left hippocampal body, and memory tests demonstrated severe short-term recall deficits. We diagnosed her with a left hippocampal infarction and administered a course of 75mg of clopidogrel. She gradually became less confused over the course of a week, and a follow-up memory test revealed partial improvement in some domains. No abnormalities were found on a follow-up brain scan. However, despite rehabilitation, memory impairments remain. CONCLUSIONS: It is important to note that had the symptom of short-term memory been absent or less severe, she might have been misdiagnosed with depression or another psychiatric illness. Although a computed tomography scan failed to detect hippocampal lesions, a diffusion-weighted magnetic resonance imaging scan clearly revealed a lesion within the left hippocampus. Therefore, in addition to assessments focusing on psychiatric illnesses that might be the root cause of an attempted suicide, organic factors should be considered along with radiological examination and precise memory assessments for diagnosing similar cases.
Picetti E. et al., 2017 Acta Biomedica (2017), Vol. 88, No. 4, pp. 533	This is a report about multi-organ failure after suicidal ingestion of formulated glyphosate and should not impact re-registration.	Not available
Thakur D. S. et al., 2014 Toxicology international (2014), Vol. 21, No. 3, pp. 328	This is a case report of the clinical manifestations of glyphosate-based herbicide ingestions and discusses predictors of mortality in suicidal ingestions and therefore should not impact registration decisions.	Background: Water soluble and insoluble chemicals in the pesticide formulation may be eliminated more effectively in time if hemodialysis (HD) and hemoperfusion (HP) are performed concurrently. Aim: This study is aimed at evaluating the efficacy of concurrent HP and HD in patients with acute pesticide intoxication. Methods: Between January 2011 and December 2012, we used HP and HD consecutively (HP-HD group, 347 cases), and then during the next 2 years (January 2013 to December 2014), we used concurrent HP and HD (HPD group, 383 cases). We compared the clinical outcomes between the 2 groups. Results: The mortality was higher in the HP-HD group than in the HPD group: (48.1 vs. 20.9%) for the overall mortality and (81.8 vs. 57.9%) for the paraquat (bipyridylium) mortality ($p < 0.001$). In multiple logistic analyses, age ($p = 0.013$), ingested volume ($p < 0.001$), and HP-HD ($p = 0.014$) were significant risk factors for mortality in the paraquat ingested group. Conclusion:

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		Concurrent HP and HD would be an effective and safe treatment for patients with acute pesticide intoxication, in particular, paraquat intoxication.
Veale D. J. H. et al., 2013 SAMJ (2013), Vol. 103, No. 5, pp. 293	This article summarises the chemicals used in South Africa for suicide. Glyphosate is only mentioned in a table in the article as being involved in 23 cases over a 1 year period accounting for 0.9% of the overall cases reported.	A 55 years old man self-presented to our Emergency Department (ED) reporting an attempted suicide by cutting the left forearm veins and ingesting approximately 200 mL of an herbicide (Myrtos®, containing 36% of glyphosate as isopropylamine salt). Laboratory tests showed metabolic acidosis. Hydration with normal saline and alkalinization with sodium bicarbonate was started according to suggestion of the poison control center, since an antidote was unavailable. Cardiorespiratory condition gradually worsened, so that non-invasive positive pressure ventilation (NIPPV) was applied and infusion of fluids was established. Nevertheless, the patient deteriorated and he needed to be transferred to the Intensive Care Unit (ICU), where he underwent orotracheal intubation and invasive mechanical ventilation. Noradrenaline and adrenaline were infused and fluid resuscitation with crystalloids was incremented. An esophagogastroduodenoscopy (EGD) showed diffuse mucosal erosions of upper digestive tract. No signs of visceral perforation were found during ICU stay. In the following days, the clinical conditions improved and a new EGD showed marked improvement of erosive lesions. After 12 days of ICU stay, the patient was extubated and then transferred to the Psychiatric Unit, in good clinical conditions. Gliphosate ingestion is associated with rapid development of multiple organ failure (MOF). Since an effective antidote is unavailable, major attention should be placed to aggressive life-support care and careful monitoring of complications.
Vidyadhara et al., 2014 Indian Journal of Critical Care Medicine (2014), Vol. 18, Suppl. 1, pp. S36.	This is a report about multiorgan failure after suicidal ingestion of formulated glyphosate and should not impact re-registration.	A 55 years old man self-presented to our Emergency Department (ED) reporting an attempted suicide by cutting the left forearm veins and ingesting approximately 200 mL of an herbicide (Myrtos®, containing 36% of glyphosate as isopropylamine salt). Laboratory tests showed metabolic acidosis. Hydration with normal saline and alkalinization with sodium bicarbonate was started according to suggestion of the poison control center, since an antidote was unavailable. Cardiorespiratory condition gradually worsened, so that non-invasive positive pressure ventilation (NIPPV) was applied and infusion of fluids was established. Nevertheless, the patient deteriorated and he needed to be transferred to the Intensive Care Unit (ICU), where he underwent orotracheal intubation and invasive mechanical ventilation. Noradrenaline and adrenaline were infused and fluid resuscitation with crystalloids was incremented. An esophagogastroduodenoscopy (EGD) showed diffuse mucosal erosions of upper digestive tract. No signs of visceral perforation were found during ICU stay. In the following days, the clinical conditions improved and a new EGD showed marked improvement of erosive lesions. After 12 days of ICU stay, the patient was extubated and then transferred to the Psychiatric Unit, in good clinical conditions. Gliphosate ingestion is associated with rapid development of multiple organ failure (MOF). Since an effective antidote is unavailable, major attention should be placed to aggressive life-support care and careful monitoring of complications.

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Reference	Evaluation	Abstract
Wang D. et al., 2019 Medicine (2019), Vol. 98, No. 6., pp. e14414	This article describes using ECMO to manage a patient with multiorgan failure after formulated glyphosate and diquat ingestion. Since this is describing medical management of suicidal overdoses, it should not impact re-registration	This article requires a subscription to view the full text. If you have a subscription you may use the login form below to view the article. Access to this article can also be purchased. A 66-year-old man, with a history of Wolff-Parkinson-White syndrome, coronary artery disease, left partial colectomy for polyps, and alcohol abuse (50 g/d), attempted suicide by ingestion of commercially available glyphosate (Round-Up, 200 mL). He was admitted to the emergency ward for monitoring and hyperhydration. First clinical and biological workup and gastric endoscopic examinations were normal.
Wu M-H. et al., 2015 Clinical Toxicology (2015), Vol. 53, No. 4, pp. 330	This is a report about renal failure and haemodialysis after suicidal ingestion of formulated glyphosate and should not impact re-registration.	INTRODUCTION: The mechanisms underlying early central nervous system (CNS) signs and symptoms of glyphosate-surfactant herbicide (GlySH) poisoning are unclear. CASE PRESENTATION: A 58-year-old woman ingested approximately 150 mL of GlySH containing 41% glyphosate and 15% polyoxyethyleneamine. Two days later, she was admitted in the Emergency Center in a semicomatose state. Acute respiratory distress syndrome, circulatory collapse, acute renal failure, and disseminated intravascular coagulopathy were diagnosed. Meningitis was also suspected as she demonstrated Kernig's sign and significant neck stiffness with rigidity of the extremities as well as consciousness disturbance and fever (38.4°C). Investigations of cerebrospinal fluid (CSF) revealed the presence of glyphosate (122.5 µg/mL), significant elevation of IL-6 (394 µg/mL), and pleocytosis (32 cells/µL) with monocyte dominance. All bacteriological and virological tests were later found to be negative. She recovered completely after responding to aggressive supportive care in the intensive care unit. All signs and symptoms suggesting meningitis resolved as the concentration of glyphosate in CSF decreased. She was discharged on day 39 of hospitalization. DISCUSSION: These findings suggest that the present case involved aseptic meningitis in association with GlySH poisoning. CONCLUSION: CNS signs and symptoms induced by aseptic meningitis should be considered in cases of glyphosate-surfactant herbicide poisoning.
You Y. et al., 2012 The American journal of emergency medicine (2012), Vol. 30, No. 9, pp. 2097.e1	This article is discussing the efficacy of intravenous fat emulsion as therapy for formulated glyphosate overdose. This report contributes to the evidence that intravenous fat emulsion may be a useful treatment for glyphosate overdose	Glyphosate herbicide is promoted by the manufacturer as having no risks to human health, with acute toxicity being very low in normal use. In Thailand, however, poisoning from glyphosate agricultural herbicides has been increasing. A case of rapid lethal intoxication from glyphosate-surfactant herbicide involved a 37-year-old woman, who deliberately ingested approximately 500 mL of concentrated Roundup formulation (41% glyphosate as the isopropylamine salt and 15% polyoxyethylene amine; Mosanto Company). The postmortem examination revealed that the stomach contained 550 mL of yellow fluid. The gastric mucosa of anterior fundus revealed hemorrhage and the small intestines had marked dilatation and thin walls. We used the high-performance liquid chromatography method for determination of serum and gastric content levels of glyphosate. The glyphosate levels of serum and gastric content were 3.05 and 59.72 mg/mL,

Appendix 1: Overview on case reports. No review information or epidemiologic information is listed in this table. The complete references on any kind of medical data are available in the literature search report, CA 9-01.

Reference	Evaluation	Abstract
	as it may limit the toxicity associated with large surfactant ingestions. There are no RCTs for this as it is a suicidal overdose situation.	respectively. Toxic effects of polyoxyethylene amine and Roundup were caused by their ability to erode tissues including mucous membranes and linings of the gastrointestinal and respiratory tracts. A mild degree of pulmonary congestion and edema was observed in both lungs. We proposed that the characteristic picture of microvesicular steatosis of the hepatocytes, seen predominantly in centrilobular zones of the liver, resembled drug-induced hepatic toxicity or secondary hypoxic stress.
Yu G. C. et al., 2017 Chinese journal of industrial hygiene and occupational diseases (2017), Vol. 35, No. 5, pp. 382	This is a case study describing the clinical course of 10 patients who drank formulated glyphosate. There were no long-term sequelae of ingestion, and all 10 patients survived. These were suicdal ingestions and should not impact re-registration.	The literature, particularly from India, is scarce on the renal effects of glyphosate poisoning. Glyphosate causes toxicity not only after its ingestion but also after dermal exposure by inhalation route and on eye exposure. We present a patient report of glyphosate consumption which resulted in toxic epidermal necrolysis - the first report after glyphosate consumption and acute kidney injury.
Zouaoui K. et al., 2013 Forensic science international (2013), Vol. 226, No. 1-3, pp. E20	This report demonstrates a link between higher blood and urine concentrations with formulated glyphosate overdoses and a poorer outcome. This is unsurprising as it reflects that patients drank a larger volume. Larger volumes of formulated product are associated with more toxicity due to the caustic nature of the surfactant, not the amount of active ingredient. All of the laboratory parameters are expected in critically ill patients. As these were suicidal ingestions, this paper should not impact re-registration.	An 86-year-old woman intentionally drank approximately 300 mL of a glyphosate-surfactant. She was found with consciousness disturbance and experienced several vomiting episodes. On arrival, serum biochemistry revealed a decreased level of butyrylcholinesterase (B-CHE) [11 (normal range: 180-450) IU/L]. Later, her B-CHE level further decreased to single-digit values, and she became comatose with involuntary movement and an increase in muscle tone. Her consciousness level and muscle tone improved with the recovery of her B-CHE level. Physicians should be alert for the occurrence of intermediate syndrome when the B-CHE levels of patients who have consumed a massive amount of glyphosate-surfactant show a prolonged decrease.

Glyphosate

Annex M-CA 5-01

ANNEX to the Documen M of the technical section[46]:

TOXICOLOGY AND METABOLISM

[46] Annex to the Doc ID: 110054-MCA5_GRG_Jun_2020

AIR 5 introduction

During the AIR 2 evaluation process of glyphosate, in the Renewal Assessment Report 2015 version[47], the RMS Germany included public literature articles as part of the Annex B.6. **All articles** included in this version of the RAR Vol 3 2015 have been included in this annex for the sake of completeness, with the aim of providing the EU authorities during the AIR 5 EU process with all information available for glyphosate from previous EU evaluations.

All information presented in this Annex, is an exact copy of the literature information included in the RAR Vol 3 2015 version. When reading the present annex, please note:

- This annex only present articles and not regulatory studies.
- Some references are made to the former Monograph glyphosate 1998.
- If text was strickethrough in the RAR Vol 3 2015, then those sentences were not included in the present annex.
- The numbering of tables in the present annex have not been changed and remain as original presented in the RAR Vol 3 2015 version.
- If text was highlighted in the RAR Vol 3 2015, then those sentences are also highlighted in the present annex.
- If text was given in italic style in the RAR Vol 3 2015, then those sentences are also given in italic in the present annex.

[47] Renewal Assessment Report, Revised 2015 Vol 3

Renewal Assessment Report, Revised 29 January 2015 Vol 3, annex B.6.4 Background

General introduction and explanation of the approach taken by RMS

This health evaluation of glyphosate is based on the following sources:

- Toxicological and ADME studies that were submitted by the GTF for this re- evaluation.
- Toxicological studies and ADME studies that had been reported in the previous DAR (1998, ASB2010-10302) already and, thus, were part of previous EU evaluation. However, they were subject to re-assessment by the RMS according to current quality standards and were used only when regarded as acceptable or at least supplementary. In very few cases, NOAELs/LOAELs were revised. Unacceptable (old or new) studies were usually deleted with justifications given in the respective sections of Volume 3. In exceptional cases, such studies are still mentioned, i.e., if they were formerly taken into consideration for, e.g., ADI setting.
- Scientific publications and other relevant information that were submitted either by the GTF or by third parties or of which the RMS was aware before. It must be emphasised that a large part of the publications was on formulations different from the representative one and, thus, is of limited value for the toxicological evaluation of the active ingredient. With rather few exceptions in the areas of genotoxicity and human data, mainly scientific literature published since 2000 was assessed.

Due to the large numer of submitted toxicological studies, the RMS was not able to report the original studies in detail and an alternative approach was taken instead. The study descriptions and assessments as provided by GTF were amended by deletion of redundant parts (such as the so-called ”executive summaries”) and new enumeration of tables. Obvious errors were corrected. Each new study was commented by the RMS. These remarks are clearly distinguished from the original submission by a caption, are always written in italics and may be found on the bottom of the individual study summaries.

Furthermore, in Volume 3, assessment was performed on the individual study level. Overall evaluation of the diverse toxicological endpoints was transferred into Volume 1 (section 2.6).

The technical databases that have been used for the literature search include: Web of ScienceSM, BIOSIS Previews®, CAB Abstracts® (CABI), MEDLINE®, and CA Plus (Chemical Abstracts Plus). The searches were made on glyphosate acid, glyphosate salts (including isopropyl amine, potassium, ammonium, and methylamine), and AMPA, and their related chemical names and CAS numbers. Searches based on these search terms were also found suitable to identify publications that consider glyphosate and surfactants (such as polyoxyethylenealkylamines, or POEA) in the context of glyphosate formulations.

Additional publications cited in a recent document prepared by the NGO ”Earth Open Source” (Antoniou M, *et al.*, 2011, ASB2011-7202) have also been included in the literature review.

The peer-reviewed publications identified for inclusion during the literature search were reviewed and classified into one of the categories listed below.

- Category 0 publications: These are publications in which glyphosate is only mentioned as an example substance or is discussed/studied in a context that is not relevant or related to any of the regulatory sections or the exposure/hazard assessments within this submission; the publication is therefore outside of the scope of this submission.
- Category 1 publications: These are publications which discuss glyphosate in a context relevant or related to the regulatory dossier sections and the conclusions fall within the conclusions of the exposure/hazard assessment. The publication is submitted with minimal or no comment or discussion.
- Category 2 publications: These are publications which discuss glyphosate in a context relevant or related to the regulatory dossier sections and have conclusions that call into question the

endpoints/conclusions in the exposure/hazard assessment. Additionally, Category 2 also includes publications with conclusions that support the risk/hazard assessment, and may be included in discussion of other relevant publications. For selected Category 2 publications, an OECD Tier-II type summary is provided in addition to a reliability assessment (Klimisch rating, see Klimisch *et al.*, 1997, ASB2010-14388); limited comments and critical remarks are provided, as appropriate.

- Category 3 publications: These are publications that discuss glyphosate in a context relevant or related to (1) non-regulatory endpoints that need to be addressed as per new Regulation (EC) 1107/2009; or (2) in a context relevant to sensitive allegations that have emerged or could emerge in the media; or (3) in a context relevant to the regulatory dossier sections and have conclusions that are in disagreement with endpoints/conclusions in the exposure/hazard assessment (although the experimental design seems relevant at first glance). An OECD Tier-II type summary is provided and a Klimisch rating assigned, and supplemented with critical review and discussion.
- Category 'E' publications: These are peer-reviewed publications that were cited in the Earth Open Source document. This category includes publications that were already captured by the literature search and are addressed within the appropriate discipline, as well as publications that were out of scope of the search (primarily as a result of being published prior to 2001). Publications already captured in the literature search were assigned a Category 1, 2 or 3 rating (as appropriate) in addition to a Category 'E' rating. An OECD Tier-II type summary has been prepared and a Klimisch rating assigned for each of the Category E publications. All Category 'E' publications are reviewed within the appropriate discipline, with most of the reviews provided within the toxicology dossier under Section IIA 5.10.

A full description of the literature search methodology was provided by the GTF in a separate document (Carr and Bleeke, 2012, ASB2012-11583).
Five separate publication subject areas are addressed in the literature review.

1. Developmental and Reproductive Toxicity (DART) and Endocrine Disruption (ED)
2. Neurotoxicity
3. Carcinogenicity
4. Genotoxicity
5. Category E and other publications

The publications on subject areas 1-4 are presented in the chapters on Genotoxicity, Long term toxicicity and carcinogenicity, Reproductive Toxicity and Neurotoxicity of the report.
Furthermore, publications are presented in the chapters "Further toxicological studies" and "Medical data". Important publications are presented in summaries as quoted from the articles followed by Klimisch ratings and by RMS comments on the paper.

In the process of public consultation after the submission of the first draft of this RAR PAN-Europe, PAN-Germany and PAN-UK conducted a PubMed literature search on the keywords 'glyphosate' and 'toxicity' and stated they got significant differences in comparison conducted by the notifier. The GTF repeated the PubMed search on June 11, 2014, using the same keywords (Glyphosate Task Force 2014, ASB2014-9624). Overall, a total of 504 articles were identified in the search. Of those, 349 were from the time period of 2001 to 2012, and thus were considered relevant to the glyphosate submission, and were further evaluated as the whether or not they were included in either the original literature search, included in the May 2012 submission, or as part of the ongoing update of the search. As of the time of June 11 PubMed search. There were 266 reviewed for the submission (222 were included), with an additional 34 reviewed after the submission (29 selected for submission). Of the 49 remaining articles, 43 were considered to tbe not relevant based on the subject of the article (the majority were either on GM crops, efficacy or weed resistance). The remaining 6 were added to the literature review, and of those 4 were considered to be relevan and were selected for submission in the update.

Thus, of the 349 articles identified in the search, only 4 were determined to be relevant and were not already identified in the GTF literature search process.

B.6.1 Absorption, distribution, excretion and metabolism (toxicokinetics) (Annex IIA 5.1)

B.6.1.1 Toxicokinetic and metabolism studies in rats that were not previously evaluated in the EU

B.6.1.2 Re-evaluation of previously known studies (mentioned in the 1998 DAR, ASB2010-10302) by the RMS

B.6.1.3 Published information

Toxicokinetics and metabolism of glyphosate were seldom subject to investigations of industry-indpendent researchers and, thus, experimental data in open literature is scarce. The following paragraphs were transferred from the original DAR (1998, ASB2010-10302) and slightly amended for purposes of the RAR:

Brewster et al. (1991, TOX9551791) reported an absorption rate from the gastrointestina tract of 35 - 40% of the total dose following the single oral administration of 10 mg of a mixture of ^{12}C- and ^{14}C-glyphosate per kg bw to male Sprague-Dawley rats. Urine and faeces were considered equally important routes of elimination. 7 days after application, total body burden was approximately 1% of the administered dose and was primarily associated with the bone. Two hours following a single dose of the mixed test material, traces of a minor metabolite (<0.1% of the dose applied) were detected beside the predominating parent in the colon tissue. This compound was also found in the gastrointestinal tract content of one rat at 28 hours post dosing and was considered likely to be aminomethyl phosphonic acid (AMPA) although the retention time for this metabolite was not identical to that for AMPA. The authors reported AMPA to be a product of metabolic activity of intestinal microbes.

Chan and Mahler (1992, TOX9551954) investigated (with contributions of Duerson and Sipes who are mentioned above) the elimination and tissue distribution of ^{14}C-glyphosate in male F344/N rats following oral and intravenous administration. After single low (5.6 mg/kg bw) or high (56 mg/kg bw) oral doses, more than 90% of the applied radioactivity was eliminated within 72 hours. During the first 24 hours, approximately 50% had been excreted in the faeces and nearly 30% via the urine. It was assumed that the urinary radioactivity represented the amount of glyphosate absorbed. The peak blood levels occurred at 1 (low dose) or 2 (high dose) hours after dosing. Following an i.v. dose of 5.6 mg/kg bw, 90% of the radioactivity was excreted in urine within the first 6 hours already. Glyphosate did not accumulate in the body. In a further group of rats receiving 5.6 mg/kg bw by oral gavage, only 1% of the dose remained in the tissues after 24 hour. It is also stated that pretreatment with Roundup® via drinking water did not change the elimination pattern of glyphosate.

In a more recent paper that was included in the GTF dossier submitted for current evaluation, Anadon *et al.* (2009, ASB2012-11542) reported some parts of toxicokinetics of glyphosate (obtained from SIGMA CHEMICALS) in rats after single intravenous (i.v.) administration of 100 mg/kg bw or a single oral dose of 400 mg/kg bw. The focus was on plasma characteristics and distribution to the different compartments: "Serial blood samples were obtained after i.v. and oral administration. Plasma concentrations of glyphosate and its metabolite amiomethyl phosphonic acid (AMPA) were determined by HPLC method. After i.v. and oral administration, plasma concentration-time curves were best described by a two-compartment open model. For glyphosate, the elimination half-lives ($T_{1/2ß}$) from plasma were 9.99 h after i.v. and 14.38 h after oral administration. The total plasma clearance was not influenced by dose concentration or route and reached a value of 0.995 l $h^{-1}kg^{-1}$. After i.v. administration, the apparent volume of distribution in the second

compartment (V_2) and volume of distribution at steady state (V_{ss}) were 2.39 and 2.99 l kg^{-1}, respectively, suggesting a considerable diffusion of the herbicide into tissues. After oral administration, glyphosate was partially and slowly absorbed with a T_{max} of 5.16 h. The oral bioavailability of glyphosate was found to be 23.21%. Glyphosate was converted to AMPA. The metabolite AMPA represented 6.49% of the parent drug plasma concentrations. The maximum plasma concentrations of glyphosate and AMPA were 4.62 and 0.416 µml^{-1}, respectively. The maximum plasma concentration of AMPA was achieved at 2.42 h. For AMPA, the elimination half-life ($T_{1/2ß}$) was 15.08 h after oral administration of glyphosate parent compound (*quoted from original article*)."

The RMS is not aware of any further scientific publications dealing with toxicokinetics and metabolism of glyphosate in laboratory animals or man. However, information on urinary excretion of glyphosate in humans was provided. In the original draft, this data was reported here but, for the revised version, the information was substantially amended (because more data had become available in the meantime) and transferred to section B.6.9.3 where a new sub-section on human biomonitoring was created[48].

B.6.3.2.1 90-day studies in rats

Published information

Glyphosate was tested in the 1980ies in U.S. National Toxicology Program (NTP) for oral subchronic toxicity (Chan and Mahler, 1992, TOX9551954). The following paragraph was partly copied from the previous DAR (1998, ASB2010-10302):

20 F344/N rats per sex and dose were fed glyphosate (supplied by Monsanto, approximately 99% pure) for 13 weeks at dietary levels of 0, 3125, 6250, 12500, 25000 or 50000 ppm. Ten rats/sex and group were used for evaluation of haematological and clinical chemistry parameters. All rats survived until the end of the study and there were no clinical signs of toxicity apart from diarrhea at the top dose level in both sexes. Body weight gain was markedly reduced in high dose males and slightly decreased in high dose females. There were some minor alterations in haematological and clinical chemistry parameters at least at the upper dose levels. Morphologic changes at necropsy were confined to parotid and submandibular (submaxillary) salivary glands in both sexes. This "cytoplasmatic alteration"consisted of basophilic change and hypertrophy of acinar cells. The parotid gland was more affected. Here, the normal granular, eosinophilic staining cytoplasm of the acinar epithelial cells was replaced by basophilic and finely vacuolated cytoplasm. A NOEL could not be established since these lesions were observed already at the lowest dose level but not in the control groups. The degree of change showed a clear dose response. The outcome of this study shows that glyphosate is of low toxicity when administered orally over a period of 3 months to rats since the animals tolerated daily doses as high as 50000 ppm (more than 3000 mg/kg bw/day) without mortality or clinical signs of overt toxicity and without pathological changes other than the rather equivocal salivary gland findings.

B.6.3.2.1 90-day studies in mice

[48] Text which was strickethrough in the RAR was not copied into this Annex 1

Published information

Glyphosate was tested in the 1980ies in U.S. National Toxicology Program (NTP) for oral subchronic toxicity (Chan and Mahler, 1992, TOX9551954). The following paragraph was partly copied from the previous DAR (1998, ASB2010-10302):

Groups of ten B6C3F1 mice per sex and dose were fed glyphosate (supplied by Monsanto, approximately 99% pure) for 13 weeks at dietary levels of 0, 3125, 6250, 12500, 25000 or 50000 ppm. Clinical pathology investigations were not performed but all animals were necropsied at study termination. Mice of the highest dose group and of the control group were subjected to complete histopathological examination. In addition, salivary glands were examined microscopically in all dose groups. There were no clinical signs of toxicity. However, one high dose female died from undetermined causes. Body weight gain was depressed at the two upper dose levels in both sexes. From the dose level of 6250 ppm onwards, a dose-related increase in occurrence and severity of cellular alteration of the parotid salivary gland was noted. This change consisted of basophilia of the acinar cells and in more severely affected glands, the cells and acini also appeared enlarged with an associated relative reduction in the number of ducts. A dose of 3125 ppm (ca 507 mg/kg bw/day seems to represent the NOEL for substance-related effects. However, the extent of investigations performed was rather limited.

B.6.1.4 Genotoxicity of formulations (taking into account published data that were released before 2000)

Published literature (before 2000)

During the past few years, a number of studies was published dealing with possible mutagenic effects of glyphosate formulations in different test systems. Scientific assessment of these data is very difficult for at least two reasons.

One main deficiency is the lack of precise description of the test material. Usually, source, composition and/or purity neither of the formulations nor, if tested, of the active ingredient are not stated at all or, at least, not sufficiently reported in the publications. It should be also taken into consideration that different formulations may be marketed in different countries under the same trademark, e.g. Roundup®. Further confusion comes from the fact that sometimes by- products in formulations (e.g. surfactants) were replaced by others but the name of the product was not changed. On request, data on the ingredients were submitted by the manufacturer Monsanto but even this information was not sufficient to clarify all uncertainties about the test substances. However, on the basis of the information available so far, it can be be stated that the Roundup products used in the different published studies were not identical. Thus, it is questionable whether results obtained with one product will apply to others containing different non-active ingredients in different concentrations.

A second point of concern is the frequent use of less validated test systems with no proven relevance of the findings for human health risk assessment even if such systems may be well accepted to predict special environmental hazards. With regard to health effects, there are no current guidelines for these test methods and there is no actual experience how to assess positive findings in such test systems. For other test methods used, OECD guidelines do exist but the experiments were not carried out in compliance with these recommendations.

To facilitate presentation of data, it was decided to start with those experiments for which, in principle, widely agreed guidelines are available. Because of the large background database, the SCE assays were also included here. In the subsequent part of this section, investigations in test systems less frequently used for examination of plant protection products and with no guidelines existing are reported. As a result of this approach, one and the same publication may be referred to repeatedly on different sites.

It should be mentioned that in some publications also experiments are reported which were carried out with glyphosate active substance (i.e., the acid or one of its salts) being the test material. These data were not included in the monograph since the respective publications, for different reasons, were considered unacceptable for evaluation purposes (for justification, see description of experimental conditions below) in particular when the current OECD criteria for assessment of published data were applied. However, the findings are reported in this addendum since a direct comparison between active ingredient and formulation data may be of particular interest.
Although various test systems measuring different endpoints were used, it was tried to summarize the available studies in Table B.6.4-26 (see next pages) to facilitate general overview before the individual publications were discussed in greater detail below. For practical reasons, in particular to facilitate direct comparison, the studies were divided into sections according to the test systems and methods and the experiments separately tabulated.

Table B.6.4-26: Overview on published studies on mutagenicity of glyphosate, its salts and formulations

Test method/ test system	Test material	Dose levels/ Dose range	Results	Remarks	Reference
Ames test in S.typhimurium strains TA98 and TA100 (+/- S9 mix)	Roundup (48% glyphosate IPA; polyoxyethylene tallowamine surfactant)	0 - 1440 µg/plate (calculated as glyphosate IPA salt)	Equivocal. Occasional increase in mutation rate but no clear dose response. Marked cytotoxicity from 360 or 720 µg/plate onwards.	Study not acceptable for evaluation purposes due to serious deficiencies. Reliable assessment avoided by cytotoxicity.	Rank *et al.*, 1993 Z82234
Micronucleus test in mouse bone marrow; single i.p. administration; sampling after 24 or 48 h	Glyphosate IPA salt (1:1 mixture) and Roundup (48% glyphosate IPA; tallowamine surfactant)	0, 100, 150, 200 mg/kg bw (glyphosate IPA); 0, 133, 200 mg/kg bw (Roundup, calculated as IPA salt)	Negative. Indication of dose-related bone marrow cytotoxicity with the Roundup formulation but not with glyphosate IPA.	Supplementary study confirming previous results.	Rank *et al.*, 1993 Z82234
Micronucleus test in mouse bone marrow; two i.p. administrations with a 24-h interval between; sampling after 6 and 24 h after the final dose	Glyphosate a.i. (99.9% pure) and Roundup (IPA salt with 30.4% glyphosate a.e.; alkyle sulfate surfactant)	0, 300 mg/kg bw (2X150 mg/kg bw/d) for glyphosate; 0, 450 mg/kg bw (2x225 mg/kg bw/d) for Roundup	Weakly positive for glyposate after 24 h and for Roundup at both sampling times. Some evidence of bone marrow cytotoxicity of Roundup.	Supplementary study (methodical deficiencies) revealing an increase in micronucleus frequency, data in contrast to previous results.	Bolognesi *et al.*, 1997 Z59299
SCE assay in human lymphocytes	Glyphosate a.i. (99.9% pure)and Roundup (IPA salt with 30.4% glyphosate a.e.; alkyle sulfate surfactant)	0 - 6 mg/ml for glyphosate; 0, 0.1, 0.33 mg/ml for Roundup	Positive for glyphosate from 1 mg/ml onwards and for Roundup at both concentrations. With Roundup, complete cytotoxicity at concentrations >0.33 mg/ml.	Insufficient data. In addition, a positive result in this assay is of equivocal biological significance against the background of more appropriate mutagenicity studies.	Bolognesi *et al.*, 1997 Z59299
SCE assay in human lymphocytes	Roundup (not specified)	0, 250, 2500, 25000 µg/ml	Weakly positive at the low and mid dose level (for one of two donors). Cytotoxic at the high dose.	see comment above	Vigfusson and Vyse, 1980 TOX9700576 / ASB2012-12044
Alkaline elution assay for DNA single-strand breaks and formation of alkali labile sites in DNA obtained from liver and kidneys of mice following single i.p.administration	Glyphosate a.i. (99.9% pure)and Roundup (IPA salt with 30.4% glyphosate a.e.; alkyle sulfate surfactant)	0, 300 (glyphosate a.i.), 900 (Roundup) mg/kg bw; sampling after 4 and 24 h	Weakly positive after 4 h in both organs suggesting possible transient DNA damage.	Supplementary study (methodical deficiencies). Biological significance equivocal. Results in contrast to the negative outcome of the UDS assay. Effects might be also due to toxicity.	Bolognesi *et al.*, 1997 Z59299

Table B.6.4-26: Overview on published studies on mutagenicity of glyphosate, its salts and formulations (continued)

Test method/ test system	Test material	Dose levels/ Dose range	Results	Remarks	Reference
Investigations for oxida-tive damage in liver and kidney of i.p. treated mice by measuring the number of 8-OHdG (hydroxydesoxyguanosine) adducts	Glyphosate a.i. (99.9% pure)and Roundup (IPA salt with 30.4% glyphosate a.e.; alkyle sulfate surfactant)	0, 300 (glyphosate a.i.), 900 (Roundup) mg/kg bw (single i.p. administra-tion); sampling after 4 and 24 h	Evidence of stimulation of oxidative metabolism in the liver (only glyphosate) or kidney (only Roundup) after 24 h.	Finding not indicative of mutagenicity but could indicate one possible mechanism of toxicity.	Bolognesi *et al.*, 1997 Z59299
Measuring of DNA adducts by means of ^{32}P-postla-beling technique in the liver and kidney of mice following single i.p. administration	Glyphosate IPA salt and Roundup (IPA salt with 30.4% glyphosate a.e.; alkyle sulfate surfactant)	0, 130, 270 mg/kg bw (glyphosate IPA); 0, 400, 500, 600 mg/kg bw (Roundup)	Weak dose-related increase in adducts with Roundup; n adducts seen with the IPA salt alone and in the control group.	Indication of possible DNA damage, however, biolo-gical significance of this finding equivocal. Further characterization of ad-ducts needed. Toxicity not adressed. However, non-mutagenic toxic effects can also cause DNA adducts.	Peluso *et al.*, 1998 TOX1999-318
Comet assay for single-strand DNA breaks in tadpole erythrocytes	Roundup (41% glyphosate IPA; tallowamine surfactant)	0-1.69-6.75-27-108 mg/L water	Dose-related effect on DNA at 6.75 and 27 mg/l; completely lethal at 108 mg/l.	Impact of this formulation on tadpole DNA under environmental conditions indicated. Effect could be also due to toxicity. No relevance for human health risk evaluation.	Clements *et al.*, 1997 Z101728
Test for lethal mutations in Drosophila melanogaster after treatment of larvae	Roundup (assumed to contain 41% glyphosate IPA and tallowamine surfactant); Pondmaster (probably made from 41% glyphosate IPA; alkyle sulfate surfactant)	Not specified but indicated to be around LC_{50} concentration.	Positive.	Not predictive for mutagenicity in mammals. Concentrations used were expected to exhibit high toxicity making evaluation of results very difficult.	Kale *et al.*, 1995 Z73986
Anaphase-telophase allium test for chromosome aberrations in onion root cells	Glyphosate IPA salt (1:1 mixture) and Roundup (48% glyphosate IPA; tallowamine surfactant)	0-720-1440-2880 µg/L (for Roundup calculated as IPA salt)	Roundup: increase in chro-mosome aberrations at the two upper levels indicating rather polyploidy than clastogenicity, no clear dose response Glyphosate IPA: negative	Effects in plant cells not predictive for mutagenicity in mammals. Testing a herbicide for genotoxic effects in plants generally doubtful since cytotoxicity may be expected.	Rank *et al.*, 1993 Z82234
Chromosome aberration (CA) and Sister chromatid exchange (SCE) in human lymphocytes	Glyphosate (purity ≥ 98 %)	0-5.0-8.5-17.0-51.0 µM	Increase in CA and SCE frequency	Increase of SCE not dose related in highest dose group	Lioi et al, 1998a ASB2013-9836

Table B.6.4-26: Overview on published studies on mutagenicity of glyphosate, its salts and formulations (continued)

Test method/ test system	Test material	Dose levels/ Dose range	Results	Remarks	Reference
CA and SCE in bovine lymphocytes	Glyphosate (purity ≥ 98 %)	0-17-85-170 µM	Increase in CA and SCE frequency	Increase of SCE not dose related in highest dose group	Lioi et al, 1998b ASB2013-9837

a.e. = acid equivalents

Studies in test systems for which guidelines exist and/or much experience is available (i.e. Ames test, micronucleus test, SCE assay)

Rank *et al.* (1993, Z82234) studied the mutagenic potential of the herbicide Roundup and of glyphosate isopropylamine salt in different test systems *in vitro* as well as *in vivo*. An Ames test (plate incorporation test) was performed with Roundup only in the *Salmonella typhimurium* strains TA 98 and TA 100 with and without S9 mix for metabolic activation. Evidence of mutagenicity was confined to the strain TA 98 under non-activation conditions as indicated by a slight but significant increase in the mean number of revertants at a concentration level of 360 µg/plate (calculated as IPA salt) which was also confirmed in the repeat experiment. With activation, however, no increase in mutation rate was seen up to this dose level. From the next higher concentration (720 µg/plate) onwards, cytotoxicity became apparent with and without metabolic activation avoiding meaningful evaluation. The study authors also reported a positive result for TA 100 in the presence of S9 mix at a concentation of 720 µg/plate but already the next lower dose of 360 µg had markedly reduced the number of revertants as compared to the control suggesting a cytotoxic effect. Furthermore, a dose response was lacking. Thus, the marked increase in mutation frequency at 720 µg/plate is not reliable. In the second experiment, this dose level was not included. Without activation, concentrations from 720 µg/plate onwards appeared cytotoxic. At lower concentrations, no increase in mutation rate was noted with strain TA 100.
In a micronucleus test in mouse bone marrow erythrocytes following single i.p. administration, Roundup as well as the IPA salt (i.e., a 1:1 mixture of glyphosate technical and isoproplyamine) proved negative up to the highest dose of 200 mg/kg bw. However, with Roundup but not with the glyphosate IPA salt alone, there was evidence of bone marrow cytotoxicity at this top dose level as indicated by a significantly lower percentage of polychromatic erythrocytes.

Comment: According to the publication and to further information submitted by Monsanto, it is assumed that the Roundup formulation used was made of 48% IPA salt, tallowamine surfactant, and water. The study design of the Ames test does not comply with current guideline requirements, e.g. the plate number scored was inconsistent throughout the study. The data obtained are so controversial that a reliable interpretation is not possible. Unfortunately, a complete confirmatory experiment was not performed since repeated testing was confined to the dose of 360 µg/plate and an additional concentration of 180 µg/plate was included. A more extensive study by [redacted] (1992, TOX1999-242, see above in section I) using four S. typhimurium strains including also TA 98 and TA 100 failed to elicit any indications of mutagenicity. This latter trial was conducted in compliance with OECD guideline 471 requirements and is of higher reliability, therefore. Of course the Roundup formulations tested by Rank and her group and by [redacted] were not identical but similar since both contained only the active substance formulated as IPA salt, tallowamine surfactant, and water. The cytotoxicity of Roundup was described by both groups but the respective concentrations were different.

The design of the micronucleus test was also not in compliance with guideline requirements. A direct comparison between results obtained with the IPA salt and Roundup is not feasible since not exactly the same dose levels were used and since there was a difference in sampling time (24 and 48 h post dosing for the IPA experiment versus only at 24 h after administration of Roundup). The negative outcome of previous micronucleus studies with the IPA salt (Rodeo® formulation, [redacted], 1992, TOX9552376) and with a similar Roundup formulation in mice ([redacted], 1992, TOX1999-242) was confirmed. The reported weak bone marrow cytotoxicity occurring already after single i.p. administration of 200 mg Roundup/kg bw (amount calculated as the IPA salt to facilitate comparison) may be considered a possible formulation-related effect when the observations in other micronucleus studies (see section I) are taken into consideration.

In contrast, Bolognesi *et al.* (1997, Z59299) reported positive results from a micronucleus test in mouse bone marrow erythrocytes. Either glyphosate a.i. (declared as 99.9% pure) or a Roundup formulation were administered to Swiss mice once daily by the i.p. route on two consecutive days. Cell samples were harvested at 6 and 24 hours following the final dose. A weak positive effect was observed at total dose levels of 300 mg/kg bw (2 x 150 mg/kg bw/day) after 24 hours for glyphosate and of 450 mg/kg bw (2 x

225 mg/kg bw/day) at both sampling times for Roundup.
Further data in this publication indicated for high purity glyphosate a significant and dose- dependent increase in SCE frequency in human lymphocyte cultures obtained from two female donors from a concentration of 1000 µg/mL onwards. For Roundup, this effect became apparent even at lower concentrations of 100 and 330 µg/mL.

<u>Comment:</u> The outcome of the micronucleus test with glyphosate a.i. is at least surprising since much higher doses of this compound had been tested before and did not reveal indications of clastogenicity (see section B.5.4.2.1 in the monograph). A direct comparison is not possible since the only available test using the i.p. route in which the highest dose of 1000 mg/kg bw proved negative [redacted], 1983, TOX9552369) was performed in rats. The respective study by Rank et al. (1993, Z82234, see above) was conducted in mice but the test material was glyphosate IPA salt and the dose administered was probably too low for meaningful evaluation. However, a number of well-performed micronucleus tests with oral administration to mice is available. Even when the low oral absorption rate of glyphosate (about 30%) is taken into account, the dose levels (up to 5000 mg/kg bw nominal) are much higher than those given by Bolognesi and her co-workers but no convincing evidence of a potential to cause chromosome aberrations in vivo was obtained. It should be emphasized that the increase in the incidence of micronucleated polychromatic erythrocytes as reported in this publication was rather weak only. The test was not performed according to the current OECD guideline. In particular, the number of animals used (three male mice per dose group) was too low since a group size of at least five is recommended. A dose response cannot be assessed since only one dose level was included. The basis for statistical comparison is questionable since it is not clear when the six control animals were sacrificed because only one group mean value was indicated. Due to these deficiencies, this isolated positive finding is not considered to provide sufficient evidence to contravene the previously obtained negative results regarding the active substance.
The same methodical shortcomings apply to the experiment with the Roundup fomulation. The formulation tested is reported to contain 30.4% glyphosate acid equivalents. The a.i. is formulated as the IPA salt. Alkyl sulfate surfactant (MON 8080) is also contained (source of information: Monsanto). The weak positive response is in contrast to the beforementioned GLP-like study by [redacted]. (1992, TOX1999-242) in which Roundup® proved negative. However, these two Roundup formulations were not identical since the glyphosate concentrations were nearly the same but the surfactants contained were different making a direct comparison of the study results difficult. Little is known on mutagenicity of alkyle sulfate itself, however, MON 8080 proved negative in the Ames test but was clearly cytotoxic at relatively low concentrations (see section III of this addendum). Some evidence of bone marrow cytotoxicity was obtained with both Roundup products as indicated by a decrease in the ratio between polychromatic and normochromatic erythrocytes. Cytotoxicity could have also an impact on chromosome aberration frequency. An overall, unequivocal conclusion from the experiment of Bolognesi and her group cannot be drawn, however an actual clastogenic response is not very likely. Even if a positive result could be confirmed, it would not be applicable to products containing other surfactants. A higher SCE frequency is not considered to provide evidence of mutagenicity against the large number of studies in which glyphosate proved clearly negative. The two other studies of this type which have been submitted for purposes of toxicological evaluation of glyphosate ([redacted], 1990, TOX9500269 and [redacted], 1993, TOX9500381, the latter using the IPA salt) did not reveal an increase in sister chromatid exchange frequency but, unfortunately, did not include the high concentrations as tested by the group of Bolognesi (see section B.5.4.1.3 of the monograph). Apart from general doubts about biological significance of a positive result in an SCE assay, some methodical deficiencies became obviously in this publication. For statistical reasons, the number of only two subjects to be included in the study appears too low for meaningful evaluation. Furthermore, the data from two experiments were pooled for the two donors and individual values were not given. Therefore, a possible influence of interindividual variation could not be sufficiently assessed by the reviewer. As shown below, this variation may well reach a considerable level. Again, the positive result obtained with Roundup at least might be also due to cytotoxicity of the formulation avoiding further testing at dose levels exceeding 330 µg/ml since no mitotic cells were present anymore.

Vigfusson and Vyse (1980, TOX9700576) also reported a weak but statistically significant increase in SCE frequency in human lymphocytes obtained from two donors when the cultures were exposed to Roundup

(not specified) at concentrations of 250 and 2500 µg/mL. At the next higher concentration of 25000 µg/mL, the test substance was absolutelycytotoxic.

Comment: The reported increase is doubtful since a dose response was seen in the cultures from one of the two donors only. Furthermore, this increase in SCE frequency over the control was weak only and the statistically increased values in the cultures provided from donor 1 were below the control value from donor 2. Furthermore, possible cytotoxicity was not adressed in this paper. Generally, the SCE assay is not accepted to provide convincing evidence of mutagenicity but is rather a screening test. For clarification, the study authors themselves recommended further mutagenicity tests to be conducted.

Lioi *et al.* (1998a, ASB2013-9836 and 1998b, ASB2013-9837) reported an increase in CA and SCE frequency in human lymphocytes of 3 donors in concetrations between 5 and 51 µM and in bovine lymphocytes between 17 and 170 µM.

Comment: The results are questionable because a number of well performed and validated studies in vitro im mammalian cells and in vivo in mammals did not register comparable effects even in dose levels more than 10 times higher than the doses used in the studies described by Lioi et al. (1998a and 1998b, ASB2013-9836 and 1998b, ASB2013-9837). A replication would be needed to confirm such aberrant results.

Other test systems (Comet assay in tadpole erythrocytes, tests for DNA adducts in rats and mice, *Drosophila melanogaster*, plant cells)

Clements *et al.* (1997, *Z101728*) investigated the genotoxicity of selected herbicides in *Rana catesbeiana* (bullfrog) tadpoles using the single-cell gel DNA electrophoresis test (`Comet´ assay). After a previous study had shown a higher amount of DNA damage in bullfrog tadpoles inhabiting small bodies of water in agricultural areas as compared to non-agricultural regions, the impact of Roundup and some other commonly used herbicides on the DNA of tadpole erythrocytes was investigated in this test system under alkaline conditions. This modification allows the detection of single-stranded DNA breaks which are indicated by an increase in length: width ratio of the DNA mass following electrophoresis. DNA was obtained from tadpole erythrocytes (nucleated cells in amphibians) after the animals had been exposed to different concentrations of Roundup in the surrounding water for 24 hours. Whereas the low dose of 1.69 mg/L did not cause evidence of DNA damage, a clear and dose-dependent effect became apparent at the following concentrations of 6.75 mg/L and 27 mg/L. At 27 mg/L, the effect level caused by the positive control substance methylmethanesulphonate (MMS) was already approached. The intended top dose level of 108 mg/L could not be evaluated since all tadpoles died during the exposure period. According to the study authors, the concentrations tested were well below the recommended application levels suggesting an environmental mutagenic hazard in particular for organisms living in small adjacent bodies of water that are usually the first to be affected by pesticide runoff.

Comment: Generally, information on genotoxic effects of pesticides under natural conditions is scarce and, thus, this test system may provide important information regarding environmental effects. In this special case, however, it appears equivocal whether the observed impact on the DNA was indicative of a true mutagenic effect or rather caused by cytotoxicity. It is known that a positive response in the Comet assay may be not only the result of direct interaction with cellular DNA but can be also mediated by toxic and other effects causing apoptosis or necrosis. Cytotoxicity is not addressed in the publication because it is not directly measured in this test system. A certain degree of general toxicity can be assumed since the highest dose was completely lethal to the tadpoles. This effect could be well in line with the toxicity of certain glyphosate formulations to aquatic organisms as reported in the monograph. The Roundup product tested by Clements et al. was made of 41% glyphosate IPA salt and MON 0818, i.e. the tallowamine surfactant which is already known to cause toxic effects in different test systems in vitro as well as in vivo. Of course, although there is some evidence of a cytotoxic mechanism behind the positive result in the Comet assay, a direct impact of the test compound on the DNA cannot be completely excluded.
At this time, it is not clear whether a positive result of this test obtained in tadpole erythrocytes, even if it was actually due to mutagenicity, would be of any relevance to human beings exposed. In particular, this

is doubtful when the strong body of evidence that neither glyphosate nor its formulations are mutagenic as coming from many studies in various test systems is taken into consideration. Thus, the outcome of the Comet assay should be rather used for environmental hazard evaluation only. Again, the application of results obtained with one formulation to others must be critically regarded.

A possible impact on the DNA was also investigated by Bolognesi *et al.* (1997, Z59299) in further experiments. A transient but significant effect towards DNA damage in liver and kidney was noted in the alkaline elution assay after glyphosate (300 mg/kg bw) or Roundup (900 mg/kg bw) had been administered once by the i.p. route to mice. This assay may indicate the induction of DNA single-strand breaks and alkali labile sites. A test for DNA oxidative damage suggested glyphosate and the formulation Roundup to stimulate oxidative metabolism in the liver (glyphosate) or in the kidney (Roundup) at 24 hours after application.

In a subsequent study from the same institute (Peluso *et al.*, 1998, TOX1999-318), a low incidence of DNA adducts was found by means of the very sensitive ^{32}P-postlabeling technique in the liver and kidney of mice following single intraperitoneal administration of Roundup. All tested concentrations (400, 500 and 600 mg Roundup/kg bw, corresponding to 122, 152, and 182 mg glyphosate salt/kg bw) caused DNA adducts in both organs. A dose response was to be seen. In contrast, treatment with the vehicle (i.e., a DMSO/olive oil mixture) and with doses of 130 and 270 mg glyphosate IPA salt/kg bw did not result in DNA adduct formation.

Comment: *The data from the tests for DNA damage and stimulation of oxidative metabolism (Bolognesi et al., 1997, Z59299) are hardly to interpret since the results are given in summary figures only which are based on pooled individual data. There are reporting inconsistencies, e.g. it is not clear how many animals were actually used for testing. A positive control substance was not included. Taking into account that glyphosate proved negative in the UDS assay which is generally accepted to indicate a more frequent occurrence of DNA damage and repair (see section B.5.4.1.3 in the monograph), the published findings are not considered to provide convincing evidence of an interaction with the DNA. Positive results in the alkaline elution assay may also occur as a result of toxic but not-mutagenic effects. Stimulation of oxidative metabolism is not a sign of mutagenicity but may elucidate a possible mechanism behind toxic effects.*

The results of Peluso (1998, TOX1999-318) and his group suggest a direct effect on the DNA. It has been shown that the observed effects were related to administration of the formulation only but not to glyphosate IPA salt. Biological significance of the results is equivocal. Generally, it is questionable whether findings after i.p. administration can be applied to more realistic exposure conditions. Of course, the occurrence of such effects also after oral intake would be much more relevant for human health evaluation. Furthermore, some deficiencies of this study make a definitive assessment difficult. It is rather equivocal what a low incidence of DNA adducts per animal as compared to no adducts in the control group actually means since a positive control substance was not included. The degree of variation between the animals is not known because only mean values for the groups' comprizing of 3 to 6 mice were reported and individual values are not given but would be helpful for interpretation of the results. Another point of concern is the lacking information on toxicity. At least with Roundup, one could expect marked general toxicity when the observations reported from the micronucleus tests (see section I of this addendum) and from the acute intraperitonal toxicity studies (see section B.5.2.4 in the monograph) were taken into account. It is known that DNA adducts may be formed not only as a result of direct interaction of cellular DNA with chemicals but also occur naturally or can be even related to a treatment-dependent increase in endogenous metabolites. Thus, further characterisation of these adducts and clarification of their nature would be desirable.

Kale *et al.* (1995, Z73986) examined nine agricultural chemicals in the sex-linked recessive lethal test in *Drosphila melanogaster* for their ability to cause genotoxic damage to the germ cells leading to lethal mutations in the subsequent generations. The group of test compounds included two insecticides and seven herbicides among those were the glyphosate formulations Roundup and Pondmaster. Unlike the generally used method of feeding the test substance to adult males only, larvae were treated in this experiment. This modification was expected by the study scientists to improve the sensitivity of the test system. All products tested proved positive.

Comment: This test system is not considered predictive for mutagenicity in mammals. Generally, tests in Drosophila are considered helpful for screening purposes. For glyphosate, however, a large database on the basis of much more reliable test systems does exist. Furthermore, since lethal changes in spermatogonia and spermatocytes were the relevant endpoint, it appears difficult to distinguish between mutagenicity and general toxicity. The dose level tested was not specified but it is stated in the publication that concentrations around the LC_{50} were used. At such a high dosage, some toxicity must be expected.

An anaphase-telophase allium test in onion root cells was conducted by Rank *et al.* (1993, Z82234) to detect a possible induction of chromosome aberrations. The exposure period was 24 hours. In this plant system, a significant increase in the occurrence of chromosome aberrations was noted at the two upper dose levels when Roundup was tested. However, there was no dose response, since the total incidence of aberrations at 1440 µg/L was twice that seen at 2880 µg/L. The authors attributed this lack of a clear dose response to cytotoxicity, however, mitotic index was not dramatically reduced (24.2 in the mean at 2880 µg/L versus 28.2 at 1440 µg/L). According to the investigators, the type and pattern of aberrations suggest rather spindle disturbances than clastogenicity in particular when compared to the effects caused by the positive control substance MMS. In contrast, the glyphosate IPA salt did not increase the frequency of chromosome aberrations in this experiment.

Comment: The Roundup product tested was made of the IPA salt, tallowamine surfactant, and water (for details see description of the Ames test and the micronucleus test portions of this study above). The more pronounced effect of the formulation as compared to the IPA salt could be explained by an improved uptake by the onion root cells as mediated by the surfactant. However, genotoxic or aneugenic effects in a plant system are generally not accepted to be indicative of mutagenicity in mammals. For glyphosate and its formulations, a number of well-performed studies in mammals is available for this purpose. Generally, it appears questionable whether an herbicide should be tested for mutagenicity in a plant cell system since at least a certain degree of cytotoxicity must be expected.

Assessment

In the whole, the published data are not sufficient to provide convincing evidence of mutagenic effects caused by glyphosate or its formulations. Of cours, the effects observed in different test systems cannot be totally ignored. Looking for an explanation, the data obtained in the mutagenicity studies with formulations (see section I) must be also considered. Taking all the findings together, the effects reported in the literature appear rather due to cytotoxic properties of the formulations than to a genotoxic mode of action. The same conclusion was also reached by the Danish EPA in an assessment (Rasmussen, 1997, ASB2013-9671) which was made available to the Rapporteur. It has been already known before, that cytotoxicity is much more pronounced with glyphosate formulations than with the active substance and, therefore, is probably due to by-products or impurities. In particular, surfactants are the agents to be suspected for causing such effects.

There are even data suggesting the possiblity of a direct interaction of glyphosate formulations with cellular DNA in some test systems. This is evidenced by a higher frequency of DNA adducts in mouse liver and kidneys following i.p. administration (Peluso *et al.*, 1998, TOX1999-318) as well as from the Comet assay in tadpole erythrocytes (Clements *et al.*, 1997, Z101728). Since glyphosate active ingredient is apparently devoid of a DNA damaging potential (see monograph), these effects, if occurring, can be certainly assumed to be related to co-formulants. Damage to the DNA is not essentially indicative of mutagenicity but could also result from cytotoxicity. Irrespective of the origin of these effects on DNA level, they appear to be confined to very special exposure situations only and not to represent a health hazard to human beings.

B.6.1.5 Published data (released since 2000)

B.6.4.8.1 Introduction

An earlier review of the toxicity of glyphosate and the original Roundup™ formulation concluded that neither glyphosate nor the formulation pose a risk for the production of heritable/somatic mutations in

humans (Williams *et al.*, 2000, ASB2012-12053). This review of subsequent glyphosate genotoxicity publications includes analysis of study methodology and incorporation of all the findings into a weight of evidence for genotoxicity. Two publications provided limited additional support for the conclusion that glyphosate and glyphosate based formulations (GBFs) are not active in the gene mutation assay category. The weight of evidence from *in vitro* and *in vivo* mammalian chromosome effects studies supports the earlier conclusion that glyphosate and GBFs are predominantly negative for this end point category. Exceptions are mostly for unusual test systems but there are also some unexplained discordant positive results in mammalian systems. Several reports of positive results for the SCE and comet DNA damage endpoints have been published for glyphosate and GBFs. The data suggest that these DNA damage effects are likely due to cytotoxic effects rather than DNA reactivity. This weight of evidence review concludes that there is no significant *in vivo* genotoxicity and mutagenicity potential of glyphosate or GBFs that would be expected under normal exposure scenarios.

B.6.4.8.1 General review and analysis considerations

The published studies for review consideration were identified by literature searches for published reports containing references to glyphosate or glyphosate based formulations (GBFs) that also contained searchable terms which indicated that genotoxicity studies were performed. Literature search utilised Chemical Abstracts (provided by Chemical Abstracts Service, a division of the American Chemical Society) and Web of Knowledge (Thompson Reuters), using the following modules: Web of ScienceSM, BIOSIS Previews®, MEDLINE®, and CAB Abstracts® (CABI) abstracting services. Search criteria were as follows (glyphosate acid and the various salts): glyphosat* OR glifosat* OR glyfosat* OR 1071-83-6 OR 38641- 94-0 OR 70901-12-1 OR 39600-42-5 OR 69200-57-3 OR 34494-04-7 OR 114370-14-8 OR 40465-66-5 OR 69254-40-6 OR (aminomethyl w phosphonic*) OR 1066-51-9. Each identified publication was evaluated to verify that it contained original results of one or more genotoxicity studies on glyphosate or GBFs. Emphasis was placed on publications in peer- reviewed journals and abstracts or other sources with incomplete information were not considered. Reviews without original data were not considered for evaluation; however, these reviews were examined to determine if there were any cited publications that had not been detected in the literature searches.

Each relevant publication was examined using several criteria to characterize the scientific quality of the reported genetic toxicology studies. Useful, objective criteria for this purpose were international guidelines for genetic toxicology studies developed by expert groups. These include principles for conducting studies, reporting results and analyzing and interpreting data. Some of the principles of the guidelines are generally applicable to categories of studies or all studies while others are specific for a particular type of test system and end point. Some of the specific types of studies encountered in the review do not yet have international guidelines; however, some of the guideline elements should be generically applicable to these studies. The guidelines for genetic toxicology tests developed for the Organisation for Economic Cooperation and Development (OECD) are a pre-eminent source of internationally agreed and expert guidelines. Other regulatory international and national regulatory genetic toxicology testing guidance are usually concordant with the OECD guidelines. Table B.6.4-28 presents some key OECD guideline criteria that were found to be relevant to analysis of the studies considered in this review.

Comparison of the published studies to the criteria in guidelines used for regulatory purposes does not represent an absolute judgment standard but it does serve to provide one means of characterization of the various published studies. Some of the criteria are rarely met in scientific publications. For example, data for individual cultures and individual animals are not commonly included in publications in scientific journals. These data are presumably collected but are usually summarised as means with a measure of variance for the treatment and control groups. This is not considered to be a significant omission in a scientific publication. However, other guideline features are more essential in demonstrating scientific quality standards and should be considered as having greater weight in evaluating a study. For example, there are consistent recommendations that assays involving visual scoring (e.g. chromosome aberration, micronucleus and sister chromatid exchange) should use slides that are independently coded so that scoring is performed without knowledge of the treatment or control group being scored. This guidance is good scientific practice and studies that do not include a description of coding or "blind" scoring in the methodology would appear to have a deficiency either in the methodology or the description of the methodology used. Other examples of guideline features that have clear experimental scientific value are

the use of concurrent negative and positive controls and concurrent measurement and reporting of toxicity endpoints in main experiments, especially in in vitro mammalian cell assays.
Test materials, as described in the publications, were reviewed by industry experts to identify any publicly available and useful information on composition for the reported formulations to assist in interpreting the relevance of findings to glyphosate and/or formulation components. It should be noted that a common problem encountered in the published literature is the use of the terms "glyphosate", "glyphosate salt" or "Roundup" to indicate what may be any GBF that contains additional components such as surfactants. Published results from studies with different formulations have sometimes been incorrectly or inappropriately attributed to the active ingredient. The original Roundup formulation (MON 2139), containing 41% isopropyl amine glyphosate salt and 15.4% MON 0818 (a polyethoxylated tallowamine based surfactant blend), is no longer sold in many markets. However, other glyphosate based formulations are sold under the Roundup brand name with varying glyphosate forms, concentrations and surfactant systems. Clear identification of the test material is very important in toxicology studies because toxicity of formulations can be dramatically different than the active ingredient. The fact that test materials identified as Roundup formulations may actually have different compositions should be considered when comparing results of different studies. A major consideration, especially for DNA damage endpoints and for in vitro mammalian cell assays, is an assessment of whether observed effects might be due to toxicity or extreme culture conditions rather than indicating DNA-reactive mediated processes. Relevant considerations include control of medium pH and osmolality for in vitro mammalian cell studies and whether effects are observed only at cytotoxic doses or in association with severe toxicity to the test system. Other important generic considerations in evaluating experimental results of each published study are evidence of experimental reproducibility and whether a biologically plausible dose response has been demonstrated.

Table B.6.4-28: Genetic Toxicology Test Guideline Criteria

Area	Guidance	Reference
All studies	Test material purity and stability should be reported	OECD 471 (1997)
	Concurrent negative and positive controls should be included with each assay	OECD 473 (1997)
Assays with visual scoring	All slides should be independently coded before analysis (i.e. scored without knowledge of the treatment or control group)	OECD 473 (1997) OECD 479 (1986)
In vitro mammalian cell assays	Assay should be usually be conducted in the presence and absence of an appropriate exogenous metabolic activation system	OECD 473 (1997)
	Cytotoxicity should be determined in the main experiment	
	At least three analyzable concentrations should be used	
	Maximum dose determined by toxicity or 5 µg/mL, 5 mg/mL or 10 mM for soluble non-toxic test materials	
	Individual culture data should be provided	
In vivo mammalian assays	Five analyzable animals per group. Single sex may be used if there are no substantial difference in toxicity between sexes	OECD 475 (1997) OECD 474 (1997)
	Limit dose for non-toxic substances of 2000 mg/kg for treatments up to 14 days and 1000 mg/kg for treatments longer than 14 days	
In vitro chromosome aberration	Treatment for 3-6 hours in one experiment and harvest at 1.5 cell cycles. If negative a second experiment with continuous treatment for 1.5 cell cycles	OECD 473 (1997)
	Scoring of at least 200 metaphases ideally divided between duplicate cultures	
In vitro sister chromatid exchange	Treatment for 1-2 hours up to two cell cycles with harvest after two cell cycles in the presence of bromodeoxyuridine	OECD 479 (1986)
	Scoring of 25 metaphases per culture (50 per treatment group)	
In vitro micronucleus	Most active agents detected by treatment for 3-6 hours with harvest at 1.5-2 cell cycles after treatment. An extended treatment for 1.5-2 cycles in the absence of metabolic activation is also used	OECD 487 (2010)
	Scoring of at least 2000 binucleated cells or cells for micronuclei for each treatment or control group	

In vivo bone marrow chromosome aberration	Single treatment with first harvest at 1.5 cell cycles after treatment and second harvest 24 hour later or single harvest 1.5 cycles after last treatment for multiple daily treatments	OECD 475 (1997)
	Three dose levels usually recommended except when limit dose produces no toxicity	
	Concurrent measures of animal toxicity and toxicity to target cells	
	At least 100 cells analyzed per animal	
	Individual animal data should be reported	
In vivo erythrocyte micronucleus	Three dose levels for first sampling time	OECD 474 (1997)
	Treatment once with at least 2 harvests usually at 24 and 48 h after treatment or one harvest 18-24 h after final treatment if two or more daily treatments are used	
	Scoring of 2000 immature erythrocytes per animal or 2000 mature erythrocytes for treatments of 4 weeks or longer	

Table B.6.4-29 presents a summary of genotoxicity test results for glyphosate and GBFs published subsequent to Williams *et al.* (2000, ASB2012-12053). Test results are organised by the major genotoxicity assay categories of gene mutation, chromosome effects and DNA damage and other end points. Major features presented for each publication are the assay endpoint, the test system, the test material, the maximum dose tested and comments relevant to the reported conduct and results of the assay. For brevity, earlier reviewed individual publications of genotoxicity study results are referred to by citation of (Williams *et al.*, 2000, ASB2012-12053) rather than the original references reviewed in (Williams *et al.*, 2000, ASB2012-12053).

Genetic toxicology studies of glyphosate and glyphosate formulations published on or after 2000

End point	Test System	Test Material	Maximum Dose	Result	Comment[a]	Reference
In Vitro Gene Mutation						
Point mutation	Ames strains	Perzocyd 10 SL formulation	2 µg/plate (toxic)	Negative	TA1535 not used	Chrusciels ka *et al.*, 2000, (ASB2013-9830)
Wing spot test	Drosophila	Glyphosate (96%)	10 mM in larval stage	Negative/ inconclusive[c]	Negative or inconclusive in crosses not sensitive to recombination events	Kaya *et al.*, 2000, (ASB2013-9832)
In Vitro Chromosome Effects—Mammalian Systems						
Cytokinesis block micronucleus	Bovine lymphocytes	Glyphosate formulation (62% glyphosate Monsanto source)	560 µM 48 h –S9	Positive?	PH, MA, SC, TO	Piesova, 2004 (ASB2012-12001)
Cytokinesis block micronucleus	Bovine lymphocytes	Glyphosate formulation (62% glyphosate Monsanto source)	560 µM 48 h –S9 2 h –S9 2 h +S9	Positive? Negative Negative	PH, SC, TO	Piesova, 2005 (ASB2012-12000)
Chromosome aberration	Mouse spleen cells	herbazed formulation	50 µM?	Positive	Concentrations used not clear. PH, MA, SC, TO, RE	Amer *et al.*, 2006 (ASB2012-11539)

Genetic toxicology studies of glyphosate and glyphosate formulations published on or after 2000

Chromosome aberration	Bovine lymphocytes	Glyphosate formulation (62% glyphosate) Monsanto source	1.12 mM (toxic) (24 h)	Negative	Chromosome 1 FISH analysis. PH, MA, PC, SC, TO, RE	Holeckova, 2006 (ASB2012-11847)
Chromosome aberration	Bovine lymphocytes	Glyphosate formulation (62% glyphosate) Monsanto source	1.12 mM (toxic) (24 h)	Negative	PH, MA, SC, RE	Sivikova and Dianovsky, 2006 (ASB2012-12029)
Chromosome aberration	Human lymphocytes	Glyphosate (96%)	6 mM (not toxic)	Negative	MA, IC, RE	Manas *et al.*, (2009 ASB2012-11892)
Cytokinesis block micronucleus	Human lymphocytes	Glyphosate (technical, 96%)	580 µg/mL (toxic) (est. 3.43 mM)	Negative (-S9) Positive (+S9)	SC, RE	Mladinic *et al.*, 2009 (ASB2012-11906)
Cytokinesis block micronucleus	Human lymphocytes	Glyphosate (technical, 96%)	580 µg/mL (toxic) (est. 3.43 mM)	Negative (-S9) Positive (+S9)	SC, RE	Mladinic *et al.*, 2009 (ASB2012-11907)
In Vitro Chromosome Effects— Non Mammalian Systems						
Chromosome aberration	Onion root tip meristem	Roundup formulation (Bulgaria)	1% active ingredient (estimated 4.4-5.9 mM)	Negative	TO, IC, RE	Dimitrov *et al.*, 2006 (SB2012-11607)
Micronucleus	Onion root tip meristem	Roundup formulation (Bulgaria)	1% active ingredient (estimated 4.4-5.9 mM)	Negative	TO, RE	Dimitrov *et al.*, 2006 (SB2012-11607)
In Vivo Chromosome Effects—Mammalian Systems						
Bone marrow erythrocyte micronucleus	Mouse	Glyphosate	300 mg/kg i.p. Perzocyd 10 SL formulatio n	Negative Negative	DL, TO, SC, IM, RE DL, TO, SC, IM, RE	Chrusciels ka *et al.*, 2000, (ASB2013-9830)
Bone marrow erythrocyte micronucleus	Mouse	Roundup 69 formulation	2 x 200 mg/kg i.p.	Negative	TO, SC, IE, RE	Coutinho do Nasciment o and Grisolia, 2000 (ASB2013-11477)
Bone marrow erythrocyte micronucleus	Mouse	Roundup™ formulation (Monsanto)	2 x 200 mg/kg i.p.	Negative	TO, SC, IE, RE	Grisolia, 2002 (SB2012-11834)
Bone marrow Chromosome aberration	Rabbit	Roundup™ formulation	750 ppm in drinking water	Positive?	DL, PC, TO, SC, IC	Helal and Moussa, 2005 (ASB2012-11841)
Bone marrow Chromosome aberration	Mouse	Herbazed formulation (84%	50 mg/kg i.p. (1,3, 5 days)	Negative	TO, SC, RE	Amer *et al.*, 2006 (ASB2012-

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		glyphosate)	100 mg/kg oral (1,7, 14, and 21 days)	Positive		11539)
Spermatocyte Chromosome aberration	Mouse	Herbazed formulation (84% glyphosate)	50 mg/kg i.p. (1,3, 5 days) 100 mg/kg oral (1,7, 14, and 21 days)	Negative Positive	TO, SC, RE	Amer *et al.*, 2006 (ASB2012-11539)
Bone marrow Chromosome aberration	Mouse	Roundup formulation (Bulgaria)	1080 mg/kg p.o. (1/2 LD50)	Negative	DL, TO, IC, RE	Dimitrov *et al.*, 2006 (ASB2012-11607)
Bone marrow erythrocyte micronucleus	Mouse	Analytical glyphosate (96%)	2 x 200 mg/kg i.p.	Positive	Erythrocytes scored? TO, SC, IC, RE	Manas *et al.*, 2009 (ASB2012-11892)
Bone marrow Chromosome aberration	Mouse	Roundup™ formulation (Monsanto)	50 mg/kg i.p.	Positive	DL, SC, IC, RE	Prasad *et al.*, 2009 (ASB2012-12005)
In Vivo Chromosome Effects—Non-Mammalian Systems						
Erythrocyte micronucleus	Oreochromis niloticus (Tilapia)	Roundup 69	170 mg/kg i.p. (maximum tolerated)	Negative?[c]	TO, RE	Coutinho do Nasciment o and Grisolia, 2000 (ASB2013-11477)
Wing spot test	Drosophila	Glyphosate (96%)	10 mM in larval stage	Positive/inconclusive[b]		Kaya *et al.*, 2000 (ASB2013-9832)
Erythrocyte micronucleus	Tilapia	Roundup™ formulation (Monsanto)	170 mg/kg (abdominal injection)	Positive	TO, RE	Grisolia, 2002 (ASB2012-11834)
Erythrocyte micronucleus	Crasseus auratus (goldfish)	Roundup formulation	15 ppm glyphosate in water (2, 4 and 6 days)	Positive	TO, IE, RE	Cavas and Konen, 2007 (ASB2012-11587)
	Prochilodus lineatus (tropical fish)	Roundup™ formulation (75% of 96 h LC50)	10 mg/l (6, 12 and 24 h) in water	Negative	DL, TO, SC, RE	Cavalcante *et al.*, 2008 (ASB2012-11586)
Erythrocyte micronucleus	Caiman eggs	Roundup® Full II formulation	1750 µg/egg	Positive	RE	Poletta *et al.*, 2009 (ASB2012-12002)
Erythrocyte micronucleus	Caiman eggs	Roundup® Full II formulation	Sprayed 2x with 100 litres of 3%/ha 30 days apart	Positive	DL, TO, RE	Poletta *et al.*, 2009 (ASB2012-12002)

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Micronucleus (and alkaline SCGE)	Fish (Guppy)	Roundup® Transorb	5.65 µg/l	Positive		De Souza Filho *et al.*, 2013 (ASB2014-7617)
In Vitro DNA Damage Mammalian Systems						
Alkaline SCGE	GM38 human fibroblasts and HT1090 human fibrosarcoma	Glyphosate (technical grade)	6.5 mM	Positive	MA, PH, TO, SC, RE	Monroy *et al.*, 2005 (ASB2012-11910)
Sister chromatid exchange	mouse spleen cells	herbazed formulation	50 µM?	Positive	Concentrations used not clear MA, PH, TO, SC, RE	Amer *et al.*, 2006 (ASB2012-11539)
Sister chromatid exchange	bovine lymphocytes	Glyphosate formulation (62% glyphosate, Monsanto)	1.12 mM (toxic)	Positive	PH, SC, RE	Sivikova and Dianovsky, 2006 (ASB2012-12029)
Alkaline single cell gel electrophoresi s (SCGE, comet)	Hep-2 cells	Glyphosate (analytical, 96%)	7.5 mM (limited by toxicity)	Positive	MA, PH, RE	Manas *et al.*, 2009 (ASB2012-11892)
Alkaline SCGE	Human lymphocytes	Glyphosate (technical, 96%)	580 µg/ml (toxic) (est. 3.43 mM)	Positive (- S9) Positive (+S9)		Mladinic *et al.*, 2009 (ASB2012-11906)
SCGE	Human lymphocytes (compared with Tilapia erythrocytes and Tradescantia nuclei)	Glyphosate (96%)	700 µM	Positive (according to authors)	Inconsitent and not clear dose dependent	Alvarez- Moya *et al.*, 2014 (ASB2014-6902)
SCGE	Human buccal epithelial cells	Glyphosate (95%) and Roundup Ultra Max	200 mg/l	Positive	Higher activity of formulation than pure a. s.	Koller *et al.*, 2012 (ASB2014-7618)
In Vitro DNA Damage Non-Mammalian Systems						
SOS	E. coli	Roundup BIO formulation	2.5 µg/sample	Positive		Raipulis *et al.* 2009 (ASB2012-12008)
Alkaline SCGE	Tradescantia flowers and nuclei	Glyphosate(technical, 96%)	700 µM	Positive	PH, SC	Alvarez- Moya *et al.*, 2011 (ASB2012-11538)
In Vivo DNA Damage Mammalian Systems						
Spermatocytes and bone marrow	Mouse	herbazed formulation (84% glyphosate)	200 mg/kg p.o.	Positive	TO, SC, RE	Amer *et al.*, 2006 (ASB2012-11539)

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SCGE blood cells, liver cells,	Mouse	Glyphosate (96%) and AMPA	400 mg/kg bw/day Glyphosate or 100 mg/kg bw/day AMPA	Glyphosate and AMPA positive		Manas *et al.*, 2013 (ASB2014-6909)
In Vivo DNA Damage Non-Mammalian Systems						
Erythrocyte alkaline SCGE	Crasseus auratus (goldfish)	Roundup formulation	15 ppm glyphosate in water (2, 4 and 6 days)	Positive	TO, RE	Cavas and Konen, 2007 (ASB2012-11587)
Erythrocyte and gill cell alkaline SCGE	Prochilodus lineatus (tropical fish)	Roundup™ formulation (75% of 96 h LC_{50})	10 mg/l (6, 12 and 24 h) in water	Positive	DL, TO, RE	Cavalcante *et al.*, 2008 (ASB2012-11586)
Erythrocyte alkaline SCGE	Caiman eggs/hatchlin gs	Roundup® Full II formulation	1750 µg/egg	Positive	RE	Poletta *et al.*, 2009 (ASB2012-12002)
Erythrocyte alkaline SCGE	European eel	Roundup formulation	166 µg/liter	Positive	DL, SC, RE	Guilherme *et al.*, 2010 (ASB2012-11836)
Erythrocyte alkaline SCGE	Caiman eggs/hatchlings	Roundup® Full II formulation	Sprayed 2x with 100 l of 3%/ha 30 days apart	Positive	DL, RE	Poletta *et al.*, 2009 (ASB2012-12002)
SCGE blood cells	European eel	Roundup Ultra and Glyphosate and POAE	116 µg/l 35.7 µg/l 18.6 µg/l	positive	No increased effect of glyphosate in combination with POAE	Guilherme *et al.*, 2012 (ASB2014-7619)
SCGE	Fish (Prochilidus)	Roundup Transorb and Glyphosate	5 mg/l 2.4 mg/l	positive	Inconsistent and not clearly dose dependent	Moreno *et al.*, 2014 (ASB2014-7522)

[a] MA, Mammalian metabolic activation system not used and short exposure not used; PH, no indication of pH or osmolality control;
DL, less than three dose levels used; PC, no concurrent positive control;
TO, no concurrent measurement of toxicity reported or toxicity not observed for highest dose level;
SC, independent coding of slides for scoring not indicated for visually scored slides;
IC, less than 200 cells scored per treatment or less than 100 metaphases scored per animal for chromosome aberrations;
IE, less than 2000 erythrocytes scored per animal;
RE, results not reported separately for replicate cultures or individual animals;

[b] Positive for small wing spots only in one cross. Negative or inconclusive for all spot categories for three other crosses.

[c] Statistically significant increase in micronucleated PCE frequency only at mid dose level but overall result judged negative.

A new comprehensive review on genotoxicity studies of glyphosate and glyphosate-based formulations was submitted by Kier and Kirkland (2013, ASB2014-9587). The authors concluded that an overwhelming preponderance of negative results in well-conducted bacterial reversion and *in vivo* mammalian micronucleus and chromosomal aberration assays indicates that glyphosate and typical GBFs are not genotoxic in these core assays. Negative results for *in vitro* gene mutation and a majority of negative results for chromosomal effect assays in mammalian cells add to the weight of evidence that glyphosate is not typically genotoxic for these endpoints in mammalian systems. Mixed results were observed for micronucleus assays of GBFs in non-mammalian systems. Reports of positive results for DNA damage

endpoints indicate that glyphosate and GBFs tend to elicit DNA damage effects at high or toxic dose levels, but the data suggest that this is due to cytotoxicity rather than DNA interaction with GBF activity perhaps associated with the surfactants present in many GBFs. Glyphosate and typical GBFs do not appear to present significant genotoxic risk under normal conditions of human or environmental exposures.

B.6.4.8.1 Structure Activity Analysis

B.6.4.8.1 Gene Mutation

As reviewed by Williams *et al.*, (2000, ASB2012-12053), most gene mutation studies for glyphosate and GBFs were negative. Gene mutation assays included numerous Ames/*Salmonella* and *E. coli* WP2 bacterial reversion assays, *Drosophila* sex-linked recessive lethal assays and a CHO/HGPRT *in vitro* mammalian cell assay. Of fifteen gene mutation assays reported, there were only two positive observations. A reported positive Ames/*Salmonella* result for Roundup formulation was not replicated in numerous other studies. There was one report of a positive result for a GBF in the *Drosophila* sex-linked recessive lethal assay but this was contradicted by a negative result for the same GBF in this assay reported by another laboratory. Further, the positive study had some features that hampered interpretation, including the lack of concurrent negative controls (Williams *et al.*, 2000).

Subsequent to the Williams *et al.* (2000, ASB2012-12053) review only two gene mutation studies have been reported (Table B.6.4-29). One negative Ames/*Salmonella* assay result was reported for a GBF of undefined composition, Percozyd 10 SL (Chruscielska *et al.*, 2000, ASB2013-9820). Although this result is consistent with a large number of negative Ames/*Salmonella* results for glyphosate and GBFs, the reported study results have significant limitations. One of the recommended test strains, TA1535, was not used and results were only presented as "-" without presentation of revertant/plate data. A positive result for glyphosate was reported in the *Drosophila* wing spot assay which can indicate both gene mutation and mitotic recombination endpoints (Kaya *et al.*, 2000, ASB2013-9832). Small increases in small wing spot frequencies were observed in one of four crosses of larvae treated with up to 10 mM glyphosate. The lack of a positive response in the balancer-heterozygous cross offspring, which are insensitive to mitotic recombination events, suggests that there is no evidence for effects on gene mutation endpoint events such as intragenic mutations or deletions in this publication.

These gene-mutation publications add very limited data to the weight of evidence conclusion that glyphosate and GBFs do not pose significant risk for gene mutation

B.6.4.8.1 Chromosome effects

Assays to detect chromosome effects such as structural chromosome aberrations and micronucleus incidence constitute a second major genotoxicity end point category. A large number of publications with chromosome effects endpoints have been reported since the Williams *et al.* (2000, ASB2012-12053) review. These are described in Table B.6.4-29 and are separated into various test system categories which include *in vitro* cultured mammalian cell assays, *in vitro* tests in non-mammalian systems, *in vivo* mammalian assays and *in vivo* assays in non-mammalian systems. A *Drosophila* wing spot test (discussed previously) is also included in this category because results are relevant to somatic recombination.

B.6.4.8.1.1 *In vitro* chromosome effects

Two human and one bovine *in vitro* peripheral lymphocyte chromosome aberration studies of glyphosate were considered in the earlier review (Williams *et al.*, 2000, ASB2012-12053). One human lymphocyte *in vitro* study had negative results for glyphosate tested up to approximately 2-3 mM (calculated from reported mg/ml) in the absence and presence of an exogenous mammalian activation system. The other two studies with human and bovine lymphocytes and no metabolic activation system reported positive results at concentrations more than two orders of magnitude lower. The earlier review noted several other unusual features about the positive result studies including an unusual exposure protocol and discordant positive results for another chemical found negative in other laboratories.

As indicated in Table B.6.4-29 both positive and negative results have been reported for glyphosate and GBFs in the nine *in vitro* chromosome effects assays published after the Williams *et al.* (2000, ASB2012-12053) review. It is noteworthy that many of these studies have various deficiencies in conduct or reporting

compared to internationally accepted guidelines for conduct of *in vitro* chromosome aberration or micronucleus studies (see Table B.6.4-28). Perhaps the most significant deficiency was that coding and scoring of slides without knowledge of the treatment or control group was not indicated in seven of nine publications. This could be a deficiency in conducting the studies or perhaps a deficiency in describing methodology in the publications. Other common deficiencies included failure to indicate control of exposure medium pH, no use of exogenous metabolic activation and no reporting of concurrent measures of toxicity.

Results for glyphosate active ingredient

Three publications reported testing of technical glyphosate for micronucleus or chromosome aberration endpoints in cultured human lymphocytes (Manas *et al.*, 2009, ASB2012-11892; Mladinic *et al.*, 2009, ASB2012-11906; Mladinic *et al.*, 2009, ASB2012-11907). Negative results for the micronucleus or chromosome aberration end points were observed in the absence of exogenous metabolic activation (S9) in all three publications. The maximum exposure concentration in the absence of S9 was in the range of 3-6 mM in these studies.

Two publications by one author reported cytokinesis block micronucleus results for cultured bovine lymphocytes treated with what was reported as 62% by weight isopropyl amine salt of glyphosate from a Monsanto Belgium source (Piesova, 2004, ASB2012-12001; Piesova, 2005, ASB2012-12000). This test material appears to be a manufacturing batch of the isopropylamine salt of glyphosate in water without surfactants, which is not sold as a GBF. In one publication no statistically significant increases in binucleated cell micronucleus frequency were observed with 24 hours of treatment (Piesova, 2004, ASB2012-12001). For 48 hours of treatment a statistically significant increase in micronucleus frequency was observed in one donor at 280 µM but not at 560 µM and in a second donor at 560 µM but not 280 µM. The second publication reported negative results for the cytokinesis block micronucleus assay in bovine lymphocytes incubated with glyphosate formulation up to 560 µM for two hours in the absence and presence of a mammalian metabolic activation system (Piesova, 2005, ASB2012-12000). This publication also reported positive results for 48 hours of treatment without S9. Curiously, in this second publication the same inconsistent dose response pattern was observed in which a statistically significant increase in micronucleus frequency was observed in one donor at 280 µM but not at 560 µM and in a second donor at 560 µM but not 280 µM. The lack of a consistent dose response pattern between donors suggests that the results with 48 hours of treatment are questionably positive.

Two other publications found negative results for the chromosome aberration endpoint in cultured bovine lymphocytes treated with what appears to be the same test material of 62% by weight isopropylamine salt of glyphosate from a Monsanto Belgium source, (Holeckova, 2006, ASB2012-11847; Sivikova and Dianovsky, 2006, ASB2012-12029). Both the studies used a maximum concentration of 1.12 mM which was reported to cause a decrease in mitotic inhibition of >50%. These two studies have several limitations including that an exogenous mammalian metabolic activation system was not used for chromosome aberration and scoring was not reported to be on coded slides. In addition, Holeckova (2006, ASB2012-11847) only examined effects detectable by staining of chromosome 1 and did not report positive control results (Holeckova, 2006, ASB2012-11847). Despite these limitations and the variable donor results, the results from these two studies are generally consistent with a lack of chromosome aberration effects of the isopropylamine salt of glyphosate on *in vitro* cultured mammalian cells in several experiments using high, toxic dose levels and exposures of 2-24 hours in the absence of S9.

One laboratory reported increases in cytokinesis-blocked micronucleus frequency in cultured human lymphocytes exposed to glyphosate for 4 hours in the presence of an exogenous human liver metabolic activation system (S9) in two publications (Mladinic *et al.*, 2009a; Mladinic *et al.*, 2009b). In both publications a statistically significant increase in micronuclei was observed with S9 at the highest dose level of glyphosate tested (580 µg/mL, ≈ 3.4 mM). Increased proportions of centromere- and DAPI-positive micronuclei were observed for the high dose with S9 suggesting that the induced micronuclei were derived from chromosomes rather than chromosome fragments. Statistically significant increases in the frequency of nuclear abnormalities (buds and bridges) and DNA strand breakage were also observed at the highest dose tested in both publications. In parallel experiments cytotoxic effects such as early apoptosis, late apoptosis and necrosis were observed and these effects were uniquely or preferentially observed in the presence of S9 and at the highest dose level tested (Mladinic *et al.*, 2009, ASB2012-11906). Also, the

negative control level of such end points as necrosis and alkaline SCGE tail moment was significantly increased in the presence of S9 (Mladinic *et al.*, 2009, ASB2012-11906). It should be noted that glyphosate is mostly excreted unmetabolised *in vivo* in mammals with only very small levels of aminomethylphosphonic acid (AMPA) or an AMPA-related structure observed (Anadon *et al.*, 2009, ASB2012-11542; Brewster *et al.*, 1991, TOX9551791). These observations suggest that the observations of S9 mediated effects by Mladinic *et al.* are not likely to be due to *in vivo* relevant metabolites. The preponderance of *in vitro* genotoxicity studies conducted with exogenous mammalian metabolic activation systems has been negative, including a previously reviewed chromosome aberration study in human lymphocytes conducted up to a similar dose level (Williams *et al.*, 2000, ASB2012-12053) and a bovine lymphocyte cytokinesis block micronucleus study (Piesova, 2005, ASB2012-12000). Overall these results suggest the possibility of a weak aneugenic rather than clastogenic (chromosome breaking) effect occurring in the presence of S9 at high dose levels of glyphosate. The pattern of activity as well as the failure to observe activity in several other *in vitro* genotoxicity assays conducted with S9 suggests that the activity observed in the Mladinic *et al.* studies does not have a significant weight of evidence for *in vitro* genotoxicity and is not likely to be relevant to *in vivo* genotoxicity.

The recently published results for mammalian *in vitro* chromosome aberration and micronucleus assays demonstrate a weight of evidence that technical glyphosate and glyphosate salt concentrates are negative for these end points in cultured mammalian cells in the absence of an exogenous mammalian metabolic activation system. Five publications from four laboratories report negative *in vitro* mammalian cell chromosome or micronucleus results in the absence of exogenous activation while three publications from two laboratories report positive results. These results reinforce the Williams *et al.* (2000, ASB2012-12053) conclusion that positive chromosome aberration results reported for glyphosate in cultured human lymphocytes in the absence of an exogenous metabolic activation system are aberrant. Recent reports of positive chromosome aberration and micronucleus results for glyphosate in the presence of an exogenous mammalian activation system in cultured human lymphocytes in one laboratory (Mladinic *et al.*, 2009, ASB2012-11906; Mladinic *et al.*, 2009, ASB2012-11907) have no substantial reproducibility verification from other laboratories in the recent *in vitro* chromosome effects studies considered in this review because most of the studies performed by other laboratories (Table B.6.4-29) did not employ an exogenous mammalian activation system. These results are discordant with one previously reviewed result demonstrating a negative result for glyphosate in cultured human lymphocytes with mammalian metabolic activation using the chromosome aberration endpoint (Williams et al 2000, ASB2012-12053) and a negative result in the presence of S9 for the micronucleus endpoint in bovine lymphocytes (Piesova, 2005, ASB2012-12000). The numerous consistent negative results for glyphosate and GBFs in gene mutation studies which employed exogenous mammalian metabolic activation and careful examination of the data suggests that the positive results indicate a possible threshold aneugenic effect associated with cytotoxicity rather than a DNA-reactive mechanism resulting in chromosome breakage. Thus, the weight evidence for the *in vitro* chromosome effect assays indicates a lack of DNA-reactive clastogenic chromosome effects.

Results for GBFs

Amer *et al.* (2006, ASB2012-11539) reported positive *in vitro* chromosome aberration effects in mouse spleen cells for a formulation described as herbazed, which was reported to contain 84% glyphosate and 16% solvent, an unusually high glyphosate concentration for a formulation. The test material is not further characterised, lacking description of the glyphosate salt form and inert ingredients. The glyphosate concentrations used in the study are not clear because there are different descriptions of the concentration units (M or M glyphosate/ml medium) in the publication. Thus, the maximum concentration might have been 5×10^{-5} M (50 µM) or 5×10^{-5} M glyphosate/mL medium (50 mM). The former concentration, which was reported as toxic, would indicate effects at concentrations well below those typically found toxic for GBFs in cultured mammalian cells. The latter level of 50 mM would be well in excess of the limit level of 10 mM recommended in OECD guidelines (OECD473, 1997). In addition to a question about the concentration used there are several other limitations to the reported study including no indication that pH of treatment solutions was controlled, no use of a mammalian metabolic activation system, no reported concurrent toxicity measurements and no reported use of coded slides for scoring. Given these limitations,

the uncertainty about the concentrations used and the nature of the test material, these results should not be considered to have significant relevance or reliability with respect to glyphosate or GBFs.
In addition to *in vitro* mammalian cell studies there is also a report of negative results for the chromosome aberration and micronucleus endpoints in onion root tips incubated with a Roundup formulation (Dimitrov *et al.*, 2006, ASB2012-11607). The maximum exposure concentration (stated as 1% active ingredient) is estimated to be on the order of 4-6 mM. This study did not employ an exogenous mammalian metabolic activation system; however, it does provide evidence for a lack of chromosome effects for glyphosate and a GBF in a non- mammalian *in vitro* system. The result agrees with earlier reported negative onion root tip chromosome aberration results for glyphosate but is discordant with earlier reported positive results for a Roundup GBF in this system (Williams *et al.*, 2000, ASB2012-12053).

B.6.4.8.1.2 In vivo Chromosome Effects—Mammalian Systems

The Williams *et al.* (2000, ASB2012-12053) glyphosate toxicityreview presented results from *in vivo* mammalian chromosome effect assays. Results from several mouse bone marrow erythrocyte micronucleus studies of glyphosate and GBFs (e.g. Roundup, Rodeo and Direct) were negative for micronucleus induction. These included studies from different laboratories mostly following modern guidelines. The intraperitoneal (i.p.) route was used for most of the negative studies and maximum doses for many of the studies were toxic or appropriately close to LD_{50} values. In addition to i.p. studies a 13 week mouse feeding study was also negative for the micronucleus endpoint with an estimated maximum daily glyphosate dose of over 11,000 mg/kg/day. There was one published report of a weak positive mouse bone marrow micronucleus response observed for glyphosate and Roundup GBF. This study, which employed a smaller number of animals per group than other negative studies, was clearly aberrant from the numerous other negative studies not only in micronucleated cell frequency finding but also the finding of altered polychromatic erythrocyte to normochromatic erythrocyte (PCE/NCE) ratios. The overall weight of evidence from the earlier reviewed studies was that glyphosate and GBFs were negative in the mouse bone marrow erythrocyte micronucleus assay. The earlier review also noted a negative mouse dominant lethal result for glyphosate administered by gavage at a maximum dose level of 2000 mg/kg.
As indicated in Table B.6.4-29, there are numerous subsequent publications of *in vivo* mammalian chromosome effects assays. With one exception, all of the *in vivo* mammalian studies were conducted in the mouse using either the bone marrow chromosome aberration or micronucleus endpoints. It should be noted that there are some fairly consistent limitations in the reported conduct of these studies compared to OECD guidelines. In most studies concurrent indications of toxicity (other than effects on the bone marrow) are not reported, coding of slides for scoring is not reported, individual animal data are not reported and fewer than recommended cells or metaphases per animal were scored. Other limitations encountered include use of only a single or two dose levels rather than three dose levels.

Results for glyphosate active ingredient

Two publications reported results for glyphosate in the mouse bone marrow erythrocyte micronucleus assay. Negative results were reported in one study which used a dose of 300 mg/kg of glyphosate administered once i.p. with sacrifices at 24, 48 and 74 hours after dosing (Chruscielska *et al.*, 2000, ASB2013-9820). This study had some limitations including the use of only one dose level, no reporting of toxicity other than PCE/NCE ratio, no reported coding of slides for scoring and scoring of 1000 PCE's per animal (scoring of 2000 PCE's per animal is recommended by OECD guidelines). A second publication reported positive results for glyphosate administered at 50, 100 and 200 mg/kg via i.p. injections repeated at 24 hours apart with sacrifice 24 hours after the second dose (Manas *et al.*, 2009, ASB2012-11892). A statistically significant increase in micronucleated erythrocytes was observed in the high dose group. This study had limitations comparable to the negative study. A more significant potential difficulty with this second publication is that "erythrocytes" rather than polychromatic erythrocytes were indicated as scored for micronuclei. This does not appear to be a case of using "erythrocytes" to mean polychromatic erythrocytes because the term "polychromatic erythrocytes" is used elsewhere in the publication describing measurements of PCE/NCE ratios. Scoring of total erythrocytes instead of immature polychromatic erythrocytes for micronuclei would be inappropriate in an assay with the stated treatment and harvest times because of the transient nature of micronucleated PCE's in bone marrow (OECD474, 1997).
There is no definitive explanation for the discrepancy between the two publications. Although one study

used a single dose with multiple harvest times and the second used two doses and a single harvest time, both are acceptable protocols and would not be expected to lead to such discordant results (OECD474, 1997). The negative result reported for the 13 week feeding study in the earlier review (Williams *et al.*, 2000, ASB2012-12053) confirms that positive results are not simply due to repeat dosing. The reported negative result (Chruscielska *et al.*, 2000, ASB2013-9820) seems to be in accord with a majority of earlier reviewed mouse bone marrow micronucleus studies of glyphosate using similar doses and the i.p. or feeding routes (Williams *et al.*, 2000, ASB2012-12053). Also, the apparent scoring of micronuclei in erythrocytes rather than just polychromatic erythrocytes raises a significant methodological question for the reported positive study.

Results for GBFs

There are several publications reporting *in vivo* mammalian bone marrow chromosome aberration and micronucleus endpoint results for Roundup GBFs. Three publications report negative results for Roundup branded GBF in mouse chromosome aberration or micronucleus assays. Negative results were reported for two different Roundup branded GBFs administered at 2 x 200 mg/kg i.p. in mouse bone marrow erythrocyte micronucleus assays (Coutinho do Nascimento and Grisolia, 2000, ASB2013-11477; Grisolia, 2002, ASB2012-11834). The second study did not report coding of slides for scoring. Another publication reported negative results in mouse bone marrow studies for both the chromosome aberration and erythrocyte micronucleus endpoints (Dimitrov *et al.*, 2006, ASB2012-11607) using a dose of 1080 mg/kg administered orally (p.o.). In contrast, one publication reported positive results for Roundup GBF in mouse bone marrow for the chromosome aberration and erythrocyte micronucleus endpoints using a single maximum dose of 50 mg/kg i.p. (Prasad *et al.*, 2009, ASB2012-12005). Both the positive results and the magnitude of the increases in the chromosome aberration and micronucleus endpoint reported in this study are remarkably discordant with other reported results for Roundup and other GBFs in mouse bone marrow chromosome aberration and erythrocyte studies in a number of laboratories and publications (Table B.6.4-29 and Williams *et al.*, 2000, ASB2012-12053). The reasons for this discordance are not clear. One unusual feature of the positive study is that the Roundup GBF was administered in dimethylsulfoxide. This is an unusual vehicle to use in *in vivo* genotoxicity studies, particularly for glyphosate which is water soluble and especially so in a formulated product. A published toxicity study found that use of a dimethylsulfoxide/olive oil vehicle by the i.p. route produced dramatically enhanced toxicity of glyphosate formulation or the formulation without glyphosate compared to saline vehicle and that the enhanced toxicity observed with this vehicle was not observed when the oral route was used (Heydens *et al.*, 2008, ASB2012- 11845). These observations suggest that use of DMSO as a vehicle for administration of formulation components by the i.p. route might produce unusual toxic effects that are not relevant to normally encountered exposures. Regardless of the reasons for the discordant positive results it is clear that a large preponderance of evidence indicates that GBFs are typically negative in mouse bone marrow chromosome aberration and erythrocyte assays. One publication reported positive results for bone marrow chromosome aberration in rabbits administered Roundup GBF in drinking water at 750 ppm for 60 days (Helal and Moussa, 2005, ASB2012-11841). This study is relatively unique in terms of species and route of administration. The results do not report water intake in the test and control groups. Given the potential for water palatability issues with a formulated product, this is a significant shortcoming, as any effects noted may be attributable to dehydration. This study had further limitations including the use of only a single dose level and not coding slides for scoring. Examination of the chromosome aberration scoring results showed that large increases for the treated group were observed for gaps and "centromeric attenuation" which were included in the summation and evaluation of structural chromosome aberration effects. Ordinarily gaps are scored but are not recommended for inclusion in total aberration frequency and centromeric attenuation is not included in ordinary structural aberrations (OECD475, 1997). These unusual scoring and interpretive features raise significant questions about using this study to make conclusions about clastogenicity of the GBF tested.

Two other publications report *in vivo* mammalian chromosome aberration or micronucleus results for GBFs. An uncharacterisd GBF, Percozyd 10L, was reported to be negative in a mouse bone marrow erythrocyte micronucleus assay (Chruscielska *et al.*, 2000, ASB2013- 8929 and ASB2013-8931). The maximum dose level tested, 90 mg/kg i.p., was reported to be 70 of the i.p LD_{50} as determined experimentally by the authors. This study had several limitations including use of less than three dose levels

and no reported coding of slides for scoring. Positive results were reported for another uncharacterized GBF, herbazed, in mouse bone marrow and spermatocyte chromosome aberration studies (Amer *et al.*, 2006, ASB2012- 11539). No statistically significant increases in aberrant cells were observed in bone marrow cells for i.p. treatment of 50 mg/kg for 1, 3 or 5 days or in spermatocytes for 1 or 3 days treatment. Statistically significant increases in frequency of spermatocytes with aberrations were reported for 5 days of treatment with 50 mg/kg (i.p.). Oral treatment of 50 mg/kg and 100 mg/kg were reported to produce increases in aberrant cell frequency in bone marrow cells after extended treatments (14 and 21 days) but not after shorter 1 and 7 day treatments. Similarly, significant increases in aberrant cell frequencies of spermatocytes were reported at 14 and 21 days of 50 mg/kg oral treatment (negative for 1 and 7 days treatment) and at 7, 14 and 21 days of 100 mg/kg treatment (negative for 1 day treatment). Although not a genotoxic endpoint per se, it should be noted that statistically significant increases in frequency of sperm with abnormal morphology were also observed in mice treated with 100 and 200 mg/kg p.o. for 5 days. The positive results for the uncharacterized herbazed GBF were only observed after extended oral treatments (bone marrow and spermatocytes) and extended i.p. treatments (spermatocytes). The fact that positive results were not observed in an erythrocyte micronucleus test of mice treated with glyphosate up to 50,000 ppm in feed for 13 weeks (Williams *et al.*, 2000, ASB2012-12053) provides direct evidence that extended glyphosate treatment by the oral route does not induce detectable chromosome effects. This treatment was longer and up to much higher glyphosate exposures than those used for the Amer *et al.* (2006, ASB2012-11539) studies. Thus, it appears likely that these effects were due to some component(s) of the specific herbazed GBF tested rather than glyphosate. *In vivo* mammalian assays for chromosome effects are an important category for characterisng genotoxicity that complements the gene mutation category. While some positive results have been reported the preponderance of evidence and published results are negative for glyphosate and GBFs.

B.6.4.8.1.3 In vivo Chromosome Effects – Non-Mammalian Systems

The Williams *et al.* (2000, ASB2012-12053) review reported a few *in vivo* plant assays for chromosome effects in non-mammalian systems. These included negative results for glyphosate and positive results for Roundup GBFs for chromosome aberrations in an onion root tip assay and negative results for glyphosate with the micronucleus end point in a *Vicia faba* root tip assay. Subsequent to the earlier review a number of publications reported results for erythrocyte micronucleus assays conducted on GBFs in several non-mammalian fish and reptile species with discordant results. One publication reported apparently negative results for the erythrocyte micronucleus test in *Oreochromis niloticus* (Nile tilapia) administered a test material described as Roundup 69 GBF at an upper dose of 170 mg/kg i.p. (Coutinho do Nascimento and Grisolia, 2000, ASB2013-11477). Although there was an increase in micronucleated erythrocyte frequency at the mid-dose level this was not observed at the high dose level and considerable variability in frequencies in different groups was noted. Negative results were also reported in another fish species (*Prochilodus lineatus*) exposed to 10 mg/liter Roundup branded GBF for 6, 24 and 96 hours (Cavalcante *et al.*, 2008, ASB2012- 11586). This concentration was reported to be 96% of a 96 hour LC_{50}. Positive results were reported for the erythrocyte micronucleus assay conducted in the fish *Tilapia rendalii* exposed to 170 mg/kg i.p. of another Roundup GBF (Grisolia, 2002, ASB2012-11834). Examination of the micronucleus frequencies in this publication indicated that the negative control micronucleus frequency was considerably lower than the frequencies for all but one of 21 treatment groups for 7 different test materials. This suggests an unusually low control frequency and at least one treatment group was statistically significantly elevated for each of the 7 test materials, including many instances where the statistically significant increases were not consistent with a biologically plausible dose response. The possibility that the apparently significant increases were due to a low negative control value should be considered for this publication. Another publication reported positive erythrocyte micronucleus results in goldfish (*Carassius auratus*) exposed to 5 to 15 ppm of a Roundup GBF for 2 to 6 days (Cavas and Konen, 2007, ASB2012-11587). The reasons for the discordant results are not clear for these fish erythrocyte micronucleus assays of Roundup GBFs. Although different species and GBF's were used in the different studies there were pairs of studies with positive and negative results that used similar treatment conditions (170 mg/kg i.p. or 10-15 mg/litre in water).

Results for an unusual test system of exposed caiman eggs are reported by Poletta *et al.*, 2009, ASB2012-12002. Eggs were topically exposed in a laboratory setting to Roundup Full II GBF, and erythrocyte

micronucleus formation was measured in hatchlings (Poletta *et al.*, 2009, ASB2012-12002). The GBF tested was reported to contain the potassium salt of glyphosate and alkoxylated alkylamine derivatives as surfactants. Statistically significant increases in micronucleated erythrocytes were observed in hatchlings from eggs treated with 500-1750 µg/egg. This system is quite unusual in the species tested and even more so in using an egg application with measurement of effects in hatchlings. Although there is some experience with a hen's egg erythrocyte micronucleus assay using *in ovo* exposure the erythrocytes are evaluated in embryos with only a few days between treatment and the erythrocyte micronucleus end point. In the reported caiman egg assay there was presumably a single topical exposure followed by an egg incubation period of about 10 weeks before hatching. Biological plausibility raises questions whether genotoxic events *in ovo* can produce elevated micronucleated erythrocyte frequencies detectable after 10 weeks, given the number of cell divisions occurring in development of a hatchling.

One published study reported a weak positive result in a *Drosophila* wing spot assay (Kaya *et al.*, 2000, ASB2013-9832). Statistically significant positive increases were only in one of four crosses for small twin spots and not for the two other wing spot categories (large wing spots and twin wing spots). As discussed above, only negative or inconclusive results were observed for crosses that were not subject to mitotic recombination effects. If the result was actually treatment related it only would indicate an increase in recombination events and not in somatic mutations.

The above *in vivo* chromosome effect assays in non-mammalian systems give discordant results for reasons that aren't precisely defined. Typically these results would be given lower weight than mammalian systems in being predictive of mammalian effects, especially since there is little or practically no assay experience with these systems in comparison with *in vivo* mammalian chromosome effects assays, such as the rat or mouse bone marrow chromosome aberration or erythrocyte micronucleus assays.

B.6.4.8.2 DNA damage and other end points

A number of studies of glyphosate and GBFs have been published since 2000 which used various DNA damage end points in a variety of *in vitro* and *in vivo* systems. The DNA damage category includes end points such as sister chromatid exchange and DNA repair response in bacteria, but the most common DNA damage end point encountered was the alkaline single cell gel electrophoresis end point (alkaline SCGE) also commonly referred to as the "comet" assay. The alkaline SCGE end point has been applied to both *in vitro* and *in vivo* test systems.

In addition to DNA damage there are a few reports of other types of studies which can be associated with genotoxic effects even though the end points are not specific indicators of genotoxicity per se. These include sperm morphology and carcinogenicity studies.

In vitro DNA Damage Studies

Some positive results for glyphosate or GBFs in the SCE end point were reported in cultured human and bovine lymphocytes in the earlier review (Williams *et al.*, 2000,ASB2012-12053). These results tended to be weak, inconsistent and with limited evidence for dose response. A number of limitations were observed for the studies such as the failure to control pH and abnormally low control values. Additional *in vitro* DNA damage end point results described in the earlier review included negative results for glyphosate in the *B. subtilis* rec-assay and in the primary hepatocyte rat hepatocyte unscheduled DNA synthesis assay.

There are two subsequent publications using *in vitro* cultured mammalian cells and the SCE endpoint. Positive SCE results were reported for the uncharacterised herbazed GBF in mouse spleen cells (Amer *et al.*, 2006, ASB2012-11539). The dose response pattern for SCE response in this study was similar to the response for chromosome aberrations in this publication. Limitations of this study are in common to those described above for the chromosome aberration end point portion of the study; no indication that pH of treatment solutions was controlled, no use of a mammalian metabolic activation system, no reported concurrent toxicity measurements and no reported use of coded slides for scoring. Positive SCE results were also reported for cultured bovine lymphocytes treated with up to 1.12 mM glyphosate for 24 and 48 hours without exogenous mammalian metabolic activation (Sivikova and Dianovsky, 2006, ASB2012-12029). The highest dose of 1.12 mM significantly delayed cell cycle progression with 48 hour treatment. These same concentrations for 24 h exposures did not induce statistically significant increases in chromosome aberrations which provides a clear example of a differential response of the SCE endpoint

(Sivikova and Dianovsky, 2006, ASB2012-12029). This is an important consideration in these publications, as chromosome effects are considered more relevant to genotoxicity than DNA damage.
Positive results for glyphosate are reported for the alkaline SCGE end point in three publications. Positive SCGE results were observed for two mammalian cell lines exposed to glyphosate for 4 hours at concentrations of 4.5-6.5 mM (GM39 cells) and 4.75-6.5 mM (HT1080 cells) (Monroy *et al.*, 2005, ASB2012-11910). These concentrations are close to the upper limit dose of 10 mM generally recommended for *in vitro* mammalian cell assays and control of medium pH is not indicated. Characterisation of nuclear damage was done by visual scoring without coding of slides being indicated. Positive alkaline SCGE results were also reported in Hep-2 cells exposed for 4 hours to 3.5-7.5 mM glyphosate (Manas *et al.*, 2009, ASB2012-11892). Higher concentrations of glyphosate were reported to result in viability of <80% as determined by dye exclusion. As noted for the preceding publication, the concentrations employed were reasonably close to a limit dose of 10 mM and control of medium pH was not reported. This publication reported negative results for the chromosome aberration endpoint in cultured human lymphocytes exposed to up to 6 mM glyphosate for 48 hours and it should be noted that in this case an appropriate control of medium pH was reported for this human lymphocyte experiment. Positive alkaline SCGE results have also been reported for cultured human lymphocytes exposed to glyphosate at concentrations up to 580 µg/mL (estimated 3.4 mM) for 4 hours (Mladinic *et al.*, 2009, ASB2012-11906). Effects were observed both in the presence and absence of S9 with statistically significant increases in tail intensity at 3.5, 92.8 and 580 µg/mL without S9 and at 580 µg/mL with S9. A modification of the alkaline SCGE assay employing human 8-hydroxyguanine DNA-glycosylase (hOGG1) to detect oxidative damage only indicated statistically significant effects on tail length for treatment with 580 µg/mL with S9. Increases in nuclear abnormalities (nuclear buds and/or nucleoplasmic bridges) were also observed at 580 µg/mL with and without S9 and in micronucleus frequency at 580 µg/ml with S9. Measurements of total antioxidant capacity and thiobarbituric acid reactive substances showed statistically significant increases at 580 µg/ml in the presence or absence of S9. Interpretation of the significance of metabolic activation effects is complicated by the observation that several of the end points (alkaline SCGE tail intensity and nuclear abnormalities) tended to show increases in the presence of S9 in negative controls or at the very lowest concentrations of glyphosate. A reasonable summation of the results in this publication is that alkaline SCGE effects and other effects such as nuclear abnormalities, early apoptosis, necrosis and oxidative damage were consistently observed at 580 µg/mL.
In addition to mammalian cell studies there are publications reporting positive alkaline SCGE effects for glyphosate in *Tradescantia* flowers and nuclei exposed to up to 700 µM glyphosate (Alvarez-Moya *et al.*, 2011, ASB2012-11538) and in the *E. coli* SOS chromotest for DNA damage conducted on a Roundup BIO GBF (Raipulis *et al.*, 2009, ASB2012-12008). Observations of DNA damage in plants exposed to glyphosate are of questionable significance because of the herbicidal nature of glyphosate and the SOS chromotest provides only indirect evidence of DNA damage in a bacterial system.

Overall there appear to be a number of studies in which glyphosate or GBFs have been reported to produce positive responses in DNA damage endpoints of SCE or alkaline SCGE *in vitro* in mammalian cells. Most of these have occurred with exposures to mM concentrations of glyphosate. Although this dose level range is lower than the limit dose of 10 mM recommended for several *in vitro* mammalian cell culture assays (OECD473, 1997; OECD476, 1997; OECD487, 2010), an even lower limit dose of 1 mM was recently recommended for human pharmaceuticals, particularly because of concerns about relevance of positive *in vitro* findings observed at higher dose levels. In addition, many of the studies have limitations such as not indicating control of medium pH and not coding slides for visual scoring.
Concerns over the possibility of effects induced by toxicity have led to several suggestions for experimental and interpretive criteria to distinguish between genotoxic DNA-reactive mechanisms for induction of alkaline SCGE effects and cytotoxic or apoptotic mechanisms. One recommendation for the *in vitro* alkaline SCGE assay is to limit toxicity to no more than a 30% reduction in viability compared to controls. Importantly, dye exclusion measurements of cell membrane integrity, such as those reported in some of the above publications may significantly underestimate cytotoxicity that could lead to alkaline SCGE effects. Other recommendations include conducting experiments to measure DNA double strand breaks to determine if apoptotic process might be responsible for alkaline SCGE effects. Measurement of apoptotic and necrotic incidence were only performed in one publication (Mladinic *et al.*, 2009, ASB2012-11906)

and these measurements indicated both apoptotic and necrotic processes occurring in parallel with observations of alkaline SCGE effects. These direct observations as well as the reported dose responses, consistently suggest that biological effects and cytotoxicity accompany the observations of DNA damage *in vitro* in mammalian cells and therefore confirm the likelihood that the observed effects are secondary to cytotoxicity and are thresholded.

In vivo DNA damage studies

In the earlier review positive results for DNA strand breakage were reported for mice treated by the i.p. route with glyphosate and GBFs and for the alkaline SCGE endpoint in tadpoles of the frog *Rana catesbiana* exposed to a GBF (Williams *et al.*, 2000, ASB2012-12053).

Amer *et al.* (2006, ASB2012-11539) reporten an increase in SCE frequency in bone marrow cells of mice treated with uncharacterised herbazed GBF. Statistically significant positive effects were only observed at the highest dose level tested (200 mg/kg administered p.o.).

Several recent publications report alkaline SCGE results for GBFs in aquatic species. Three publications reported positive alkaline SCGE results in aquatic vertebrates exposed to Roundup GBFs in water. These publications have a common feature that alkaline SCGE results were reported as visually scored damage category incidence rather than instrumental measurements of properties such as the tail length or tail intensity. In one publication increases in nuclei exhibiting alkaline SCGE visual damage effects were observed in erythrocytes and gill cells of the tropical fish *Prochilodus lineatus* exposed to 10 mg/litre of a Roundup GBF in water (Cavalcante *et al.*, 2008, ASB2012-11586). Results were variable with cell type and incubation; statistically significant positive responses were observed for erythrocytes at 6 hours and 96 hours, but not 24 hours or for branchial cells from the gills at 6 hours and 24 hours. Measurement of erythrocyte micronucleus frequency and nuclear abnormalities did not show statistically significant increases in these endpoints. The concentration used was reported to be 75% od the 96 hour LC_{50} trypan blue dye measurements apparently indicated >80% viability of cells used in the alkaline SCGE assays. A second publication reported positive alkaline SCGE results in erythrocytes of the goldfish, *Carasseus auratus*, exposed to 5, 10 and 15 ppm of a Roundup GBF for 2, 4 or 6 days (Cavas and Konen, 2007, ASB2012-11587). Similar effects were observed for other end points (micronucleus and nuclear abnormalities). In general, effects increased with concentration and time. This publication did not report toxicity measurements or, more specifically, measurements of cell viability in the population studied. Positive results were also reported in erythrocytes of the European eel, *Anguilla anguilla*, exposed to 58 and 116 µg/L of a Roundup GBF in water for 1 or 3 days (Guilherme *et al.*, 2010, ASB2012-11836). Increasesin nuclear abnormalities were also observed in erythrocytes from animals exposed for 3 days. Measurement of toxicity was not reported for the animals or erythrocytes; however, several endpoints relevant to antioxidant responses and oxidant effects were made in whole blood samples. No statistically significant effects were observed for catalase, glutathione transferase, glutathione peroxidase, glutathione reductase or reduced glutathione content. A large statistically significant increase for thiobarbituric acid reactive substances (TBARS, a measure of lipid peroxidation) was observed for the 115 µg/L concentration group at 1 day. Statistically significant TBARS increases were not observed at 3 days, but, the 3-day negative control value appeared to be several fold higher than the 1-day value.

Significance of DNA damage end point results

DNA damage end points such as SCE or alkaline SCGE are generally regarded as supplementary to the gene mutation and chromosome effects end point categories. DNA damage endpoints do not directly measure effects on heritable mutations or events closely associated with chromosome mutations. *In vitro* DNA damage endpoints such as the SCE or alkaline SCGE can be induced by cytotoxicity and cell death processes rather than from DNA- reactive mechanisms.

The observation of effects of sodium dodecyl sulfate is also interesting because it suggests responses to surfactants which are typically components of GBFs. As a more specific example, polyoxyethylenealkalylmine (POEA), a surfactant component of some GBFs has been shown to elicit

cytotoxic effects such as perturbation of the mitochondrial membrane and disruption of mitochondrial membrane potential in cultured mammalian cells (Levine *et al.*, 2007, ASB2009-9030). Surfactant effects provide a plausible mechanism for observations of GBFs inducing DNA damage responses. Such responses would be expected to be associated with cytotoxicity-inducing exposures and exhibit a threshold.

B.6.4.8.3 Human and environmental studies

A number of human and environmental studies have been published in or after 2000 where some exposures to GBFs in the studied populations were postulated. These publications are summarised in Table B.6.4-30.

Table B.6.4-30: Studies of Human and Environmental Populations with Reported or Assumed Glyphosate Exposure

Exposed Population	End point	Exposures	Result	Reference
Human Studies				
Open field and fruit farmers	Bulky DNA adducts	glyphosate formulation use reported in only 1 of 29 fruit farmers	No effects attributed to glyphosate formulation exposure	Andre *et al.*, 2007 (ASB2012-11543)
Humans in areas where glyphosate formulation is applied	Lymphocyte cytokinesis block micronucleus (CB MN)	Aerial or manual spraying of glyphosate formulation for illicit crop control and sugar cane maturation	Increase in CB MN but no clear relationship to assumed or reported exposures	Bolognesi *et al.*, 2009 (ASB2012-11570)
Floriculturists	Lymphocyte CB MN	Glyphosate formulation use reported in 21/51 workers with average of 106.5 kg applied	Increase in CB MN but not statistically significant	Bolognesi *et al.*, 2004 (ASB2012-11572)
Floriculturists	Lymphocyte CB MN	Glyphosate formulation use reported in 57/107 workers. Numerous other pesticides reported as used by a similar number or more of workers	Statistically significant increase in CB MN	Bolognesi *et al.*, 2002 (ASB2012-11573)
Agricultural workers	Buccal cell micronucleus	Glyphosate formulation use reported along with numerous other pesticides	Statistically significant increase in MN	Bolognesi *et al.*, 2009 (ASB2012-11570)
Fruit growers	Lymphocyte Alkaline SCGE; Ames test on urine	Glyphosate use reported in 2/19 1 day before captan spraying and 1/19 on the day of captan spraying	No effects attributable to glyphosate formulation exposure	Lebailly *et al.*, 2003 (ASB2012-11878)
Agricultural workers	Lymphocyte CB MN; buccal cell micronucleus	Glyphosate formulation use reported in 16% of one of four populations studied (Hungary)	No statistically significant increases in CB MN or buccal cell micronucleus frequencies	Pastor *et al.*, 2003 (ASB2012-11991)
Individuals on or near glyphosate spraying	Lymphocyte alkaline SCGE	Glyphosate formulation aerially sprayed within 3 km	Statistically significant increases in damaged cells	Paz-y-Mino C, 2007 (ASB2012-11992)
Greenhouse Farmers	Lymphocyte SCE	Glyphosate formulation use reported in 99/102 workers; numerous other	Statistically significant increases in SCE	Shaham *et al.*, 2001 (ASB2012-12025)

		pesticides used		
Farmers	Lymphocyte CB MN	Glyphosate formulation use reported in 3/11 farmers	Statistically significant increase in micronucleus frequency but not in frequency of binucleated cells with micronuclei	Vlastos *et al.*, 2006 (ASB2012-12045)
Environmental Studies				
Meadow voles living on golf cousres	Blood cell alkaline SCGE; erythrocyte micronucleus	Glyphosate formulation use reported along with numerous other pesticides	Some effects judged possibly related to Daconil® fungicide	Knopper and Lean., 2004 (ASB2012-11871)

Many of the human studies either found no effects attributable to GBFs or the reported GBF usage by the studied population was too low to be associated with observed population effects (Andre *et al.*, 2007, ASB2012-11543; Bolognesi *et al.*, 2004, ASB2012-11572; Lebailly *et al.*, 2003, ASB2012-11878; Pastor *et al.*, 2003, ASB2012-11991; Vlastos *et al.*, 2006, ASB2012-12045).

In some studies, incidence of GBF use by the population studied was significant but high incidence of use of other pesticides was also reported (Bolognesi *et al.*, 2002, ASB2012- 11573; Shaham *et al.*, 2001, ASB2012-12025). Even though positive effects were observed in these populations, ascribing these effects to any particular environmental exposure is not scientifically justifiable and such results certainly cannot be considered as definitive evidence for GBF-induced human genotoxic effects.

Two published studies focused on populations believed to be exposed to GBFs by their presence at or near aerial or manual spraying operations. One publication reported induction of alkaline SCGE effects in blood lymphocytes of populations living within 3 km of areas sprayed with glyphosate formulation for illicit crop eradication (Paz-y-Mino C, 2007, ASB2012-11992). The populations studied were relatively small (24 exposed individuals and 21 non-exposed individuals). The sprayed material was reported to be Roundup Ultra, a GBF containing 43.9% glyphosate, polyethoxylated tallowamine surfactant and a proprietary component, Cosmoflux 411F. Specific methods for collection, storage, and transport of blood samples are not described for either the exposed population or control group. The publication also does not indicate that slides were coded for scoring which consisted of visual classification into damage categories and measurement of DNA migration (tail length). There were fairly large differences in ages and sex distribution of the exposed and control populations but these did not appear to be statistically significant. The study reported increases in damaged cell categories and statistically significant increases in DNA migration (tail length) in the presumably exposed population. Interpretation of the results of this study should consider numerous reported signs of toxicity in the exposed population and the reported application rate of 24.3 litres/ha which was stated to be 20 times the maximum recommended application rate. Some of the reported human health effects described by Paz-y- Mino (2007, ASB2012-11992) appear to be consistent with severe exposures noted in clinical reports of acute poisoning incidents with GBFs and other pesticide formulations (often self- administered) rather than typical bystander exposures. Given the considerably favorable general toxicology profile of glyphosate as reported by the WHO/FAO Joint Meeting on Pesticide Residues (WHO/FAO, 2004, ASB2008-6266) and in Williams *et al.* (2000, ASB2012-12053), factors related to either high surfactant exposure, unusual GBF components in this formulation or other undocumented variables appear to be confounding factors in this study. It appears that the reported alkaline SCGE effects could well have been secondary to the ailments reported in this study population.

A second publication reported results for a blood lymphocyte cytokinesis-block micronucleus study of individuals in areas treated with glyphosate formulation by aerial spraying or manual application (Bolognesi *et al.*, 2009, ASB2012-11570). Although the title of the publication contains the term "agricultural workers", most of the populations studied do not appear to be agricultural workers who are involved in application of GBFs. The human lymphocyte culture and scoring methodology employed in the Bolognesi *et al.* (2009, ASB2012-11570) study appear to be generally consistent with commonly used

and recommended practices for this assay. However, there is a significant question as to how long the blood samples used in the study were stored prior to initiating cultures and this may have affected the micronucleus numbers observed in the different sets of samples and populations. Also, the populations in the aerially sprayed regions had a second sampling a few days after the first sampling and this second sampling was not performed in the control populations. The publication reported a small increase in the frequency of binucleated cells with micronuclei and micronuclei per cell in samples collected from people living in three regions after spraying of GBFs compared with control values of samples collected just before spraying. However, the pattern of the increases did not correlate either with the application rate or with self-reported exposure. The largest post-spraying increase in binuclated cell micronucleus frequency was reported for a population with a much lower glyphosate active ingredient application rate and only 1 of 25 people in this region reported contact with sprayed glyphosate formulation. Increases in binucleated cell micronucleus frequency did not have a statistically significant relationship with self-reported exposure for two other populations. Some interpretative statements in Bolognesi *et al.* (2009, ASB2012-11570) suggest a small transient genotoxic effect of glyphosate formulation spraying on frequencies of binucleated cells with micronuclei, but other statements indicate that causality of the observed effects could not be determined using reasonable criteria and that lack of exposure data precluded conclusions. This study has a combination of uncontrolled or inadequately characterized variables, such as uncharacterisd exposure to ”genotoxic pesticides”, that would appear to preclude using the data to support any conclusion that exposure to GBFs affects binucleated micronucleus frequencies. Actually, the available data, while certainly limited in nature, support a conclusion that the observed effects do not appear to be attributable to glyphosate formulation exposure. This conclusion is reinforced by Acquavella *et al.* (2004, ASB2012-11528), where biomonitoring of agricultural workers applying GBFs reports systemic exposures orders of magnitude below *in vivo* model chromosome aberration and micronucleus study doses, the majority of which were negative for glyphosate and GBFs.

There are two publications related to environmental monitoring for genotoxic endpoints. One study using blood cell alkaline SCGE and micronucleus endpoints was conducted on samples from meadow voles living on or near golf courses where pesticides had been applied (Knopper & Lean, 2004, ASB2012-11871). Results were significantly inconsistent between two seasons. Although some suggestions of effects were reported, glyphosate was only one of a number of applied pesticides and the effects observed were considered as possibly attributable to exposure to Daconil® fungicide. A second publication reported results for the erythrocyte micronucleus assay applied to fish collected from several dams in Brazil (Salvagni *et al.*, 2011, ASB2012-12017). Glyphosate formulation was one of a number of pesticides reported to be used in the area of the dams. No efforts appear to have been made to measure glyphosate or other pesticide concentrations in any of the ten dams from which fish were sampled. This study reported what were considered to be high levels of micronucleated cell frequency but there were no concurrent negative controls. In the absence of these controls the results cannot be interpretted as indicating any effect of pesticide exposure.

Although there have been a fairly large number of human genotoxicity studies reported where there was some exposure to GBFs, the large majority of these studies do not allow any conclusions about possible effects of glyphosate or GBFs because the exposure incidence was low or because there were reported exposures to a large number of pesticides. One report found an increase in alkaline SCGE effects in humans living in or near areas where a GBF was sprayed but that study had a number of methodology reporting and conduct deficiencies and the reported effects could well have been due to toxicity reported in the study population. A second study found some increases in cytokinesis-block micronucleus frequency in humans possibly exposed to GBFs but the effects were not concordant with application rates or self- reported exposures and thus do not constitute reliable indications of effects for this endpoint in humans exposed to GBFs. Neither of the two environmental monitoring studies in meadow voles or fish provide any reliable evidence of exposures to glyphosate or GBFs or adverse effects resulting from potential exposures to glyphosate or GBFs.

After submission of the first draft of this RAR for public comment the following additional studies have been included.

Koureas *et al.* (2014, ASB2014-9724) performed a study aimed at estimating the oxidative damage to DNA in different subpopulations in Thessaly region (Greece) and investing its correlation with exposure to pesticides and other potential risk factors. The study produced findings that support the hypothesis that

pesticide exposure is involved in the induction of oxidative damage to DNA. A correlation was found in this study between exposure to formulations containing neonicotinoids or glufosinate ammonium and oxidative damage to DNA. However, no significant correlation was reported for glyphosate.
Gentile *et al.* (2012, ASB2014-9482) submitted results of the micronucleus assay as a biomarker of genotoxicity in the occupational exposure to agrochemicals in rural workers in Argentina. The authors found significant differences in the frequency of micronuclei between occupationally exposed (20 individuals) and unexposed (10 individuals) workers. However, no conclusion on genotoxicity of glyphosate or other specific pesticides is possible on basis of this study.
Da Silva *et al.* (2014, ASB2014-9358) performed a genotoxic assessment in tobacco farmers at different crop times. The study sought to determine genotoxic effects in farmers occupationally exposed to agrochemicals and nicotine. A significant increase of micronucleated cells in the off-season group was observed. However, no conclusion on genotoxicity of glyphosate or other specific pesticides is possible on basis of this study.
Benedetti *et al.* (2013, ASB2014-9279) studied genetic damage in soybeans workers exposed to pesticides. The evaluation was performed with the comet and buccal micronucleus assays. The results of both tests revealed DNA damage in soybean workers. No special pesticide can be identified as cause of the observed effects.

B.6.4.8.4 DNA-Reactivity and carcinogenesis

As noted in the earlier review, ^{32}P-postlabelling DNA adduct studies in mice did not indicate formation of adducts from glyphosate and questionable evidence of adducts from Roundup GBF administered as a high 600 mg/kg i.p. dose in an unusual dimethylsulfoxide/olive oil vehicle (Peluso *et al.*, 1998, TOX1999-318; Williams *et al.*, 2000, ASB2012-12053). Another earlier reviewed study reported DNA strand breakage in liver and kidneys of mice injected i.p with glyphosate and Roundup GBF. This study also reported an increase in 8- hydroxydeoxyguanosine (8-OHdG) residues in liver DNA from mice injected with glyphosate but not GBF. Increased 8-OHdG was found in kidney DNA from mice injected with GBF but not glyphosate (Bolognesi *et al.*, 1997, Z59299; Williams *et al.*, 2000, ASB2012-12053). No new direct studies of DNA reactivity of glyphosate or GBFs were encountered in publications since 2000. One publication did report on studies in mice to further investigate toxic effects and 8-OHdG levels associated with the routes, vehicles and dose levels employed in earlier ^{32}P-postlabelling and DNA strand breakage and 8-OHdG studies (Heydens *et al.*, 2008, ASB2012-11845). This publication reported that high i.p. dose levels of GBF induced significant liver and kidney toxicity that were not observed with oral administration. Statistically significant increases in 8-OHdG were not observed in this study under the same conditions as employed by the earlier study. The dimethylsulfoxide/olive oil vehicle dramatically enhanced toxicity of GBF administered by the i.p. route and the toxicity was also observed for formulation components without glyphosate. These results indicated that the effects reported in the earlier studies were associated with high liver and kidney toxicity that was primarily due to the non-glyphosate components of the formulation and which were produced by the i.p. route of exposure to very high dose levels. The enhancement of toxicity by the unusual dimethylsulfoxide/olive oil dosing vehicle further calls into question whether the ^{32}P-postlabelling finding represented effects associated with unusual toxicity rather than being indicative of adducts formed from glyphosate or glyphosate formulation components.

Carcinogenicity is not a direct endpoint for genotoxicity but it is one of the possible consequences of genotoxicity and, conversely, lack of carcinogenicity in well-conducted experimental studies provides some evidence that a significant genotoxic mode of action is not operating *in vivo*. The earlier review of glyphosate concluded that it was not carcinogenic in mouse or rat chronic studies and notes that glyphosate was not considered carcinogenic by numerous regulatory agencies and scientific organisations (Williams *et al.*, 2000, ASB2012- 12053).

B.6.4.8.4 AMPA and POEA

B.6.4.8.5 Genotoxicity of glyphosate mixtures and photoactivation

B.6.4.8.6 Genotoxicity Weight of Evidence

The earlier review applied a weight of evidence analysis to the available genotoxicity data. Various weighted components included assay system validation, test system species, relevance of the endpoint to heritable mutation, reproducibility and consistency of effects and dose- response and relationship of effects to toxicity (Williams *et al.*, 2000, ASB2012-12053). The conclusion of this analysis was that glyphosate and Roundup GBFs were not mutagenic or genotoxic as a consequence of direct chemical reaction with DNA. This was supported by a strong preponderance of results indicating no effects in *in vivo* mammalian assays for chromosome effects and consistently negative results in gene mutation assays. Although some DNA damage responses were noted, these were judged likely to be secondary to toxicity rather than DNA reactivity.

Since this earlier review, a large number of genotoxicity studies have been conducted with glyphosate and GBFs. For gene mutation, one of the two primary endpoint categories with direct relevance to heritable mutation, one subsequent publication contains a summary of results from a bacterial gene mutation endpoint assay (Ames/*Salmonella* bacterial reversion assay). Although there were very significant limitations to the information published, the negative result is consistent with the majority of negative results reported for glyphosate and GBFs in Ames/*Salmonella* bacterial reversion assays. Another publication reported results for a *Drosophila* wing spot assay of glyphosate. Results were negative or inconclusive in this assay for crosses that would have detected gene mutation as loss of heterozygosity. The new results provide some support to reinforce the earlier conclusion that glyphosate and GBFs are not active for the gene mutation endpoint category.

The second primary endpoint category with direct relevance to heritable mutation is chromosome effects. The earlier review noted mixed results for two *in vitro* chromosome effects assays in mammalian cells but concluded that the most reliable result was the negative assay. A number of *in vitro* mammalian cell chromosome aberration or micronucleus assay results have been subsequently published using bovine or human lymphocytes. These assays suffer from some technical limitations in conduct or reporting of methodology that frequently included failure to indicate control of medium for pH and failure to indicate coding of slides for visual scoring. Both positive and negative results are reported in these assays. A large preponderance of results in the absence of an exogenous mammalian metabolic activation system were negative up to high (mM) dose levels that were toxic or close to toxic levels observed in parallel experiments. The exceptions were a weak and inconsistent response reported in two publications from the same laboratory and a positive response for the uncharacterized formulation, herbazed. In addition to these findings in mammalian cells negative results were also reported for Roundup GBF in an onion root tip assay conducted without exogenous mammalian metabolic activation. Thus, the preponderance of evidence from assays not employing an exogenous mammalian metabolic activation system indicates that glyphosate and GBFs are not structural chromosome breakage inducers (clastogenic) in *in vitro* mammalian chromosome aberration or micronucleus assays.

Two publications from one laboratory reported an increase in micronucleus frequencies for glyphosate in *in vitro* cultured mammalian cells in the presence of an exogenous S9 metabolic activation system (Mladinic *et al.*, 2009, ASB2012-11906; Mladinic *et al.*, 2009, ASB2012- 11907). An enrichment for centomeric-containing micronuclei suggested that the increased micronuclei observed in these studies were derived from aneugenic processes, probably mediated through toxicity, rather than chromosome breakage. Thus, these two reports of weak micronucleus responses in the presence of exogenous mammalian metabolic activation appear to result from toxicity-associated aneugenic rather than clastogenic mechanisms. A number of other gene mutation and *in vitro* chromosome effect genotoxicity studies are negative with exogenous metabolic activation which supports the conclusion that the weight of evidence does not indicate a DNA-reactive clastogenic activity in *in vitro* assays using mammalian cells.

All except one of a number of *in vivo* mouse bone marrow chromosome aberration or micronucleus assays of glyphosate and GBFs were reported as negative in the earlier review. In the updated review both positive and negative results were reported for glyphosate and GBFs in these types of assays. Many of these studies had limitations or deficiencies compared to international guidelines with the most common and significant being no indication of slide coding for visual scoring. Four publications from three laboratories reported negative results in mouse bone marrow erythrocyte micronucleus assays of glyphosate and GBFs which are consistent with the earlier reviewed studies. These studies used high, peri-lethal dose levels administered by the i.p. or oral routes.

Two publications from two laboratories reported positive results for glyphosate and GBFs in the mouse bone marrow erythrocyte micronucleus assay. One positive result for glyphosate was encountered using dose levels and routes that were similar to those employed in the negative glyphosate studies in the same assay system. The publication reporting this result indicates that erythrocytes rather than polychromatic erythrocytes were scored which would be inappropriate for the treatment protocol but it is possible that this is a misreporting of what types of cells were actually scored. Although there is no definitive explanation for the discordance, the preponderance of mouse bone marrow erythrocyte micronucleus studies of glyphosate are clearly negative. The reported positive result for Roundup GBF is discordant with a number of negative results for Roundup or other GBFs conducted at higher dose levels. The most unique feature of this study was the use of dimethylsulfoxide as a vehicle. The preponderance of mouse bone marrow erythrocyte micronucleus studies for Roundup and other GBF studies is negative.

Positive results were reported in an unusual test system (rabbit) and route (drinking water), but water intake was not reported and effects may therefore be attributable to dehydration. Furthermore, most of the effects were on endpoints not usually considered as indicators of clastogenicity and structural chromosome aberration. One laboratory reported positive results for chromosome aberration effects in bone marrow and spermatocytes after extended dosing. However, the herbazed formulation test material was not characterised. While more discordant results in the important *in vivo* mammalian chromosome effect assay category have been reported in publications subsequent to the earlier 2000 review the preponderance of evidence continues to indicate that glyphosate and GBFs are not active in this category of end point.

Several *in vivo* erythrocyte micronucleus assay results for GBFs in non-mammalian systems (fish and caiman eggs) have been published since the earlier review. These test systems have relatively little experience and are largely unvalidated in comparison to the mouse bone marrow erythrocyte micronucleus assay. Two publications report negative results and two publications report positive results in different fish species and there is no definitive explanation for the discordance. Both the positive and negative studies employed maximum dose levels that were toxic or close to toxic dose levels. One possible explanation for the discordance is that the positive effects were associated with toxicity that only occurred beyond an exposure threshold and over a fairly narrow dose range. Positive results in hatchlings derived from caiman eggs exposed to Roundup formulation are given relatively little weight because of extremely limited experience with this assay system and because of significant questions about how DNA damage effects induced in embryos can persist and be evident in cells of hatchlings after several weeks and numerous cell divisions. The reported weak and inconsistent response in one of four crosses for somatic recombination in the *Drosophila* wing spot assay is also accorded relatively low weight. These non-mammalian test systems are generally considered of lower weight for predicting mammalian effects than mammalian test systems. Also, the environmental significance of effects for GBFs should consider the relationship between concentrations or exposures producing effects and likely environmental concentrations or exposures. This is particularly important if the effects are produced by threshold mediated toxic processes.

There have been a significant number of publications since the earlier review of results for assays in the DNA damage category with some SCE and a large number of alkaline SCGE endpoint publications. In general, the DNA damage end point category is considered supplementary to the gene mutation and chromosome effect categories because this endpoint category does not directly measure heritable events or effects closely associated with heritable events. Regulatory genotoxicity testing recommendations and requirements focus on gene mutation and chromosome effect end points for initial core testing, particularly for *in vitro* testing. This consideration is underscored by the observation of some cases of compounds where positive effects are observed in these assays that are not observed for gene mutation or chromosome effect assays. Also, there are numerous examples of responses in these endpoints that do not appear to result from mechanisms of direct or metabolite DNA-reactivity. The unique response consideration is reinforced in this data set by observations of responses in DNA damage endpoints but not in chromosome effect end points.

Many DNA damage endpoint assays of glyphosate or GBFs have produced positive results at high, toxic or peri-toxic dose levels for the SCE and alkaline SCGE endpoints in a variety of test systems including cultured mammalian cells, several aquatic species and caiman eggs. The only new report of positive *in vivo* mammalian DNA damage effects are for an uncharacterised formulation, herbazed. There are several examples of negative results for a chromosome aberration or micronucleus endpoint and positive results for the alkaline SCGE or SCE endpoint in the same publication (Cavalcante *et al.*, 2008, ASB2012-11586;

Manas *et al.*, 2009, ASB2012-11892; Mladinic *et al.*, 2009, ASB2012-11906; Sivikova and Dianovsky, 2006, ASB2012-12029). These examples confirm the impression that the DNA damage endpoints are not necessarily predictive of heritable mutation effects and are also consistent with the DNA damage endpoints reflecting toxic effect mechanisms. While the number of reported positive responses in these endpoints does suggest that effects in these endpoints can be induced by glyphosate or GBFs, comparison with results for gene mutation and chromosome effects endpoints, examination of the dose response and association of the effects with toxic endpoints indicates that these effects are likely secondary to toxicity and are threshold mediated. Surfactants in GBFs increase toxicity compared to the active ingredient of glyphosate salts and are shown to induce effects such as membrane damage and oxidant stress which are likely capable of inducing DNA damage effects at cytotoxic doses. These factors as well as other considerations presented in Section 6.3 indicate that these DNA damage effects have negligible significance to prediction of hazard or risk at lower and more relevant exposure levels.

Most of the human studies do not provide interpretable or relevant information regarding whether there are *in vivo* human genotoxic effects of GBFs because the reported incidence of glyphosate formulation exposure in the population was low or because there were reported exposures to a relatively large number of pesticides. Two studies with focus on glyphosate exposure through presence in or near areas of glyphosate formulation spraying found increases in the DNA damage alkaline SCGE end point. In one study clinical signs of toxicity were reported in the population and spraying concentrations were reported to be many times the recommended application rate. Given the nature of the end point a reasonable interpretation is that effects might well be due to the overt toxicity that was reported in the publication. This would be a threshold mediated, non-DNA reactive mechanism and is consistent with experimental system results showing alkaline SCGE effects in animals exposed to high levels of formulation components. The low weight of evidence for significant genotoxic hazard indicated by this particular endpoint in human monitoring is reinforced by findings that exercise induces alkaline SCGE effects in humans. The other study found increases in binucleated micronucleated cell frequency in population in spraying areas but the increases were not consistent with spraying levels or self-reported exposure. These latter observations are not consistent with the study presenting clear evidence of GBF effects on this endpoint. In sum, the available human data do not provide any clear indications that exposed humans are substantially different in response than mammalian animal models or that exposure to GBFs produces DNA-reactive genotoxicity.

Carcinogenicity is an adverse effect that is a possible consequence of genotoxic and mutagenic activity. Conversely, lack of carcinogenicity in properly conducted animal models is supportive for lack of significant *in vitro* mammalian genotoxicity. The updated review provides one new study of glyphosate formulation which is negative for either initiator or complete carcinogenesis activity which provides additional evidence to reinforce the conclusion from earlier mammalian carcinogenicity assays that glyphosate and GBFs are non- carcinogenic. These findings support the conclusion that glyphosate and GBFs do not have *in vivo* mammalian genotoxicity or mutagenicity.

In addition to considering the results relevant to genotoxicity hazard assessment, an important additional perspective on risk can be provided by comparing levels used in experimental studies with expected human and environmental exposure levels. A study of farmers indicated a maximum estimated systemic glyphosate dose of 0.004 mg/kg for application without protective equipment and a geometric mean of 0.0001 mg/kg (Acquavella *et al.*, 2004, ASB2012-11528). When compared with *in vivo* mammalian test systems that utilize glyphosate exposures on the order of 50-300 mg/kg, the margins of exposure between the test systems and farmers is 12,500-75,000 for the maximum farmer systemic exposure and 0.5-3 million for the geometric mean farmer systemic exposure. These margins are quite substantial, especially considering that many of the *in vivo* genotoxicity studies are negative. Assuming reasonable proportionality between exposure to glyphosate and GBF ingredients, similar large margins of exposure would exist for GBF components. The margins of exposure compared to *in vitro* mammalian cell exposures are estimated to be even larger. Assuming uniform distribution, the systemic concentration of glyphosate from the Aquavella *et al.* (2004, ASB2012-11528) farmer biomonitoring study would be on the order of 24nM for the maximum and 0.59 nM for the geometric mean exposure. A typical maximum *in vitro* mammalian exposure of 1-5 mM represents a margin of exposure of 42,000-211,000 for the maximum farmer exposure and 1.7-8.4 million for the geometric mean farmer systemic exposures, respectively.

Overall, the weight of evidence of the studies considered in the earlier review as well as the studies considered in this review indicates that glyphosate and GBFs are not genotoxic in the two general endpoint categories most directly relevant to heritable mutagenesis, gene mutation and chromosome effects. This conclusion results from a preponderance of evidence; however, there are reports of positive discordant results in both end point categories. The new studies considered in this review provide some evidence for DNA damage effects induced by high, toxic exposures, particularly for the alkaline SCGE end point and for GBFs containing surfactant. Several considerations, including the lack of response in other endpoint categories, suggest that these effects result from toxic and not DNA-reactive mechanisms and that they do not indicate *in vivo* genotoxic potential under normal exposure levels.
Regulatory and authoritative reviews of glyphosate supporting registrations and registrations in all regions of the world over the last 40 years have consistently determined that glyphosate is nongenotoxic (Commission, 2002, ASB2009-4191; WHO/FAO, 2004, ASB2008-6266). Scientific publications contrary to these regulatory reviews should be evaluated using a weight of evidence approach with consideration for reliability of the assay used and data quality presented.

Abbreviations
AMPA, aminomethylphosphonic acid ; CB MN, cytokinesis block micronucleus; GBF, glyphosate based formulation; i.p., intraperitoneal ; NCE, normochromatic erythrocyte; OECD, Organization for Economic Cooperation and Development; PCE, polychromatic erythrocyte; POEA, polyethoxylated tallow amine, tallowamine ethoxylate; SCE, sister chromatid exchange; SCGE, single cell gel electrophoresis (comet).

Author(s)	Year	Study title
Alvarez-Moya, C., Silva, M.R., Arambula, A.R.V., Sandoval, A.I., Vasquez, H.C., Gonzales Montes, R.M.	2011	Evaluation of genetic damage induced by glyphosate isopropylamine salt using *Tradescantia* bioassays Genetics and Molecular Biology Volume: 34 Number: 1 Pages: 127-130 ASB2012-11538

Abstract*
Glyphosate is noted for being non-toxic in fishes, birds and mammals (including humans). Nevertheless, the degree of genotoxicity is seriously controversial. In this work, various concentrations of a glyphosate isopropylamine salt were tested using two methods of genotoxicity assaying, *viz*., the pink mutation assay with *Tradescantia* (4430) and the comet assay with nuclei from staminal cells of the same plant. Staminal nuclei were studied in two different forms, namely nuclei from exposed plants, and nuclei exposed directly. Using the pink mutation assay, isopropylamine induced a total or partial loss of color in staminal cells, a fundamental criterion utilised in this test. Consequently, its use is not recommended when studying genotoxicity with agents that produce pallid staminal cells. The comet assay system detected statistically significant ($p < 0.01$) genotoxic activity by isopropylamine, when compared to the negative control in both the nuclei of treated plants and directly treated nuclei, but only the treated nuclei showed a dose-dependent increase. Average migration in the nuclei of treated plants increased, when compared to that in treated nuclei. This was probably due, either to the permanence of isopropylamine in inflorescences, or to the presence of secondary metabolites. In conclusion, isopropylamine possesses strong genotoxic activity, but its detection can vary depending on the test systems used.

Klimisch evaluation

Not reliable
Exposure conditions of plants (immersion) not representative for glyphosate. Inappropriate test model as herbicides are toxic to plants. Presentation of results not sufficient for assessment. Reporting deficiencies (e.g. positive controls).
Not relevant (Due to reliability, and exposure conditions of plants and inappropriate test model).

Klimisch code: 3

Author(s)	Year	Study title
Bolognesi, C. Bonatti, S. Degan, P. Gallerani, E. Peluso, M. Rabboni, R. Roggieri, P. Abbondandolo,A.	1997	Genotoxic activity of glyphosate and its technical formulation roundup Journal of Agricultural and Food Chemistry Volume: 45 Pages: 1957-1962 Z59299

Abstract*
Glyphosate (N-phosphonomethylglycine) is an effective herbicide acting on the synthesis of aromatic amino acids in plants. The genotoxic potential of this herbicide has been studied: the results available in the open literature reveal a weak activity of the technical formulation. In this study, the formulated commercial product, Roundup, and its active agent, glyphosate, were tested in the same battery of assays for the induction of DNA damage and chromosomal effects in vivo and in vitro. Swiss CD1 mice were treated intraperitoneally with test substances, and the DNA damage was evaluated by alkaline elution technique and 8- hydroxydeoxyguanosine (8-OHdG) quantification in liver and kidney. The chromosomal damage of the two pesticide preparations was also evaluated in vivo in bone marrow of mice as micronuclei frequency and in vitro in human lymphocyte culture as SCE frequency. A DNA-damaging activity as DNA single-strand breaks and 8-OHdG and a significant increase in chromosomal alterations were observed with both substances *in vivo* and *in vitro*. A weak increment of the genotoxic activity was evident using the technical formulation.

Klimisch evaluation

Reliability of study: Not reliable
Basic data given, however, the study is performed with methodological and reporting deficiencies (only data without metabolic activation generated in *in vitro* tests, no positive controls included in *in vitro* SCE and *in vivo* experiments, in some experiments only two test substance concentrations tested).
Not relevant (Due to methodological and reporting deficiencies data considered to be supplemental information; i.p. exposure route is not relevant for human exposure)
Klimisch code: 3

Author(s)	Year	Study title
Bolognesi, C., Perrone, E., Landini, E.	2002	Micronucleus monitoring of a floriculturist population from western Liguria, Italy Mutagnesis Volume: 17 Number: 5 Pages: 391-397 ASB2012-11573

Abstract*
A biomonitoring study was carried out to investigate whether exposure to complex pesticide mixtures in ornamental crop production represents a potential genotoxic risk. Exposed and control subjects were selected in western Liguria (Italy). The area was chosen for its intensive use of pesticides. The main crops produced were roses, mimosas, carnations and chrysanthemums, as ornamental non-edible plants, and tomato, lettuce and basil, as edible ones. The levels of micronuclei (MN) were analysed in peripheral blood lymphocytes of 107 floriculturists (92 men and 15 women) and 61 control subjects (42 men and 19 women). A statistically significant increase in binucleated cells with micronuclei (BNMN) was detected in floriculturists with respect to the control population (4.41 +/- 2.14 MN/1000 cells versus 3.04 +/- 2.14, $P < 0.001$). The mean number of BNMN varied as a function of sex and age. Smoking habit had no effect on MN frequency. A positive correlation between years of farming and MN frequency in peripheral blood lymphocytes was observed ($r = 0.30$, $P = 0.02$). The conditions of exposure were also associated with an increase in cytogenetic damage, with a 28 % higher MN frequency in greenhouse workers compared with subjects working only outdoors in fields. Workers not using protective measures during high exposure activities showed an increase in MN frequency. Our findings suggest a potential genotoxic risk due to pesticide exposure.

* Quoted from article

Klimisch evaluation

Reliability of study: Not reliable for glyphosate
Comment: MN-test comparable to OECD guidelines, but not equal. Exposures to multiple pesticides with no information on exposure concentrations to individual pesticides make result unreliable for glyphosate.
Relevance of study: Not relevant (Due to the exposure of multiple pesticides, only general conclusions about pesticide exposure and cytogenicity possible. Not relevant for glyphosate.
Klimisch code: 3

Author(s)	Year	Study title
Bolognesi, C., Landini, E., Perrone, E., Roggieri, P.	2004	Cytogenetic biomonitoring of a floriculturist population in Italy: micronucleus analysis by fluorescence in situ hybridization (FISH) with an all-chromosome centrometric probe Mutation Research Volume: 557 Number: 2 Pages: 109-117 ASB2012-11572

Abstract*
Flower production in greenhouses associated with a heavy use of pesticides is very wide- spread in the western part of the Ligurian region (Italy). The formation of micronuclei in peripheral blood lymphocytes is a valuable cytogenetic biomarker in human populations occupationally exposed to genotoxic compounds. In the present study we investigated the micronucleus frequency in peripheral blood lymphocytes of 52 floriculturists and 24 control subjects by use of the cytokinesis-block methodology associated with fluorescence in situ hybridization with a pan-centromeric probe that allowed to distinguish centromere-positive (C+) and centromere-negative (C−) micronuclei. The comparison between floriculturists and controls did not reveal any statistically significant difference in micronucleus frequency, although an increase was observed with increasing pesticide use, number of genotoxic pesticides used and duration of exposure. An increase in C+ as well as in C− micronuclei and in the percentage of C+ micronuclei with respect to the total number of micronuclei was detected in floriculturists, suggesting a higher contribution

of C+ micronuclei in the total number scored. The percentage C+ micronuclei was not related to the duration of exposure or to the number of genotoxic pesticides used, but a higher percentage (66.52% versus 63.78%) was observed in a subgroup of subjects using benzimidazolic compounds, compared with the floriculturist population exposed to a complex pesticide mixture not including benzimidazolics. These results suggest a potential human hazard associated with the exposure to this class of aneuploidy-inducing carcinogens.

Klimisch evaluation

Reliability of study: Not reliable for glyphosate
Comment: Well-documented study. MN-test comparable to OECD guidelines, but not equal. No information on exposure concentrations to individual pesticides.
Relevance of study: Not relevant (Due to the exposure of multiple pesticides, only general conclusions about pesticide exposure and cytogenic non-statistically significant differences possible. No statistically relevant findings reported for glyphosate alone).
Klimisch code: 2

Author(s)	Year	Study title
Cavas, T., Könen S.	2007	Detection of cytogenetic and DNA damage in peripheral erythrocytes of goldfish (*Carassius auratus*) exposed to a glyphosate formulation using the micronucleus test and the comet assay Mutagenesis 22 263-268 ASB2012-11587

Abstract*
Glyphosate is a widely used broad-spectrum weed control agent. In the present study, an in vivo study on the genotoxic effects of a technical herbicide (Roundup®) containing isopropylamine salt of glyphosate was carried out on freshwater goldfish *Carassius auratus*. The fish were exposed to three doses of glyphosate formulation (5, 10 and 15 ppm). Cyclophosphamide at a single dose of 5 mg/L was used as positive control. Analysis of micronuclei, nuclear abnormalities and DNA damage were performed on peripheral erythrocytes sampled at intervals of 48, 96 and 144 h post treatment. Our results revealed significant dose-dependent increases in the frequencies of micronuclei, nuclear abnormalities as well as DNA strand breaks. Our findings also confirmed that the alkaline comet assay and nuclear deformations in addition to micronucleus test on fish erythrocytes in vivo are useful tools in determining the potential genotoxicity of commercial herbicides.

* Quoted from article

Klimisch evaluation

Reliability of study: Not Reliable
Comment: Methodological and reporting deficiencies (e.g. test substance source, no concurrent measurement of toxicity reported, less than 2000 erythrocytes scored per animal and results not reported separately for replicates).
Relevance of study: Relevant with restrictions (Due to reliability. Discussion confuses glyphosate with glyphosate formulated products.)
Klimisch code: 3

Author(s)	Year	Study title
Guilherme, S. Gaivao, I. Santos, M.A. Pacheco, M.	2010	European eel (*Anguilla Anguilla*) genotoxic and pro-oxidant responses following short-term exposure to Roundup® - a glyphosate-based herbicide. Mutagenesis Volume: 25 Number: 5 Pages: 523-530 ASB2012-11836

Abstract*

The glyphosate-based herbicide, Roundup®, is among the most used pesticides worldwide. Due to its extensive use, it has been widely detected in aquatic ecosystems representing a potential threat to non-target organisms, including fish. Despite the negative impact of this commercial formulation in fish, as described in literature, the scarcity of studies assessing its genotoxicity and underlying mechanisms is evident. Therefore, as a novel approach, this study evaluated the genotoxic potential of Roundup® to blood cells of the European eel (*Anguilla anguilla*) following short-term (1 and 3 days) exposure to environmentally realistic concentrations (58 and 116 mg/L), addressing also the possible association with oxidative stress. Thus, comet and erythrocytic nuclear abnormalities (ENAs) assays were adopted, as genotoxic end points, reflecting different types of genetic damage. The prooxidant state was assessed through enzymatic (catalase, glutathione-S-transferase, glutathione peroxidase and glutathione reductase) and non-enzymatic (total glutathione content) antioxidants, as well as by lipid peroxidation (LPO) measurements. The Roundup® potential to induce DNA strand breaks for both concentrations was demonstrated by the comet assay. The induction of chromosome breakage and/or segregational abnormalities was also demonstrated through the ENA assay, though only after 3-day exposure to both tested concentrations. In addition, the two genotoxic indicators were positively correlated. Antioxidant defences were unresponsive to Roundup®. LPO levels increased only for the high concentration after the first day of exposure, indicating that oxidative stress caused by this agrochemical in blood was not severe. Overall results suggested that both DNA damaging effects induced by Roundup® are not directly related with an increased pro-oxidant state. Moreover, it was demonstrated that environmentally relevant concentrations of Roundup® can pose a health risk for fish populations.

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* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	No positive controls were included, which significantly detracts from the utility of a non-validated, non- standard test method. Less than the standard of a minimum of three dose levels used, independent coding of slides for scoring and results not reported separately for replicates.
Relevance of study:	Not relevant (Non-standard test system, no positive controls to verify test method/study validity.)
Klimisch code:	3

Author(s)	Year	Study title
Kale, P.G. Petty, B.T. Jr. Walker, S. Ford, J.B. Dehkordi, N. Tarasia, S. Tasie, B.O. Kale, R. Sohni, Y.R.	1995	Mutagenicity Testing of Nine Herbicides and Pesticides Currently Used in Agriculture. Environmental and Molecular Mutagenesis Volume: 25 Pages: 148-153 Z73986, ASB2012-11860

Abstract*
Nine herbicides and pesticides were tested for their mutagenicity using the *Drosophila* sex- linked recessive lethal mutation assay. These are Ambush, Treflan, Blazer, Roundup, 2,4-D Amine, Crossbow, Galecron, Pramitol, and Pondmaster. All of these are in wide use at present. Unlike adult feeding and injection assays, the larvae were allowed to grow in medium with the test chemical, thereby providing long and chronic exposure to the sensitive and dividing diploid cells, i.e., mitotically active spermatogonia and sensitive spermatocytes. All chemicals induced significant numbers of mutations in at least one of the cell types tested. Some of these compounds were found to be negative in earlier studies. An explanation for the difference in results is provided. It is probable that different germ cell stages and treatment regimens are suitable for different types of chemicals. Larval treatment may still be valuable and can complement adult treatment in environmental mutagen testing.

Klimisch evaluation

Reliability of study: Not reliable
Comment: Comparable to 1984 OECD guideline, but with several deficiencies (no positive controls reported and thus study validity not verifiable; wild type male treatment age different than recommended, purity of test substances not reported, tested formulation other ingredients such as surfactants not reported.)
Relevance of study: Not relevant for glyposate (Glyphosate not tested; formulation tested)
Klimisch code: 3

Author(s)	Year	Study title
Manas, F. Peralta, L. Raviolo, J. Garcia Ovando, H. Weyers, A. Ugnia, L. Gonzalez Cid, M. Larripa, I. Gorla, N.	2009	Genotoxicity of AMPA, the environmental metabolite of glyphosate, assessed by the Comet assay and cytogenetic tests. Ecotoxicology and Environmental Safety Volume: 72 Pages: 834-837 ASB2012-11891

Abstract*
Formulations containing glyphosate are the most widely used herbicides in the world. AMPA is the major environmental breakdown product of glyphosate. The purpose of this study is to evaluate the in vitro genotoxicity of AMPA using the Comet assay in Hep-2 cells after 4h of incubation and the chromosome aberration (CA) test in human lymphocytes after 48 h of exposition. Potential in vivo genotoxicity was evaluated through the micronucleus test in mice. In the Comet assay, the level of DNA damage in exposed cells at 2.5-7.5 mM showed a significant increase compared with the control group. In human lymphocytes we found statistically significant clastogenic effect AMPA at 1.8 mM compared with the control group. In vivo, the micronucleus test rendered significant statistical increases at 200-400 mg/kg. AMPA was

genotoxic in the three performed tests. Very scarce data are available about AMPA potential genotoxicity.

Klimisch evaluation

Reliability of study: Not reliable
Comment: Reporting deficiencies (purity of AMPA not specified, several parameters in the MNT not reported, only 2 dose levels used in both CA and MNT). Exposure route used in the MNT is not relevant for human exposure; methodological deficiencies (see guideline deviations).
Relevance of study: Not relevant (Due to reliability)
Klimisch code: 3

Author(s)	Year	Study title
Manas, F. Peralta, L. Raviolo, J. Garcia Ovando, H. Weyers, A. Ugnia, L. Gonzalez Cid, M. Larripa, I. Gorla, N.	2009	Genotoxicity of glyphosate assessed by the comet assay and cytogenic tests Environmental Toxicology and Pharmacology Volume: 28 Pages: 37-41 ASB2012-11892

Abstract*
It was evaluated the genotoxicity of glyphosate which up to now has heterogeneous results. The comet assay was performed in Hep-2 cells. The level of DNA damage in the control group (5.42±1.83 arbitrary units) for tail moment (TM) measurements has shown a significant increase ($p < 0.01$) with glyphosate at a range concentration from 3.00 to 7.50mM. In the chromosome aberrations (CA) test in human lymphocytes the herbicide (0.20–6.00 mM) showed no significant effects in comparison with the control group. In vivo, the micronucleus test (MNT) was evaluated in mice at three doses rendering statistical significant increases at 400 mg/kg (13.0±3.08 micronucleated erythrocytes/1000 cells, $p < 0.01$). In the present study glyphosate was genotoxic in the comet assay in Hep-2 cells and in the MNT test at 400 mg/kg in mice. Thiobarbituric acid reactive substances (TBARs) levels, superoxide dismutase (SOD) and catalase (CAT) activities were quantified in their organs. The results showed an increase in these enzyme activities.
* Quoted from article

Klimisch evaluation

Reliability of study: Not reliable
Comment: Guideline deviations and reporting deficiencies. Several parameters in the MNT not reported. Blind scoring reported for the CA but not MNT. Exposure route used in the MNT is not relevant for human exposure. (see guideline deviations). No indication of pH or osmolality control for the comet assay. Results not reported separately for replicates.
Relevance of study: Not relevant (Due to guideline deviations and reporting deficiencies).
Klimisch code: 3

Author(s)	Year	Study title

Mladinic, M. Berend, S. Vrdoljak, A.L. Kopjar, N. Radic, B. Zeljezic, D.	2009	Evaluation of Genome Damage and Its Relation to Oxidative Stress Induced by Glyphosate in Human Lymphocytes *in Vitro* Environmental and Molecular Mutagenesis Volume: 50 Number: 9 Pages: 800-807 ASB2012-11906

Abstract*
In the present study we evaluated the genotoxic and oxidative potential of glyphosate on human lymphocytes at concentrations likely to be encountered in residential and occupational exposure. Testing was done with and without metabolic activation (S9). Ferric-reducing ability of plasma (FRAP), thiobarbituric acid reactive substances (TBARS) and the hOGG1 modified comet assay were used to measure glyphosate's oxidative potential and its impact on DNA. Genotoxicity was evaluated by alkaline comet and analysis of micronuclei and other nuclear instabilities applying centromere probes. The alkaline comet assay showed significantly increased tail length (20.39 µm) and intensity (2.19%) for 580 µg/mL, and increased tail intensity (1.88%) at 92.8 µg/mL, compared to control values of 18.15 µm for tail length and 1.14% for tail intensity. With S9, tail length was significantly increased for all concentrations tested: 3.5, 92.8, and 580 µg/mL. Using the hOGG1 comet assay, a significant increase in tail intensity was observed at 2.91 µg/mL with S9 and 580 µg/mL without S9. Without S9, the frequency of micronuclei, nuclear buds and nucleoplasmic bridges slightly increased at concentrations 3.5 µg/mL and higher. The presence of S9 significantly elevated the frequency of nuclear instabilities only for 580 µg/mL. FRAP values slightly increased only at 580 µg/mL regardless of metabolic activation, while TBARS values increased significantly. Since for any of the assays applied, no clear dose-dependent effect was observed, it indicates that glyphosate in concentrations relevant to human exposure do not pose significant health risk.

* Quoted from article

Klimisch evaluation

Reliability of study:	Reliable with restrictions
Comment:	Non-GLP, non-guideline *in vitro* study, meeting scientific principles
Relevance of study:	Relevant with restrictions (Assessment ofGenotoxicity *in vitro* at concentrations relevant to human exposure levels; authors state that no clear dose-dependent effect was observed, and results indicate that glyphosate in concentrations relevant to human exposure do not pose significant health risk.
Klimisch code:	2

Author(s)	Year	Study title
Mladinic, M., Perkovic, P., Zeljezic, D.	2009b	Characterization of chromatin instabilities induced by glyphosate, terbuthylazine and carbofuran using cytome FISH assay Toxicology Letters Volume: 189 Number: 2 Pages: 130-137 ASB2012-11907

Abstract*
Possible clastogenic and aneugenic effects of pesticides on human lymphocytes at concentrations likely to be encountered in residential and occupational exposure were evaluated with and without the use of

metabolic activation (S9). To get a better insight into the content of micronuclei (MN) and other chromatin instabilities, lymphocyte preparations were hybridized using pancentromeric DNA probes. Frequency of the MN, nuclear buds (NB) and nucleoplasmic bridges (NPB) in cultures treated with glyphosate slightly increased from 3.5 µg/mL onward. Presence of S9 significantly elevated cytome assay parameters only at 580 µg/mL. No concentration-related increase of centromere (C+) and DAPI signals (DAPI+) was observed for glyphosate treatment. Terbuthylazine treatment showed a dose dependent increase in the number of MN without S9 significant at 0.0008 µg/mL and higher. At concentration lower than 1/16 LD_{50} occurrence of C + MN was significantly elevated regardless of S9, but not dose related, and in the presence of S9 only NBs containing centromere signals were observed. Carbofuran treatment showed concentration dependent increase in the number of MN. The frequency of C + MN was significant from 0.008 µg/mL onward regardless of S9. Results suggest that lower concentrations of glyphosate have no hazardous effects on DNA, while terbuthylazine and carbofuran revealed a predominant aneugenic potential.

* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Non-GLP, non-guideline study *in vitro*. Positive and negative control results almost indistinguishable for MN assay without metabolic activation. Negative control NB and NBP results not reported.
Relevance of study:	Not relevant (Proposed mechanism of genotoxicity (*in vitro*) is not relevant to human exposure levels. Authors express confidence that estimated maximum human exposure levels correspond to acceptable safety levels based on evaluated *in vitro* endpoints, and that their findings need to be verified *in vivo*.)
Klimisch code:	3

Author(s)	Year	Study title
Paz-Y-Mino, C. Sanchez, M. E. Arevalo, M. Munoz, M. J. Witte, T. De-La-Carrera, G.O. Leone, P. E.	2007	Evaluation of DNA damage in an Ecuadorian population exposed to glyphosate. Genetics and Molecular Biology Volume: 30 Number: 2 Pages: 456-460 ASB2012-11992

Abstract*
We analyzed the consequences of aerial spraying with glyphosate added to a surfactant solution in the northern part of Ecuador. A total of 24 exposed and 21 unexposed control individuals were investigated using the comet assay. The results showed a higher degree of DNA damage in the exposed group (comet length = 35.5 mu m) compared to the control group (comet length = 25.94 mu m). These results suggest that in the formulation used during aerial spraying glyphosate had a genotoxic effect on the exposed individuals.

* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Documentation of Comet assay insufficient for assessment.
Relevance of study:	Not relevant (Glyphosate formulation was applied at much

higher dose rates than recommended for the intended uses in the EU. In addition, the herbicide was combined with the adjuvant (Cosmoflux 411F) that can increase the biological action of the herbicide. This adjuvant will not be used in the EU.

Klimisch code: 3

Author(s)	Year	Study title
Peluso, M. Munnia, A. Bolognesi, C. Parodi, S.	1998	32P-postlabeling detection of DNA adducts in mice treated with the herbicide Roundup. Environmental and Molecular Mutagenesis Volume: 31 Number: 4 Pages: 55-59 TOX1999-318

Abstract*
Roundup is a postemergence herbicide acting on the synthesis of amino acids and other important endogenous chemicals in plants. Roundup is commonly used in agriculture, forestry, and nurseries for the control or destruction of most herbaceous plants. The present study shows that Roundup is able to induce a dose-dependent formation of DNA adducts in the kidneys and liver of mice. The levels of Roundup-related DNA adducts observed in mouse kidneys and liver at the highest dose of herbicide tested (600 mg/kg) were 3.0 +/- 0.1 (SE) and 1.7 +/- 0.1 (SE) adducts/10(8) nucleotides, respectively. The Roundup DNA adducts were not related to the active ingredient, the isopropylammonium salt of glyphosate, but to another, unknown component of the herbicide mixture. Additional experiments are needed to identify the chemical specie(s) of Roundup mixture involved in DNA adduct formation. Findings of this study may help to protect agricultural workers from health hazards and provide a basis for risk assessment.

* Quoted from article

Klimisch evaluation

Reliability of study: Not Reliable

Comment: A non-guideline study with confounding results based on testing a surfactant containing formulation. Reporting deficiencies (statistical methods). Toxic surfactant effects subsequently verified in Heydens *et al.* (2008, ASB2012-11845) reporting the same study type with a glyphosate formulated product and an appropriate control; formulation blank without glyphosate.

Relevance of study: Not relevant (i.p. administration of high doses of a surfactant containing formulation a relevant exposure scanario for human risk assessments. In addition, the DNA adducts observed were not related to the active ingredient (isopropylammonium salt of glyphosate), but to another, unknown component of the herbicide mixture.)

Klimisch code: 3

Author(s)	Year	Study title

Poletta, G.L. Larriera, A. Kleinsorge, E. Mudry, M.D.	2009	Genotoxicity of the herbicide formulation Roundup® (glyphosate) in broad-snouted caiman (*Caiman latirostris*) evidenced by the Comet assay and Micronucleus test Mutation Research Volume: 672 Number: 2 Pages: 95-102 ASB2012-12002

Abstract*
The genotoxicity of pesticides is an issue of worldwide concern. The present study was undertaken to evaluate the genotoxic potential of a widely used herbicide formulation, Roundup® (glyphosate), in erythrocytes of broad-snouted caiman (*Caiman latirostris*) after in ovo exposure. Caiman embryos were exposed at early embryonic stage to different sub-lethal concentrations of Roundup® (50, 100, 200, 300, 400, 500, 750, 1000, 1250 and 1750 µg/egg). At time of hatching, blood samples were obtained from each animal and two short-term tests, the Comet assay and the Micronucleus (MN) test, were performed on erythrocytes to assess DNA damage. A significant increase in DNA damage was observed at a concentration of 500 µg/egg or higher, compared to untreated control animals ($p<0.05$). Results from both the Comet assay and the MN test revealed a concentration-dependent effect. This study demonstrated adverse effects of Roundup® on DNA of *C. latirostris* and confirmed that the Comet assay and the MN test applied on caiman erythrocytes are useful tools in determining potential genotoxicity of pesticides. The identification of sentinel species as well as sensitive biomarkers among the natural biota is imperative to thoroughly evaluate genetic damage, which has significant consequences for short- and long-term survival of the natural species.

* Quoted from article

Klimisch evaluation

Reliability of study: Not reliable

Comment: Non-GLP studies in a unique test model. Micronucleus assay followed guideline, Comet assay similar to guideline. Test methods have been modified to be applied caiman species. Methodological deficiencies: housing and feeding conditions of parents not specified; sex not distinguished, stability and homogeneity assessment of test substance preparations not reported. Results not reported separately for replicate individual animals.

Relevance of study: Not relevant. Highly artificial *in ovo* exposure scenario not relevant to real world environmental exposures. Caiman eggs are covered and not exposed to the surface. Any glyphosate in a potential herbicide overspray would sorb to sediment and organic matter and not transport to the egg surface.

Klimisch code: 3

Author(s)	Year	Study title
Rodrigues, H.G. Penha-Silva, N. Ferreira Pereira de Araujo, M. Nishijo, H. Aversi-Ferreira, T.A.	2011	Effects of Roundup® Pesticide on the Stability of Human Erythrocyte Membranes and Micronuclei Frequency in Bone Marrow Cells of Swiss Mice The Open Biology Journal, Volume: 4 Pages: 54-59 ASB2012-12010

Abstract*
Pesticides can affect the health of living organisms through different mechanisms such as membrane denaturation. The evaluation of the deleterious effects of chemical agents on biological membranes can be performed through the analysis of the stability of erythrocytes against a concentration gradient of certain chemical agent in physiologic saline solution. This work analyzed the effect of the herbicide Roundup® on the membrane of human erythrocytes in blood samples collected with EDTA or heparin as anticoagulant agent. The results were analyzed through spectrophotometry at 540 nm and light microscopy. There was an agreement between spectrophometric and morphologic analyses. At the concentration limit recommended for agricultural purposes, Roundup® promoted 100% of hemolysis. The D50Roundup® values obtained for human blood samples collected with EDTA were not significantly different from those obtained for samples collected with heparin. However, the lysis curves presented lower absorbance values at 540 nm in the presence of blood collected with EDTA in relation to that collected with heparin, probably due to haemoglobin precipitation with EDTA. This work also analyzed the effects of three different Roundup® doses (0.148, 0.754 and 1.28 mg/kg) on the micronuclei frequency in bone marrow cells of Swiss mice in relation to a positive control of cyclophosphamide (250 mg/kg). The two highest Roundup® doses showed the same genotoxicity level as the positive control.
* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable. Determination of the stability of human erythrocytes: Results are not surprising because surfactants are known to compromise cell membrane integrity. Doses not reflective of physiological concentrations of either glyphosate or surfactant.
Micronucleus test *in vivo*:	Irrelevant route of exposure for surfactant containing formulated products. Results confounded by presence of surfactant toxicity; refer to Heydens *et al.* (2008, ASB2012-11845)
Comment:	Non-guideline, non-GLP studies. Determination of the stability of human erythrocytes Results attributable to surfactant induced cytotoxicity Micronucleus test *in vivo*. Major reporting deficiencies (no information on number of cells evaluated, only graphical documentation of results, no information on absolute MN frequencies).
Relevance of study:	Not relevant (Test material containing surfactant is not appropriately evaluated in either model).
Klimisch code:	3

Author(s)	Year	Study title
Vigfusson, N.V. Vyse, E.R.	1980	The effect of the pesticides Dexon, Captan and Roundup on sister chromatid exchanges in human lymphocytes in vitro. Mutation Research Volume: 79 Pages: 53-57 TOX9700576, ASB2012-12044

Abstract*
Three pesticides at varying concentration were tested for the induction of SCE [sister chromatid exchanges] in human lymphocytes in vitro. The fungicide, Dexon, sodium (4- (dimethylamino)phenyl)diazene sulfonate, caused the greatest increase in SCE frequency and the response was dose related. The herbicide,

Roundup, isopropylamide salt of N- (phosphonomethyl)glycine, had the least effect on SCE requiring the use of much higher concentrations to produce an effect. Limited results were obtained with the fungicide Captan, cis-N-((trichloromethyl)thio)-4-cyclo-hexene-1, 2-dicarboximide, because of toxic levels of the fungicide or solvent used.

* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Test material was a formulated product containing surfactant. Authors acknowledge cytotoxicity was a confounding factor for data interpretation; since the time of this study, around 1980, surfactant effects on *in vitro* test systems have been well documented. Only very minor changes in SCE were reported, with a limited data set of two donors and a lack of dose-response. Statistical analysis was not feasible with this very limited data set.
Relevance of study:	Not relevant (Limited data set, internally consistent findings, no statistics conducted and no dose-response)
Klimisch code:	3

B.6.5 Long-term toxicity and carcinogenicity (Annex IIA 5.5)
B.6.5.1 Long-term toxicity and carcinogenicity in the rat
B.6.5.1.1 New studies in rats
B.6.5.1.2 Previously known long-term studies in rats (already subject to EU evaluation)
B.6.5.2 Carcinogenicity in the mouse
B.6.5.3 Published data on carcinogenicity (released since 2000)

Epidemiology studies

A number of epidemiology studies over the last decade have focused on pesticide exposure and associated health outcomes. Publications vary in the specificity of their conclusions regarding pesticides in general, classes of pesticides and in some cases individual insecticides, herbicides or fungicides. While some of these publications specifically mention glyphosate, few draw tenable associations with any specific cancer outcome. Publications suggesting glyphosate is associated with any cancer outcome are discussed below.

An essential consideration in both, risk assessment and interpreting the relevance of toxicology data is exposure assessment. An inherent low level of confidence exists for epidemiological studies where tenuous links to exposure exist. Suggested associations between health outcomes and any possible causative agent are merely speculation if exposures are not identifiable. Pivotal to the understanding of glyphosate exposure are data published by Acquavella *et al.* (2004, ASB2012-11528; 2005, ASB2012-11530), which quantified human systemic glyphosate exposure levels in farmer applicators and their families. The geometric mean systemic dose for farmers applying glyphosate, some of whom applied glyphosate to areas up to 400 acres, was 0.0001 mg/kg/day, approximately 0.03% of the EU glyphosate acceptable operator exosure Level (AOEL) according to EU Review Report 6511/VI/99-final (21 January 2008, ASB2009-4191). The highest systemic dose, skewed well above the geometric mean, was 0.004 mg/kg/day, which is 1.95% EU glyphosate AOEL according to EU Review Report 6511/VI/99-final (21 January 2008, ASB2009-4191) and 1.3% of the current EU glyphosate attapcable daily intake (ADI) according to EU Review Report 6511/VI/99-final (21 January 2008, ASB2009-4191). Even lower systemic doses were determined for spouses and children, 0.00004 mg/kg and 0.0008 mg/kg, respectively. Multiple carcinogenicity studies have since been conducted by numerous glyphosate registrants demonstrating NOAELs of at least ten-fold higher than the highest dose tested in the study driving the current EU ADI calculation.

The largest epidemiological study of pesticide exposure and health outcomes in the United States is the Agricultural Health Study (AHS), which included glyphosate. Dozens of publications have resulted from data generated in this study of approximately 57,000 enrolled farmer applicators. Blair *et al.* (2009, ASB2012-11566) provided an overview of cancer endpoints associated with different agricultural chemicals reported in earlier AHS publications. Glyphosate was not reported to be associated with leukemia, melanoma, or cancers of the prostate, lung, breast, colon or rectum. De Roos *et al.* (2005, ASB2012-11605) reported AHS data evaluating glyphosate use and multiple cancer endpoints; no association was noted for glyphosate with all cancers, including cancer of the lung, oral cavity, colon, rectum, pancreas, kidney, bladder, prostate, melanoma, all lymphohematopoietic cancers, non- Hodgkin's lymphoma (NHL) and leukemia. In an earlier publication based on another data set, however, De Roos *et al.*, (2003, ASB2012-11606) reported an association between NHL and glyphosate use. McDuffie *et al.* (2001, ASB2011-364) reported a non-significant positive association between self-reported glyphosate exposure and NHL in a Canadian study. Blair *et al.* (2009, ASB2012-11566) did not report an association between glyphosate use and NHL in the AHS data, but a "possible association" between glyphosate use and multiple myeloma was mentioned. The AHS publication reporting this refers to a "suggested association" between glyphosate use and multiple myeloma (De Roos *et al.*, 2005, ASB2012-11605), yet it did not demonstrate significant increase in relative risk for multiple myeloma. Both De Roos papers will be discussed in more detail below. Interestingly, a subsequent AHS review paper for the President's Cancer Panel (Freeman, 2009, ASB2012-11623) specifically references De Roos (2005 ASB2012-11605) as providing no observed incidents of cancers of any type being associated with glyphosate.

Lee *et al.* (2005, ASB2012-11882) reported a glyphosate association with gliomas, with the odds ratio differing between self-respondents (OR = 0.4) and proxy respondents (OR = 3.1). The authors expressed concern that higher positive associations observed for proxy respondents with glyphosate and several other pesticides, and suggested perhaps more accurate reporting of proxies for cases, and underreporting by proxies for controls; proxy respondents were spouses in 62 % of cases versus 45% of controls, lending to lower reported incidents in the control group.

Monge et al (2007, ASB2012-11909) investigated associations between parental pesticide exposures and childhood Leukaemia in Costa Rica. Results are not interpretable for glyphosate as exposure was estimated with "other pesticides", including paraquat, chlorothalanil and "others". No association was noted for paternal exposures, but elevated leukaemias were associated with maternal exposures to "other pesticides" during pregnancy. Similarly, glyphosate is captured under "other pesticides" being associated with NHL by Fritschi *et al.* (2005, ASB2012-11624) and therefore should not be interpreted as an association with glyphosate.

Some further epidemiologic studies are focused on an association between pesticide exposure and Non-Hodgkin`s Lymphoma (NHL). Hardell and Eriksson (1999, ASB2012-11838) investigated in a case-control study the incidence of NHL in relation to pesticide exposure in Sweden. 404 cases and 741 controls have been included. The authors discussed an increased risk for NHL especially for phenoxyacetic acids. Glyphosate was included in the uni-variate and multi-variate analyses. However, only 7 of 1145 subjects in the study gave exposure histories to this agent. The authors reported a moderately elevated odds ratio (OR) of 2.3 for Glyphosate. This OR was not statistically significant oand was based on only 4 "exposed" cases and 3 "exposed" controls. The major limitations of this study were: the reliance on reported pesticide use (not documented exposure) information, the small number of subjects who reported use of specific pesticides, the possibility of recall bias, the reliance on secondary sources (next-of-kin interviews) for approximately 43% of the pesticide use information, and the dificulty in the controlling for potential confounding factors given the small number of exposed subjects.

A further study was submitted by Hardell *et al.* (2002, ASB2012-11839). This study pools data from the above mentioned publication by Hardell and Eriksson (1999, ASB2012-11838) with data from a previously submitted publication from Nordström, Hardell at al. (1998, TOX1999-687).

The authors found increased risks in an uni-variate analysis for subjects exposed to herbicides, insecticides, fungicides and impregnating agents. Among herbicides, significant associations were found for glyphosate and MCPA. However, in multi-variate analyses the only significantly increased risk was for a heterogenous category of other herbicides than above, not for glyphosate. No information is given about exposure

duration, exposure concentration, as well as medical history, lifestyle factors (e.g. smoker, use of prescribed drugs etc.). In all, the above mentioned limitations of the publication from Hardell and Eriksson (1999, ASB2012-11838) are also the limitions of the publication from Hardell *et al.* (2002, ASB2012-11839).
Fritschi *et al.* (2005, ASB2012-11624) submitted a case-control study with 694 cases of NHL and 694 controls in Australia. Substantial exposure to any pesticide was associated with an increase of NHL. However, no association between NHL and glyphosate can be made on basis of this study. No information was given about exposure duration, used glyphosate products, exposure duration and application rates. Therefore, the documentation is considered to be insufficient for assessment.
Eriksson *et al.* (2008, ASB2012-11614) reported a case-control study which included 910 cases of NHL and 1016 controls living in Sweden. The highest risk was calculated for MCPA. Glyphosate exposure was reported by 29 cases and 18 controls, and the corresponding odds ratio (OR) was 2.02. Results and reliability of the study are discussed below.
Alavanja *et al.* (2013, ASB2014-9174) reviewed studies on cancer burden among pesticide applicators and others due to pesticide exposure. In this article the epidemiological, molecular biology, and toxicological evidence emerging from recent literature assessing the link between specific pesticides and several cancers including prostate cancer, non-Hodgkin lymphoma, leukemia, multiple myeloma, a breast cancer were integrated. Glyphosate was reported to be the most commonly used in conventional pesticide active ingedient worldwide. The only association between the use of glyphosate and cancer burden described in this review was the result of Eriksson *et al.* (2008, ASB2012-11614) which was described above.

The following epidemiology publications report a lack of association between glyphosate and specific cancer types.

- Alavanja *et al.* (2003, ASB2012-11535) reported on prostate cancer associations with specific pesticide exposures in the AHS; glyphosate did not demonstrate a significant exposure-response association with prostate cancer.
- Multigner et al, (2008, ASB2012-11917) also reported a lack of association between glyphosate use and prostate cancer. This data appears to have also been reported by Ndong *et al.* (2009, ASB2012-11922).
- The lack of association between glyphosate use and prostate cancer was also supported recently in an epidemiology study of Farmers in British Columbia, Canada by Band *et al.* (2011, ASB2012-11555).
- Lee *et al.* (2004, ASB2012-11883) reported a lack of association between glyphosate use and stomach and esophageal adenocarcinomas.
- Carreon *et al.* (2005, ASB2012-11585) reported epidemiological data on gliomas and farm pesticide exposure in women; glyphosate had no association with gliomas.
- Engel *et al.* (2005, ASB2012-11613) reported AHS data on breast cancer incidence among farmers' wives, with no association between breast cancer and glyphosate.
- Flower et al (2004, ASB2012-11620) reported AHS data on parental use of specific pesticides*a* and subsequent childhood cancer risk among 17,280 children, with no association between childhood cancer and glyphosate.
- Andreotti *et al.* (2009, ASB2012-11544) reported AHS data where glyphosate was not associated with pancreatic cancer.
- Landgren *et al.* (2009, ASB2012-11875) reported AHS data on monoclonal gammopathy of undetermined significance (MGUS), showing no association with glyphosate use.
- Karunanayake *et al.* (2011, ASB2012-11865) reported a lack of association between glyphosate and Hodgkin's lymphoma.
- Pahwa *et al.* (2011, ASB2012-11987) reported a lack of association between glyphosate and multiple myeloma.
- Schinasi and Leon (2014, ASB2014-4819) published the results of epidemiologic research on the relationship between non-Hodgkin lymphoma (NHL) and occupational exposure to pesticides. Phenoxy herbicides, carbamate insecticides, organophosphorus insecticides and lindane were positively associated with NHL. However, no association between NHL an glyphosate was reported.
- Kachuri *et al.* (2013, ASB2014-8030) investigated the association between lifetime use of multiple

pesticides and multiple myeloma in Canadian men. Excess risks of multiple myeloma were observed among men reported using at least one carbamate pesticide, one phenoxy herbicide and ≥ organochlorines. However, no excess risk was observed for glyphosate.

- Cocco *et al.* (2014, ASB2014-7523) investigated the role of occupational exposure to agrochemicals in the aetiology of lymphoma overall, B cell lymphoma and its most prevalent subtypes. No increased CLL risk in relation to glyphosate was evidenced.
- Alavanja and Bonner (2012, ASB2014-9173) reviewed studies on occupational pesticide exposure and cancer risk. Twenty one pesticides identified subsequent to the last IARC review showed significant exposure-response associations in studies of specific cancers. No significant association was observed for glyphosate
- El-Zaemy and Heyworth (2012, ASB2014-9473) reported a case control study on the association between pesticide spray drift from agricultural pesticide application areas and breast cancer in Western Australia. The findings support the hypothesis that woman who ever noticed spray drift or who first noticed spray drift at a younger age had increased risk of breast cancer. However, it was not possible to examine whether the observed associations are the result of a particular class of pesticides.
- Pahwa *et al.* (2011, ASB2014-9625) investigated the putative association of specific pesticides with soft-tissue sarcoma (STS). A Canadian population-based case-control study conducted in six provinces was used on this analysis. The incidence of STS was associated with insecticides aldrin and diazinon after adjustment for other independent predictors. However, no statistically significant association between STS and exposure to glyphosate or other herbicides was observed.
- Koutros *et al.* (2011, ASB2014-9594) studied associations between pesticide and prostate cancer. No statistically significant positive association between pesticides and prostate cancer were observed. There was suggestive evidence on an increased risk (OR>1.0) with an increasing number of days of use of petroleum oil/petroleum distillate used as herbicide, terbufos, fonofos, phorate and methyl bromide. However, no increased risk (OR>1.0) was observed for glyphosate.

In summarizing AHS publications, Weichenthal *et al.* (2010, ASB2012-12048) noted that increased rates in the following cancers were not associated with glyphosate use; overall cancer incidence, lung cancer, pancreatic cancer, colon or rectal cancer, lymphohematopoietic cancers, leukemia, NHL, multiple myeloma, bladder cancer, prostate cancer, melanoma, kidney cancer, childhood cancer, oral cavity cancers, stomach cancer, esophagus cancer and thyroid cancer.
Mink *et al.* (2012, ASB2014-9617) submitted a comprehensive review of epidemiologic studies of glyphosate and cancer. To examine potential cancer risks in humans they reviewed the epidemiologic literature to evaluate whether exposure to glyphosate is associated causally with cancer risk in humans. They also reviewed relevant methodological and biomonitoring studies of glyphosate. The review found non consistent pattern of positive assciations indicating a causal relationship between total cancer (in adults or in children) or any site- specific cancer and exposure to glyphosate.

Animal studies
Just recently (*i.e.*, after submission of the GTF dossier), a two-year study in rats was published (Séralini *et al.*, 2012, ASB2012-15514). Its main objective was to show a possible impact of long-term feeding of genetically modified (and glyphosate treated) maize to rats but three of the test groups were administered a commercially available formulation (Roundup GT Plus, apparently authorised at least in Belgium) containing 450 g glyphosate/L at different concentrations ranging from 0.1 ppb (50 ng glpyphosate/L) to 0.5% (2.25 g glyphosate/L) in drinking water. In these groups, the authors reported alterations in some clinical chemistry (blood and urine) parameters and hormone levels and histopathological lesions concerning the liver and the gastrointestinal tract but also a higher incidence of mammary tumours in females resulting in a shorter lifespan. This study was heavily discussed in the scientific community as well as in the general public where it gained remarkable attention due to massive promotion although it was clearly flawed by many serious deficiencies. A major point of concern was the small group size of only 10 males and 10 females per dose, *i.e.*, the test design was that one of a subchronic study. Such a small number of animals is not appropriate for a long-term study because age-related changes cannot be adequaetely

taken into account. Following the receipt of contributions from many MS authorities, a comprehensive critical assessment was published by EFSA (2012, ASB2012-15513, EFSA Journal, 2012, 10 (11), 2986). The conclusion was that "the currently available evidence does not impact on the ongoing re- evaluation of glyphosate…". This opinion on the Séralini study is agreed with and supported by the RMS.
In reaction to this publication a large number of letters was send to the editor: Barale-Thomas (2012, ASB2013-10998), Berry (2012, ASB2013-10988), Grunewald (2012, ASB2013-11001), Hammond *et al.* (2012, ASB2013-10995), Heinemann (2012, ASB2013-10987), Langridge (2012, ASB2013-10986), Ollivier (2012, ASB2013-11000), Panchin (2013, ASB2013-10937), Pilu (2012, ASB2013-10992), Schorsch (2013, ASB2013-10996), Tester (2012, ASB2013-10994), Tien & Huy (2012, ASB2013-10984), Trewavas (2012, ASB2013- 10989), Tribe (2012, ASB2013-10997), Wager (2012, ASB2013-10993), de Souza (2012, ASB2013-10999).

Chruszielska *et al.* (2000, ASB2013-9829) published a combined long term toxicity and carcinogenicity study in rats. The active substance glyphosate was used in the study and the study was performed on basis of OECD guideline 453. The number of animals per dose group and sex (85 animals) was even higher than required in guideline 453. Therefore, the study is considered to be relevant. No carcinogenic effects have been registered in the study.

George *et al.*, (2010, ASB2012-11829) used a 2-stage cancer model in mice to evaluate a glyphosate formulation for tumor promotion. A known tumor promoter, 12-o-tetradecanoyl- phorbol-13-acetate (TPA) was used as a positive control and for comparison with glyphosate effects after exposure to a tumor initiator, 7, 12-dimethylbenz[a]anthracene. Proteomics were later applied to extrapolate a basis for glyphosate formulation tumor promotion. The results are considered by the authors to indicate a tumor promoting potential of glyphosate. However, the formulation Roundup was used in the study and not the active substance glyphosate. Furthermore, the up- and down-regulation of protein expression is not sufficient to prove a carcinogenic effect.

Mechanistic studies
Andreotti *et al.* (2012, ASB2014-9198) investigated the interaction between pesticide use and genetic variants involved in lipid metabolism on prostate cancer risk. The authors examined the interactions between 39 pesticides and 220 single nucleotide polymorphisms (SNPs) in 59 genes. They found 17 interactions that displayed a significant monotonic increase in prostate cancer risk with pesticides exposure in one genotype and no significant association in the other genotype. The most noteworthy association was for ALOXE3 rs 3027208 and terbufos. A higher risk was also reported with this method for glyphosate and other pesticides. However, the authors emphasize that glyphosate was not associated with prostate cancer risk in the main effect studies (Agricultural Health Study AHS.

Barry *et al.* (2011, ASB2014-9247) evaluated interactions between 39 pesticides and 394 tag single-nucleotide polymorphisms (SNPs) for 31 BER genes among 776 prostate cancer cases and 1444 male controls in a nested case-control study of Agricultural Healt Study (AHS) pesticide applicators. The authors used likelihood ratio tests from logistic regression models to determine p-values for interactions between three-level pesticide variables and SNP (assuming a dominant model) and the false discovery rate multiple comparison adjustment approach. The authors observed notable interactions between several pesticides and BER gene variants with respect to prostate cancer. However, only fonofos x NEIL3 rs 1983132 showed an interaction fitting an expected biological pattern that remained significant after adjustment for multiple comparisons. No significant association was observed for glyphosate.

Author(s)	Year	Study title

Hardell, L. Eriksson, M.	1999	A Case-Control Study of Non-Hodgkin Lymphoma and Exposure to Pesticides. Cancer, Volume: 85, Number: 6, Pages: 1353-1360 ASB2012-11838

The following studies are described more detailed:

Abstract*

Background. The incidence of non-Hodgkin lymphoma (NHL) has increased in most Western countries during the last few decades. Immunodefective conditions are established risk factors. In 1981, the authors reported an increased risk for NHL following exposure to certain pesticides. The current study was designed to further elucidate the importance of phenoxyacetic acids and other pesticides in the etiology of NHL.

Methods. A population-based case–control study in northern and middle Sweden encompassing 442 cases and twice as many controls was performed. Exposure data were ascertained by comprehensive questionnaires, and the questionnaires were supplemented by telephone interviews. In total, 404 cases and 741 controls answered the questionnaire. Uni- variate and multi-variate analyses were performed with the SAS statistical data program.

Results. Increased risk for NHL was found for subjects exposed to herbicides (odds ratio [OR], 1.6; 95% confidence interval [CI], 1.0 –2.5) and fungicides (OR, 3.7; 95% CI, 1.1–13.0). Among herbicides, the phenoxyacetic acids dominated (OR, 1.5; 95% CI, 0.9 –2.4); and, when subclassified, one of these, 4-chloro-2-methyl phenoxyacetic acid (MCPA), turned out to be significantly associated with NHL (OR, 2.7; 95% CI, 1.0–6.9). For several categories of herbicides, it was noted that only exposure during the most recent decades before diagnosis of NHL was associated with an increased risk of NHL. Exposure to impregnating agents and insecticides was, at most, only weakly related to NHL.

Conclusions. Exposure to herbicides in total, including phenoxyacetic acids, during the decades before NHL diagnosis resulted in increased risk for NHL. Thus, the risk following exposure was related to the latency period. Fungicides also increased the risk for NHL when combined, but this group consisted of several different agents, and few subjects were exposed to each type of fungicide.

* Quoted from article

Klimisch evaluation

Reliability of study: Not reliable

Comment: Study prone to selection and recall bias. No evidence of relevant glyphosate exposures. Medical history was assessed, but not reported.

Relevance of study: Not relevant (Exposure to multiple chemicals and though glyphosate exposure data were convincing (7/1145 subjects) and statistically non-significant positive associations reported.)

Klimisch code: 3

Additional comments:

Hardell and Eriksson (1999, ASB2012-11838) conducted a case control study to look for associations between reported pesticide use and non-Hodgkin's lymphoma (NHL). The study included 404 NHL cases and 741 controls. The measure of association in this study was the odds ratio (OR), a statistic that estimates of the ratio of disease rates (in this case NHL rates) for exposed and unexposed populations.

The authors reported statistically significant associations for NHL with: reported use of any herbicide (OR = 1.6), reported use of any fungicide (OR = 3.7), and reported use of 4-chloro- 2-methylphenoxyacetic acid (OR = 2.7). The major limitations of this study were: the reliance on reported pesticide use (not documented exposure) information, the small number of subjects who reported use of specific pesticides, the possibility of recall bias, the reliance on secondary sources (next-of-kin interviews) for approximately 43% of the

pesticide use information, and the difficulty in controlling for potential confounding factors, given the small number of exposed subjects.
The authors also reported a moderately elevated OR of 2.3 for glyphosate. This OR was not statistically significant and was based on only four "exposed" cases and three "exposed" controls.
This study has several important limitations: no exposure assessment, dependence on next-of- kin's recollections of study subjects' pesticide use for approximately 43% of study subjects, potential recall bias, and the very small number of subjects who reported using specific herbicides. The latter leads to findings that are statistically imprecise. Due to the potential for bias and the statistical imprecision, the results of this study are not convincing.

Author(s)	Year	Study title
Hardell, L. Eriksson, M. Nordstrom, M.	2002	Exposure to pesticides as risk factor for non-Hodgkin's lymphoma and hairy cell leukemia: Pooled analysis of two Swedish case-control studies. Leukemia & Lymphoma Volume: 43 Number: 5 Pages: 1043-1049 ASB2012-11839

Abstract*
Increased risk for non-Hodgkin's lymphoma (NHL) following exposure to certain pesticides has previously been reported. To further elucidate the importance of phenoxyacetic acids and other pesticides in the etiology of NHL a pooled analysis was performed on two case-control studies, one on NHL and another on hairy cell leukemia (HCL), a rare subtype of NHL. The studies were population based with cases identified from cancer registry and controls from population registry. Data assessment was ascertained by questionnaires supplemented over the telephone by specially trained interviewers. The pooled analysis of NHL and HCL was based on 515 cases and 1141 controls. Increased risks in uni-variate analysis were found for subjects exposed to herbicides (OR 1.75, CI 95% 1.26-2.42), insecticides (OR 1.43, CI 95% 1.08-1.87), fungicides (OR 3.11, CI 95% 1.56-6.27) and impregnating agents (OR 1.48, CI 95% 1.11-1.96). Among herbicides, significant associations were found for glyphosate (OR 3.04, CI 95% 1.08-8.52) and 4-chloro-2-methyl phenoxyacetic acid (MCPA) (OR 2.62, CI 95% 1.40-4.88). For several categories of pesticides the highest risk was found for exposure during the latest decades before diagnosis. However, in multi-variate analyses the only significantly increased risk was for a heterogeneous category of other herbicides than above.

* Quoted from article

Klimisch evaluation
Reliability of study: Not reliable
Comment: This publication combines the results of two previous studies by the authors on HNL (Hardell and Eriksson, 1999, ASB2012-11838) and HCL (Nordström, *et al.*, 1998, TOX1999-687). No information about exposure duration, exposure concentration, as well as medical history, lifestyle factors (e.g. smoker, use ofprescribed drugs etc). Study documentation is insufficient for assessment.
Relevance of study: Not relevant (Due to reliability of data set drawn from Hardell and Eriksson, 1999, ASB2012-11838)
Klimisch code: 3

Additional comments:
This study pools data from the previously reviewed publication by Hardell and Eriksson (1999, ASB2012-11838) with data from Nordström *et al.* (1998, TOX1999-687). Therefore the discussion of limitations of Hardell and Eriksson (1999, ASB2012-11838) also applies to Hardell *et al.* (2002, ASB2012-11839) (see above).

Author(s)	Year	Study title
Fritschi, L. Benke, G. Hughes, A. M. Kricker, A. Turner, J. Vajdic, C. M. Grulich, A. Milliken, S. Kaldor, J. Armstrong, B.K.	2005	Occupational exposure to pesticides and risk of non-Hodgkin's lymphoma American Journal of Epidemiology Volume: 162, Pages: 849-857 ASB2012-11624

Abstract*
Pesticide exposure may be a risk factor for non-Hodgkin's lymphoma, but it is not certain which types of pesticides are involved. A population-based case-control study was undertaken in 2000-2001 using detailed methods of assessing occupational pesticide exposure. Cases with incident non-Hodgkin's lymphoma in two Australian states (n = 694) and controls (n = 694) were chosen from Australian electoral rolls. Logistic regression was used to estimate the risks of non-Hodgkin's lymphoma associated with exposure to subgroups of pesticides after adjustment for age, sex, ethnic origin, and residence. Approximately 10% of cases and controls had incurred pesticide exposure. Substantial exposure to any pesticide was associated with a trebling of the risk of non-Hodgkin's lymphoma (odds ratio = 3.09, 95 % confidence interval: 1.42, 6.70). Subjects with substantial exposure to organochlorines, organophosphates, and "other pesticides" (all other pesticides excluding herbicides) and herbicides other than phenoxy herbicides had similarly increased risks, although the increase was statistically significant only for "other pesticides." None of the exposure metrics (probability, level, frequency, duration, or years of exposure) were associated with non- Hodgkin's lymphoma. Analyses of the major World Health Organization subtypes of non- Hodgkin's lymphoma suggested a stronger effect for follicular lymphoma. These increases in risk of non-Hodgkin's lymphoma with substantial occupational pesticide exposure are consistent with previous work.

* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	No information about exposure duration, used glyphosate products, exposure duration and application rates. Documentation is insufficient for assessment.
Relevance of study:	Not relevant (Multiple pesticide exposures. No definitive association between NHL and glyphosate can be made.)
Klimisch code:	3

Additional comments:
No information about exposure duration, used glyphosate products, exposure duration and application rates. Only multiple pesticide exposures are reported. No association between NHL and glyphosate can be made on basis of this study.

Author(s)	Year	Study title
De Roos, A. J. Zahm, S. H. Cantor, K. P. Weisenburger, D. D. Holmes, F. F. Burmeister, L. F. Blair, A.	2003	Integrative assessment of multiple pesticides as risk factors for non-Hodgkin's lymphoma among men. Occupational and Environmental Medicine Volume: 60, Number: 9, Pages: -E11 ASB2012-11606

Abstract*
Background: An increased rate of non-Hodgkin's lymphoma (NHL) has been repeatedly observed among farmers, but identification of specific exposures that explain this observation has proven difficult.
Methods: During the 1980s, the National Cancer Institute conducted three case-control studies of NHL in the midwestern United States. These pooled data were used to examine pesticide exposures in farming as risk factors for NHL in men. The large sample size (n = 3417) allowed analysis of 47 pesticides simultaneously, controlling for potential confounding by other pesticides in the model, and adjusting the estimates based on a prespecified variance to make them more stable.
Results: Reported use of several individual pesticides was associated with increased NHL incidence,

including organophosphate insecticides coumaphos, diazinon, and fonofos, insecticides chlordane, dieldrin, and copper acetoarsenite, and herbicides atrazine, glyphosate, and sodium chlorate. A subanalysis of these "potentially carcinogenic" pesticides suggested a positive trend of risk with exposure to increasing numbers.
Conclusion: Consideration of multiple exposures is important in accurately estimating specific effects and in evaluating realistic exposure scenarios.

[k] Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	No useful information about exposure duration, exposure concentration, as well as medical history, lifestyle factors (e.g. smoker, use of prescribed drugs etc were reported. Specific lymphomas are not identified (NHL captures all types of lymphoma other than Hodgkin's lymphoma). Documentation is insufficient to associate exposures with specific NHL diseases.
Relevance of study:	Not relevant (No report of identifying various types of lymphoma under the NHL umbrella; no definite association between specific NHL diseases and glyphosate can be made)
Klimisch code:	3

Additional comments:
No useful information about exposure duration, exposure concentration, as well as medical history, lifestyle factors (e.g. smoker, use of prescribed drugs etc) were reported. Specific lymphomas are not identified. The reported hierarchical regression did not find a statistically significant odds ratio for ever use of glyphosate and NHL.

Author(s)	Year	Study title
De Roos, A.J. Blair, A. Rusiecki, J.A. Hoppin, J.A. Svec, M. Dosemeci, M. Sandler, D.P. Alavanja, M.C.	2005	Cancer Incidence among Glyphosate-Exposed Pesticide Applicators in the Agricultural Health Study Environmental Health Perspectives Volume: 113, Number: 1, Pages: 49-54 ASB2012-11605

Abstract*
Glyphosate is a broad-spectrum herbicide that is one of the most frequently applied pesticides in the world. Although there has been little consistent evidence of genotoxicity or carcinogenicity from *in vitro* and animal studies, a few epidemiologic reports have indicated potential health effects of glyphosate. We evaluated associations between glyphosate exposure and cancer incidence in the Agricultural Health Study (AHS), a prospective cohort study of 57,311 licensed pesticide applicators in Iowa and North Carolina. Detailed information on pesticide use and other factors was obtained from a self-administered questionnaire completed at time of enrolment (1993–1997). Among private and commercial applicators, 75.5% reported having ever used glyphosate, of which > 97% were men. In this analysis, glyphosate exposure was defined as *a*) ever personally mixed or applied products containing glyphosate; *b*) cumulative lifetime days of use, or "cumulative exposure days" (years of use × days/year); and *c*) intensity-weighted cumulative exposure days (years of use × days/year × estimated intensity level). Poisson regression was used to estimate exposure–response relations between glyphosate and incidence of all cancers combined and 12 relatively

common cancer subtypes. Glyphosate exposure was not associated with cancer incidence overall or with most of the cancer subtypes we studied. There was a suggested association with multiple myeloma incidence that should be followed up as more cases occur in the AHS. Given the widespread use of glyphosate, future analyses of the AHS will allow further examination of long-term health effects, including less common cancers.

[k] Quoted from article

Klimisch evaluation

Reliability of study:	Reliable without restrictions
Comment:	Well documented publication. Study included glyphosate exposure, as well as demographic and lifestyle factors. However, adjusted relative risk calculations eliminated a significant proportion of the data set without justification.
Relevance of study:	Relevant (Evaluation focussed on glyphosate, although other pesticides were also considered in the data evaluation)
Klimisch code:	2

Additional comments:
Study included glyphosate exposure, as well as demographic and lifestyle factors. However, adjusted relative risk calculations eliminated a significant proportion of the data set without justification.

Response 1 – summary from Letter to the Editor by Farmer et al. (2005, ASB2012- 11616)
Authors provided an incomplete genotoxicity review which was inconsistent with opinions of regulatory agencies and experts around the world, that glyphosate is not genotoxic. An extensive toxicology review of glyphosate was cited by the authors, mentioning a lack of carcinogenicity with glyphosate exposures, yet neglected to cite the extensive genotoxicity review in the same publication by Williams *et al.* (2000, ASB2012-12053)
Biological plausibility of a cancer effect should be considered in the light of exposure. Acquavella et al (2004, ASB2012-11528) reported the maximum systemic dose to resulting from application of glyphosate to areas as large as 400 acres was 0.004 mg/kg, and the geometric mean systemic dose was 0.0001 mg/kg in farmers. If these glyphosate applications and exposures continued daily over the course of a lifetime, the systemic dose would be at least 250,000-fold lower than the cancer no-effect level in rodents.
The authors were requested to further evaluate their models for confounding and selection bias in the multiple myeloma analysis.

Response 2 – summary from Lash (2007, ASB2012-11877)
Table 2 of De Roos *et al.* (2005, ASB2012-11605) noted 32 cases of multiple myeloma associated with "ever-use" of glyphosate and when compared with "never-use" (adjusted for age only) yielded a rate ratio of 1.1 (95% CI 0.5-2.4). However, when the data set was adjusted for age, demographic and lifestyle factors and other pesticide use, the rate ratio increased to 2.6 (95% CI 0.7-9.4).
The adjusted estimate merits careful inspection and can only be undertaken with access to the primary data, not made available by the authors.
Bias analysis was conducted, accounting for confounding and exposure misclassification.
Adjustment for confounders in De Roos *et al.* (2005, ASB2012-11605), which resulted in limiting the data set by 25 % because of missing data on the adjustment variables, likely introduced selection bias and produced the a rate ratio of 2.6 that was substantially biased.

Author(s)	Year	Study title

Eriksson, M. Hardell, L. Carlberg, M. Akerman, M.	2008	Pesticide exposure as risk factor for non-Hodgkin lymphoma including histopathological subgroup analysis International Journal of Cancer Volume: 123, Pages: 1657-1663 ASB2012-11614

Abstract*
We report a population based case-control study of exposure to pesticides as risk factor for non-Hodgkin lymphoma (NHL). Male and female subjects aged 18-74 years living in Sweden were included during December 1, 1999, to April 30, 2002. Controls were selected from the national population registry. Exposure to different agents was assessed by questionnaire. In total 910 (91%) cases and 1016 (92%) controls participated. Exposure to herbicides gave odds ratio (OR) 1.72, 95% confidence interval (CI) 1.18-2.51. Regarding phenoxyacetic acids highest risk was calculated for MCPA; OR 2.81, 95% CI 1.27-6.22, all these cases had a latency period >10 years. Exposure to glyphosate gave OR 2.02, 95% CI 1.10-3.71 and with
>10 years latency period OR 2.26, 95% CI 1.16-4.40. Insecticides overall gave OR 1.28, 95% CI 0.96-1.72 and impregnating agents OR 1.57, 95% CI 1.07-2.30. Results are also presented for different entities of NHL. In conclusion our study confirmed an association between exposure to phenoxyacetic acids and NHL and the association with glyphosate was considerably strengthened.

* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Multiple avenues for bias were introduced in study design, execution and data processing. No information about exposure duration, used glyphosate products and application rates. Other factors (i.e. smoking habits, medication etc.) were assessed but not included in the evaluation.
Relevance of study:	Relevant with reservation
Klimisch code:	3

Additional comments:
The authors (Eriksson *et al*. 2008, ASB2012-11614) conducted a population-based case- control study of exposure to a variety of pesticides and non-Hodgkin lymphoma (NHL), including separate analyses of histopathological categories of NHL. Study subjects were males and females, ages 18-74, living in Sweden between December 1, 1999 and April 30, 2002. The final study group included 910 cases and 1016 controls. Exposure, ascertained via an interviewer-administered questionnaire, focused on pesticide and other chemical agents, and included a total work history (although a job-exposure matrix was not used). For pesticide exposure, information on number of years, number of days per year, and approximate length of exposure per day was also obtained. A minimum of one full day of exposure was required for categorization as "exposed."

The authors reported a statistically significant positive association between "herbicide exposure" and NHL (OR = 1.72; 95% CI: 1.18-2.51). Glyphosate exposure was reported by 29 cases and 18 controls, and the corresponding odds ratio (OR) was 2.02 (95% CI: 1.10-3.71). The ORs for glyphosate exposure of <10 days and >10 days were 1.69 (95% CI: 0.70-4.07) and 2.36 (1.04-5.37), respectively. The ORs for glyphosate were 1.11 (95% CI: 0.24-5.08) and 2.26 (95% CI: 1.16-4.40) for "latency" periods of 1-10 years and >10 years, respectively. In analyses of glyphosate and type of NHL, statistically significant positive associations were observed for small lymphocytic lymphoma/chronic lymphocytic leukemia (SLL/CLL) (OR = 3.35; 95% CI: 1.42-7.89) and for "unspecified NHL" (OR = 5.63; 95% CI: 1.44-22.0). Odds ratios for the other types (total B-cell lymphomas, grade I-III follicular lymphoma, diffuse large B-cell

lymphoma, other specified B-cell lymphoma, unspecified B- cell lymphoma, and T-cell lymphomas) were above 1.0, but were not statistically significant (i.e., the 95% confidence intervals were relatively wide and included the null value of 1.0).

The authors concluded, "Glyphosate was associated with a statistically significant increased OR for lymphoma in our study, and the result was strengthened by a tendency to dose- response effect…" (p. 1662). The authors suggested that their findings are consistent with results of a previous case-control study (Hardell and Eriksson 1999, ASB2012-11838) and pooled analysis (Hardell *et al.* 2002, ASB2012-11839) that they conducted. In the case-control study, an OR of 2.3 (95% CI: 0.4-13.0), based on 4 exposed cases and 3 exposed controls, was reported for glyphosate and NHL. In the pooled analysis of two case-control studies, which included data from Hardell and Eriksson (1999, ASB2012-11838), an OR of 3.04 (95% CI: 1.08- 8.52) was reported, based on 8 exposed cases and 8 exposed controls. The authors also cited three studies (De Roos *et al.* 2003, ASB2012-11606; McDuffie *et al.* 2001; ASB2011-364, De Roos *et al.* 2005, ASB2012-11605) by other groups as being consistent with their results in that they "also associate glyphosate with different B-cell malignancies such as lymphomas and myelomas." It should be noted, however, that the relative risk (RR) reported by De Roos *et al.* (2005, ASB2012-11605) for the highest versus lowest category of cumulative exposure days of glyphosate and NHL in the prospective Agricultural Health Study was 0.9.

Interpretation Issues

Identification of Cases and Potential Referral Bias. It is noteworthy that the cases in the current analysis were identified from some of the same hospitals as the authors' prior publication; thus, referral bias may have been an issue. In particular, the researchers approached the patients after diagnosis if the physicians deemed it appropriate. Therefore, if the physicians were concerned that their patient's NHL was associated with agricultural exposures, they may have suggested participation in the study.

Participation Rates and Potential Selection Bias. The authors report a participation rate of 91% and 92% for cases and controls, respectively; however, these figures are based on completed questionnaires out of those who had previously said they would participate in the study. The number of eligible patients (i.e., prior to physician approval to "approach") was not reported, so the computation of an exact participation rate is difficult. Based on information provided in the paper, participation among cases is estimated to be about 80%. Nonparticipation is a concern for several reasons. First, in a case-control study, an odds ratio will be an accurate representation of the exposure-disease association when the cases are representative of all cases and the controls are representative of the exposure experience of the population that gave rise to the cases. If the final study sample is not representative of this "target population" then measures of effect (e.g., the odds ratio) may not be valid. In addition, one must be concerned about selection bias. Selection bias occurs in a case-control study when the exposure distribution for cases and controls differ for those who participate in the study compared to those who are eligible but do not participate in the study. It is not possible to determine whether there is selection bias without information about nonparticipants.

Strengths and Limitations of Using Living Cases Only versus All Cases (Living + Dead).

The authors noted that 88 potential cases died before they could be interviewed and were therefore excluded from the study. It is also stated in the Discussion that restricting the study to living cases and controls was an "advantage" of the study, as interviewing cases and controls directly compared to interviewing next-of-kin was preferable. While it is generally true that this would be an advantage, the following statement by the authors, therefore, is not accurate, "The study covered all new cases of NHL during a specified time" (p. 1660). The study did not include all new cases; it included only those cases who survived until the time of the interview. Thus, while there may have been an advantage to restricting the study to living cases, there was a trade-off in that the study population did not represent all cases, specifically those cases with more aggressive disease. This disadvantage was not discussed by the authors, nor was the potential bias that could have resulted from excluding many eligible cases.

Exposure Measurement and Information Bias. Exposure was ascertained via a questionnaire oriented towards pesticide and other chemical agents. In addition, interviewers collected information by telephone

if "important" data were lacking, incomplete, or unclear. It is unknown what is meant by "important," and the proportion of cases and controls who received phone calls was not reported. Thus, information bias may be a concern. Even though interviewers were blinded to case and/or control status, they may have been able to determine this information during the course of the interview. Furthermore, recall bias may be an issue because exposure information was based on participant response and cases and controls may recall and/or report past pesticide exposures differently. No exposure validation techniques were implemented, nor did an industrial hygienist (or any other type of personnel trained in assessing occupational exposures) independently validate/estimate the frequency and/or intensity of exposure. The authors assumed that "some misclassification regarding quantity of exposure has probably occurred, but such misclassification would most probably be nondependent of case/control status, and therefore only weaken any true risks" (p. 1660). They do not provide any explanation as to why they believe that exposure misclassification would be "most probably" nondifferential. If NHL cases believe that pesticides may be related to their disease, then it is certainly possible that they may recall and/or report pesticide exposure differently than NHL-free controls, which could result in odds ratios that are inflated as a result of bias.

Interpretation of "dose-response" analyses. The referent group in the statistical analyses consisted of participants who were unexposed to all pesticides. The dose-response analyses were based on a dichotomy of the median number of days exposed to a particular agent. It is difficult to analyze "dose-response" when only two exposure categories are considered. Furthermore, the dose-response analyses were based on median values of exposure but heterogeneity of cut-points is evident across agents. For example, glyphosate was analyzed as < 10 days and > 10 days, whereas, "other" herbicides were analyzed as < 32 days and > 32 days. Although analytical cut-points were data driven, interpretation across the wide varietyof exposures is complicated by the variability in exposure cut-points. In addition, even though though the OR for the higher category of exposure days was greater than the OR for the lower category, the two 95% confidence intervals were wide and overlapped considerably (0.70-4.07 and 1.04-5.37).
Thus, it is not clear whether the two point estimates reported (1.69 and 2.36) are significantly different from each other. Finally, this result cited in the "dose-response" analyses may have been confounded by exposure to other herbicides. In Table II (Eriksson *et al.* 2008, ASB2012- 11614), the authors observed elevated associations for other herbicides, including MCPA, 2,4,5-T and/or 2,4-D. The correlation between exposure to glyphosate and other herbicides was not provided nor were analyses of glyphosate-exposed individuals after accounting for the collinear relation between this agent and other agents. The odds ratio for "ever" exposure to glyphosate was attenuated after additional adjustment for other pesticides (Table VII, Eriksson *et al.* 2008, ASB2012-11614), but multi-variate -adjusted estimates for the "dose-response" odds ratios were not reported.
Unusual Pattern of Positive Associations. The authors conducted multiple comparisons, and one would expect a certain proportion of their findings to be statistically significant (whether in the positive or inverse direction) simply as a result of chance. It is somewhat surprising, therefore, that the vast majority of the ORs presented in this manuscript are greater than 1.0, regardless of the statistical significance. The authors do note that for some of the analyses (e.g., latency), only chemicals for which ORs were greater than 1.5 and for which there were at least 10 exposed cases, or for which there was a statistically significant OR were evaluated. On the other hand, dose-response was evaluated based on the number of exposed subjects and not on the strength or significance of the findings. The authors do not address this directly, but do state in their Discussion, "…several pesticides are chemically related and may exert their effects on humans through a similar mechanism of action, which may explain the wide range of pesticides that have been related to NHL over time in different countries and with different exposure conditions" (p. 1661). On the other hand, this pattern of positive findings could be a result of bias, including recall bias (or other information bias), selection bias, uncontrolled confounding, or a combination of these and other factors.

Interpretation of Eriksson *et al.* (2008, ASB2012-11614) in Context of Other Studies. Despite the statement by the authors that, "Recent findings from other groups also associate glyphosate with different B-cell malignancies such as lymphomas and myeloma" (p. 1662), most multi-variate analyses of glyphosate and NHL do not report statistically significant associations (De Roos *et al.* 2005, ASB2012-11605; De Roos *et al.* 2003; ASB2012-11606, Hardell and Eriksson 1999, ASB2012-11838; Hardell *et al.* 2002; ASB2012-11839, Lee *et al.* 2004; ASB2012-11883, McDuffie *et al.* 2001; ASB2011-364, Nordström *et al.* 1998,

TOX1999-687) (Tables B.6.5-62 and B.6.5-63). It is notable that Hardell *et al.* (2002, ASB2012-11839) reported a significant positive association between glyphosate association and NHL, but the multi-variate -adjusted odds ratio was attenuated and not statistically significant. Similar findings were reported by Eriksson *et al.* (2008, ASB2012-11614). Specifically, the association reported by the authors in the abstract (OR = 2.02; 95% CI: 1.10- 3.71) was adjusted for age, sex and year of diagnosis or enrollment. When other pesticides were added to that model (i.e., agents with statistically significant increased odds ratios, or with an odds ratio greater than 1.5 and with at least 10 exposed subjects), the adjusted odds ratio was 1.51 (95% CI: 0.77-2.94). Thus, the authors' final statement, "Furthermore, our earlier indication of an association between glyphosate and NHL has been considerably strengthened" is questionable. Their previous findings showed a non-significant association after multi-variate adjustment (OR = 1.85; 95% CI: 0.55-6.20). The 2008 study similarly reported a statistically non-significant association between glyphosate and NHL after multi- variate adjustment (OR = 1.51; 95% CI: 0.77-2.94). The results reported for analyses of duration of exposure and latency of exposure did not adjust for other pesticides, and one would expect that those ORs would also be attenuated.

Summary of Findings: Cohort and Case-Control Studies of Exposure to Glyphosate and Non-Hodgkin Lymphoma

Table B.6.5-62: Cohort Studies

Author Year	Description	No. of Exposed Cases	Type of Relative Risk Estimate	Relative Risk Estimate	95% Confidence Limits	Variables Included in Statistical Model
De Roos *et al.* 2005 (ASB2012 -11605)	57-2,678 vs. 1-20 Cumulative Exposure Days[a]	17	RR	0.9	0.5-1.6	Age at enrollment, education, pack-years of cigarette smoking, alcohol consumption in the past year, family history of cancer in first-degree relatives, and state of residence
	337.2-18,241 vs. 0.1-79.5 Intensity-Weighted Exposure Days[b]	22	RR	0.8	0.5-1.4	Also adjusted for other pesticides

[a] Years of use x days per year; categorized by tertiles
[b] Years of use x days/year x estimated intensity level; categorized by tertiles

Table B.6.5-63: Case Control Studies

Author Year	Exposure Evaluated	Subgroup Description	No. of Exposed Cases	No. of Exposed Controls	OR	95% CI	Variables Included in Statistical Model
De Roos *et al.* 2003 (ASB201 2-11606)	Ever exposure to specific pesticide; men only (all 47 pesticides were regressed simultaneously)	Glyphosate (Logistic Regression)	36	61	2.1	1.1-4.0	Age, study site and other pesticides
		Glyphosate (Hierarchical Regression)	36	61	1.6	0.9-2.8	Second-level model incorporated what was known about each true effect parameter prior to seeing the study data
Hardell and Eriksson 1999 (ASB201 2-11838)	Exposure to specific pesticides (ever/never exposed to the specific pesticide vs. no exposure to any pesticide)	Glyphosate (conditional logistic regression; uni-variate analysis)	4	3	2.3	0.4-13	Age and country (matching factors)
		Glyphosate (conditional logistic regression; multi-variate analysis)	4	3	5.8	0.6-54	Multi-variate variables not listed by authors
Hardell *et al.* 2002 (ASB201 2-11839)	Exposure to specific pesticides (ever/never exposed to the specific pesticide vs. no exposure to any pesticide)	Glyphosate (conditional logistic regression; uni-variate analysis)	8	8	3.04	1.08-8.52	Age and county (matching factors); study, study area (county), and vital status
		Glyphosate (conditional logistic regression; multi-variate analysis)	8	8	1.85	0.55-6.20	Multi-variate variables not listed by authors
Lee *et al.* 2004 (ASB201 2-11883)	Exposure to individual pesticides	Glyphosate use, Non-asthmatics	53	91	1.4	0.98-2.1	Age, state, vital status
		Glyphosate use, Asthmatics	6	12	1.2	0.4-3.3	

Author Year	Exposure Evaluated	Subgroup Description	No. of Exposed Cases	No. of Exposed Controls	OR	95% CI	Variables Included in Statistical Model
McDuffie *et al.* 2001 (ASB201 1-364)	Exposure to individual active chemicals	Glyphosate (Round-Up)	51	133	1.26	0.87-1.80	Strata for age and province of residence
		Glyphosate (Round-Up)	NR	NR	1.20	0.83-1.74	Plus statistically significant medical variables
Nordst-röm *et al.* 1998 (TOX199 9-687)	Exposure to specific herbicides, insecticides, and fungicides	Glyphosate	4	5	3.1	0.8-12	Age and country (matching factors)
Eriksson *et al.* 2008 (ASB201 2-11614)	Exposure to specific herbicides regardless if they also had been exposed to phenoxyacetic acids or not	Glyphosate	29	18	2.02	1.10-3.71	Age, sex, and year of diagnosis or enrollment
			29	18	1.51	0.77-2.94	Age, sex, and year of diagnosis or enrollment and pesticides with statistically significant increased odds ratios, or with an odds ratio greater than 1.5 and with at least 10 exposed subject
	Exposure to herbicide stratified by median number of days among exposed controls	Glyphosate ≤ 10 days	12	9	1.69	0.70-4.07	Age, sex, and year of diagnosis or enrollment
		Glyphosate >10 days	19	9	2.36	1.04-5.37	
	Exposure to specific herbicides according to different lymphoma entities	Glyphosate: B-Cell lymphomas	NR	NR	1.87	0.998-3.51	Age, sex, and year of diagnosis or enrollment
		Lymphocytic	NR	NR	3.35	1.42-7.89	

Author Year	Exposure Evaluated	Subgroup Description	No. of Exposed Cases	No. of Exposed Controls	OR	95% CI	Variables Included in Statistical Model
		lymphoma/B-CLL					
		Follicular grade I-III	NR	NR	1.89	0.62-5.79	
		Diffuse large B-cell Lymphoma	NR	NR	1.22	0.44-3.35	
		Other specified B-cell lymphoma	NR	NR	1.63	0.53-4.96	
		Unspecified B-cell Lymphoma	NR	NR	1.47	0.33-6.61	
		T-cell lymphomas	NR	NR	2.29	0.51-10.4	
		Unspecified NHL	NR	NR	5.63	1.44-22.0	

Author(s)	Year	Study title
George, J. Prasad, S. Mahmood, Z. Shukla, Y.	2010	Studies on glyphosate-induced carcinogenicity in mouse skin: A proteomic approach Journal of Proteomics Volume: 73, Pages: 951-964 ASB2012-11829

Abstract*

Glyphosate is a widely used broad spectrum herbicide, reported to induce various toxic effects in non-target species, but its carcinogenic potential is still unknown. Here we showed the carcinogenic effects of glyphosate using 2-stage mouse skin carcinogenesis model and proteomic analysis. Carcinogenicity study revealed that glyphosate has tumor promoting activity. Proteomic analysis using 2-dimensional gel electrophoresis and mass spectrometry showed that 22 spots were differentially expressed (>2 fold) on glyphosate, 7, 12- dimethylbenz[a]anthracene (DMBA) and 12-O-tetradecanoyl-phorbol-13-acetate (TPA) application over untreated control. Among them, 9 proteins (translation elongation factor eEF- 1 alpha chain, carbonic anhydrase III, annexin II, calcyclin, fab fragment anti-VEGF antibody, peroxiredoxin-2, superoxide dismutase [Cu–Zn], stefin A3, and calgranulin-B) were common and showed similar expression pattern in glyphosate and TPA-treated mouse skin. These proteins are known to be involved in several key processes like apoptosis and growth- inhibition, anti-oxidant responses, etc. The up-regulation of calcyclin, calgranulin-B and down-regulation of superoxide dismutase [Cu–Zn] was further confirmed by immunoblotting, indicating that these proteins can be good candidate biomarkers for skin carcinogenesis induced by glyphosate. Altogether, these results suggested that glyphosate has tumor promoting potential

in skin carcinogenesis and its mechanism seems to be similar to TPA.

[k] Quoted from article

Klimisch evaluation

Reliability of study:	Reliable with restrictions
Comment:	Non-guideline mechanistic study. Scientifically acceptable study with deficiencies (controls with glyphosate alone, and co-formulants were not included)
Relevance of study:	Relevant with restrictions (Glyphosate formulation not glyphosate alone was tested.)
Klimisch code:	2

Additional comments:

The authors use glyphosate as a synonym for what is really a glyphosate based formulated product. Doses in this study are not representative of human exposures to glyphosate or glyphosate based formulations. Mice in the tumor promoting group VIII received topical applications of concentrated glyphosate formulated product three times per week for over thirty weeks without washing after an initial treatment with the potent tumor initiator DMBA. Glyphosate had been shown to have very low dermal absorption, even in formulated products, and since is non-volatile, would likely accumulate on mouse skin. Surfactants are typically irritating and non-volatile. Given the irritation potential of the unwashed exposed mouse skin over the course of thirty or more weeks, tumor promotion may be a physical response to substantial localized dermal irritation. Epidemiological studies reported above note no association with glyphosate and either skin or lip cancers.

Label directions outline appropriate personal protective equipment such as gloves and long sleeves. Furthermore, any dermal exposure of concentrated product to human skin would prove irritating and prompt handlers to wash off soon after dermal exposure.

Human *in vitro* dermal absorption studies reported for a range of glyphosate based formulations containing different surfactant systems all demonstrate extremely low dermal absorption of glyphosate active ingredient for concentrated products, of less than 0.2%. Test material recovery in each of the four reported dermal absorption studies was very good, close to 100%. Most of the glyphosate was removed during skin surface washing at either eight or twenty four hours of in vitro human skin exposure. This also suggests significant potential for accumulation of glyphosate on the surface of the mice skin in George *et al.* (2010, ASB2012- 11829).

The up-regulation / down-regulation of protein expression reported after a single dermal dose of a glyphosate formulated product (proteomics experiment, group II), while interesting, does not demonstrate any toxicological endpoint. Rather, perturbations may well represent normal homeostatic fluctuations and be a natural response to insult.

Author(s)	Year	Study title
Seralini, G.-E. Clair, E. Mesnage, R. Gress, S. Defarge, N. Malatesta, M. Hennequin, D. Spiroux de Vendomois, J.	2012	Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. Food and Chemical Toxicology 50, 4221-4231 ASB2012-15514

Abstract*

The health effects of a Roundup-tolerant genetically modified maize (from 11% in the diet), cultivated with or without Roundup, and Roundup alone (from 0.1 ppb in water), were studied 2 years in rats. In females, all treated groups died 2–3 times more than controls, and more rapidly. This difference was visible in 3 male groups fed GMOs. All results were hormone and sex dependent, and the pathological profiles were comparable. Females developed large mammary tumors almost always more often than and before controls, the pituitary was the second most disabled organ; the sex hormonal balance was modified by GMO and Roundup treatments. In treated males, liver congestions and necrosis were 2.5–5.5 times higher. This pathology was confirmed by optic and transmission electron microscopy. Marked and severe kidney nephropathies were also generally 1.3–2.3 greater. Males presented 4 times more large palpable tumors than controls which occurred up to 600 days earlier. Biochemistry data confirmed very significant kidney chronic deficiencies; for all treatments and both sexes, 76% of the altered parameters were kidney related. These results can be explained by the non linear endocrine-disrupting effects of Roundup, but also by the overexpression of the transgene in the GMO and its metabolic consequences.

[k] Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	The study was performed to investigate the long term toxicity and carcinogenicity. However the study design does not agree with the OECD guidelines on long term toxicity and carcinogenicity.
Relevance of study:	Relevant with restrictions (Glyphosate formulation not glyphosate alone was tested.)
Klimisch code:	3

Comments:

Seralini *et al.* (2012, ASB2012-15514) submitted a report of long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. The health effects have been studied 2 years in rats. Six groups of rats were fed with 11, 22 and 22% of genetically modified NK603 maize either treated or not with Roundup. Three further groups of rats were fed with control diet and had access to water supplemented with 50 ng/L, 400 mg/L and 2.25 g/L of the commercial product Roundup (GT Plus, 450 g/L of glyphosate). The pure active substance glyphosate was not tested in this study.

The study is not considered reliable because of several important limitations. According to the authors the studies have been performed to investigate the long term toxicity and carcinogenicity. However, the number of animals per dose and sex was only 10 and also the further study design does not agree with the OECD guidelines on long term toxicity and carcinogenicity. The spontaneous incidence of mammary tumors in the used Sprague Dawley rats is much higher than in most other rat strains. Therefore, a higher number of animals would be necessary for the differentiation between treatment related carcinogenicity and accidental aberrations. Also for the assessment of mortality and further described toxic effects a higher number of animals would be needed.

The presented results in the publication are incomplete and therefore, an evaluation of the presented results was complicated.

The study was extensively discussed and criticized in the public. In an additional paper Seralini *et al.* (2013, ASB2013-10985) gave some answers to the critics. The authors admit that the study "should not be considered as a final point in knowing the toxicological effects of NK603 and Roundup)" and that the study has limits.

Jany (2012, ASB2014-9580) submitted a critical review of the study by Seralini *et al.* (2012). The authors conclude that the scientific value of this publication would be limited and non conclusions are possible concerning maize NK603 with and without Roundup treatment.

Ollivier (2012, ASB2013-11000) proposes to use the Chi-square test to compare mortality rates in the study of Seralini *et al.* (2012). In result of this test there would be no statistical significance.

In a further paper Seralini *et al.* (2014, ASB2014-9632) discuss criticisms which have been published in

reaction on the study by Seralini *et al.* (2012, ASB2012-15514).
John (2014, ASB2014-9584) reacts in a letter on the decision of the publisher to retract the article of Seralini *et al.* (2012). John concludes that there would be no grounds for retraction
Wallace-Hayes (2014, ASB2014-9559), the editor-in-chief of Food and Chemical Toxicology, gives answers on questions on the retraction of the paper of Seralini *et al.* (2012). He concludes once more that “a careful and time-consuming analysis found that the data were inconclusive, and therefore the conclusion described in the article were unreliable. Accordingly, the article was retracted.”
Folta (2014, ASB2014-9478) writes in a letter to the editor that he would see this work of Seralini (2012) as a manipulation of the scientific process to achieve activist gains. He stands behind the journal`s decision to retract the work.
Rosanoff (2014, ASB2014-9397) proposes in a letter concerning the Seralini (2012) study that the raw data should be published.
Roberfroid (2014, ASB2014-9393) writes in a letter concerning the Seralini (2012) study that he is ashamed about the decision to retract this paper.
In a further letter Roberfroid (2014, ASB2014-9392) writes that in his understanding the study of Seralini (2012) remains an important scientific (not a regularory) observation that can not be ignored.
Pilu (2012, ASB2014-9387) writes in a letter to the editor on the Seralini (2012) study that mycotoxins in maize could have influenced the results of the study. Therefore, he asks for further information on the mycotoxin content in the maize used in the Seralini study.

Author(s)	**Year**	**Study title**
Chruscielska, K. Brzezinski, J. Kita, K. Kalhorn, D. Kita, I. Graffstein, B. Korzeniowski, P.	2000	Glyphosate Evaluation of chronic activity and possible far-reaching effects. Part 1. Studies on chronic toxicity Pestycydy 2000, (3-4), 11-20 ASB2013-9829

Abstract*:
The combined test of chronic toxicity and carcinogenicity of glyphosate was performed on Wistar-RIZ rats. The herbicide was administered in water at concentrations: 0, 300, 900, 2700 m/L. The examination of the peripheral blood parameters and the smears of bone marrow did not reveal harmful effect of the herbicide on haematopoietic system of rats. The biochemical parameters determined on blood and urine only in some cases showed significant deviations in comparison with the control group, but in any examined indices dose-effect-time occurred what could manifest the toxic influence of glyphosate. In pathomorphological studies on the organs no correlation was stated between the number of observed tumours and the concentrations of the herbicide. It indicates lack of pathogenic influence of glyphosate on neoplastic pathogenesis.

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* Quoted from article

Klimisch evaluation

Reliability of study:	Reliable with restrictions
Comment:	The published details of the study are limited. However, according to the authors the study was performed on basis of OECD guideline No. 453
Relevance of study:	Relevant

Klimisch code: 2

Comments:
The active substance glyphosate was used in the study and the study was performed on basis of OECD guideline 453. The number of animals per dose group and sex (85 animals) was even higher than required in guideline 453. Therefore, the study is considered to be relevant. No carcinogenic effects have been registered in the study.

B.6.5 Reproductive toxicity (Annex IIA 5.6)

B.6.5.1 Published data (released since 2000)

A large number of studies on developmental and reproductive toxicity (DART) was published since 2000. These studies are reported and discussed below. Furthermore, also studies on endocrine disruption (ED) have been included in this chapter because they are mainly related to developmental and reproductive toxicity.

Published studies on developmental toxicity, reproductive toxicity and an endocrine disrupting potential of glyphosate and glyphosate based formulations include *in vitro* studies, *in vivo* studies and epidemiological studies. Many studies since 2000 are specifically discussed in a comprehensive glyphosate DART review publication by Williams *et al.* (2012, ASB2012-12052). Further discussions of significant papers follow.

In addition, glyphosate was included on the US EPA Endocrine Disruptor Screening Program's (EDSP) first list of 67 compounds to Tier 1 Screening. The US EPA published the criteria for inclusion on List 1 was strictly based on exposure potential, not hazard, specifically stating in the Federal Register (2009, ASB2012-12041); "This list should not be construed as a list of known or likely endocrine disruptors".

A consortium of glyphosate registrants in North America, the Joint Glyphosate Task Force, LLC (JGTF), coordinated the conduct of the glyphosate battery of Tier 1 screening assays under the EDSP and submitted these assays to the US EPA. The US EPA will evaluate the full battery of Tier 1 screening assays together using a weight of evidence approach, for glyphosate's potential to interact with the estrogen, androgen and thyroid endocrine pathways. The following below were submitted by the JGTF to the US EPA in early 2012 and are reviewed. However, the Agency has announced they will not release their Data Evaluation Records (DERs) for individual EDSP studies until a weight of evidence review has been completed for List 1 compounds.

In Vitro EDSP Glyphosate Studies submitted to the US EPA

- Androgen Receptor Binding (Rat Prostate Cytosol); OCSPP 890.1150
- Aromatase (Human Recombinant); OCSPP 890.1200
- Estrogen Receptor Binding Assay Using Rat Uterine Cytosol (ER-RUC); OCSPP 890.1250
- Estrogen Receptor Transcriptional Activation (Human cell Line, HeLa-9903); OCSPP 890.1300; OECD 455
- Published OECD Validation of the Steroidogenesis Assay (Hecker *et al.*, 2010, ASB2012-11840)

In Vivo EDSP Glyphosate Studies submitted to the US EPA

- Amphibian Metamorphosis (Frog) OCSPP 890.1100; OECD 231
- *In Vivo* Hershberger Assay (Rat); OCSPP 890.1600; OECD 441
- Female Pubertal Assay; OCSPP 890.1450; OECD None
- Male Pubertal Assay; OCSPP 890.1500
- Uterotrophic Assay (Rat); OCSPP 890.1600; OECD 440
- Fish Short-Term Reproduction Assay; OCSPP 890.1350; OECD 229

The glyphosate Tier 1 screening assay study reports are owned by the JGTF. The European Glyphosate

Task Force (GTF) is negotiating to procure access rights to the battery of glyphosate EDSP Tier 1 screening study reports. Results of the Hershberger and Uterotrophic *in vivo* rat studies, now in the public domain, as are the published results of the OECD validation of the Steroidogenesis assay, in which glyphosate clearly had no impact on steroidogenesis, are discussed below.
Recently, the first publicly data available from the glyphosate Tier 1 assays under the US EPA Endocrine Disruptor Screening Program, were reported at the 2012 Society of Toxicology meeting (Saltmiras & Tobia 2012, ASB2012-12016) for the Hershberger and Uterotrophic assays. No effects were noted for any potential for glyphosate to interact with androgenic or estrogenic pathways under these GLP studies following the US EPA 890 Series Test Guidelines.
Bailey *et al.* (2013, ASB2013-3464) summarized the first results of the male and female Pubertal assay of this programm. Based on these results, glyphosate does not exhibit endocrine disruption in Male and Female Pubertal assays.
Levine *et al.* (2012, ASB2014-9609) published a short summary of the results of tests with glyphosate in the EPA`s Endocrine Disruptor Screening Program (EDSP). They conclude that from the weight of evidence provided by the Tier 1 assays, performed at independent labs, under the EDSP along with the higher Tier regulatory safety studies, with a high level of confidence glyphosate would not be an endocrine disruptor.

In Vitro Glyphosate DART/ED Publications

Many *in vitro* research publications have characterised pesticide formulations, including glyphosate based formulations, as toxic and endocrine disrupting products. Researchers and editorial boards did in some cases not consider the fact that surfactants (which are often components of formulated pesticide products), by their physico-chemical nature, are not suitable test substances using *in vitro* cell models. Surfactants compromise the integrity of cellular membranes, including mitochondrial membranes, and thus confound endpoint measurements considered as representative of specific toxicological modes of action or pathways.

A laboratory at the University of Caen, France, has multiple recent publications of *in vitro* research with glyphosate and glyphosate based formulations (Richard *et al.*, 2005, ASB2009- 9024; Benachour *et al.*, 2007, ASB2009-9018; Benachour and Seralini, 2009, ASB2012- 11561; Gasnier *et al.*, 2009, ASB2012-11629; Gasnier *et al.*, 2010, ASB2012-11628; Gasnier *et al.*, 2011, ASB2012-11630; Clair *et al.*, 2012, ASB2012-11592; Mesnage *et al.*, 2012, ASB2012-11900), with proposed extrapolations to an array of *in vivo* effects including potent endocrine disruption, aromatase inhibition, estrogen synthesis, placental toxicity, foetotoxicity, embryotoxicity and bioaccumulation. These publications are in some cases replicates of earlier studies, using different cell lines or primary cell cultures and in some cases the same data are reported again in a subsequent publication. Firstly, the *in vitro* synergism claims are conjecture, because no control groups of surfactant without glyphosate were tested. Secondly, the extrapolations to *in vivo* effects are unjustifiable based on both the unsuitability of surfactants in such test systems and the supraphysiological cytotoxic concentrations at which *in vitro* effects are reported. Again often overlooked by *in vitro* researchers and editorial boards, Levine *et al.* (2007, ASB2009-9030) presented convincing data demonstrating a lack of *in vitro* synergism for glyphosate with other formulation ingredients. Regarding Seralini's repeated claims of glyphosate induced aromatase inhibition in mircosomes (Richard *et al.*, 2005; TOX2005-1743, Benachour *et al.*, 2007, ASB2009-9018; Gasnier *et al.*, 2009, ASB2012-11629), the data are confounded and thus uninterpretable where surfactants are introduced to such *in vitro* systems. This is noted in the US EPA Aromatase Inhibition Test Guideline, OECD 890.1200, in which notes, "Microsomes can be denatured by detergents [surfactants]. Therefore, it is important to ensure that all glassware and other equipment used for microsome preparations be free of detergent residue."

Another *in vitro* publication claiming a specific developmental toxicity pathway has gained significant public attention. Paganelli *et al.* (2010, ASB2012-11986) conducted three *in vitro* assays, (i) frog embryos exposed to glyphosate formulation; (ii) frog embryos directly injected without injection blank negative controls; and (iii) fertilised chicken embryos exposed directly to a glyphosate formulation through a hole cut in the egg shell. Key issues surrounding this research include irrelevant routes of exposure as well as excessively high and environmentally unrealistic doses.

Thongprakaisang *et al.*, (2013, ASB2013-11991) submitted a study on the effects of pure glyphosate on estrogen receptors mediated transcriptional activity and their expressions. The following cell lines have been used: a hormone-dependent breast cancer, T47D, a stably EREC-luc construct tranfected hormone-dependent breast cancer T47D-KBluc and a hormone-independent human breast cancer, MDA-MB231. Glyphosate (purity ≥ 98%) was tested in concentrations from 10^{-12} to 10^{-6} M. Glyphosate exerted proliferative effects on human hormone-dependent cell lines but not in hormone-independent cell lines. Furthermore, an additive estrogenic effect between glyphosate and genistein, a phytoestrogen, was reported. The authors conclude that these in vitro results need further investigation in an animal study. It must be emphasised that no increase in mammary tumours was reported in any of the numerous long-term studies in rats or mice (see Vol. 3, B.6.5 and Vol. 1, B.2.6).
Cavalli *et al.* (2013, ASB2014-7495) studied the effects of the formulation Roundup Original in rat testis and Sertoli cells in vitro. The authors propose that Roundup toxicity, implicated in Ca2+ overload, cell signalling misregulation, stress response of the endoplasmatic reticulum, and/or depleted antioxidant defenses, could contribute to Sertoli cell disruption in spermatogenesis that could have an impact on male fertility.

***In Vivo* Glyphosate DART/ED Publications**
Relatively few *in vivo* publications on glyphosate DART and ED exist in comparison with the list of *in vitro* publications. Some lack appropriate interpretation of basic toxicology; e.g. Daruich *et al.* (2001, ASB2012-11601). Beuret *et al.* (2005, ASB2012-11564) investigated the effects of 1% Glyphosate oral exposure (a trade product from Argentina described as "Herbicygon" was used which is a commercial herbicide formulation) on lipoperoxidation and antioxidant enzyme systems in pregnant rats and in fetuses. Lipoperoxidation was higher in both maternal and fetal livers in the glyphosate treated groups. Catalase and Superoxide dismutase activity were not altered. Both studies are reviewed in Williams *et al.* (2012, ASB2012-12052).

Dallegrave *et al.* (2003, ASB2012-11600; 2007, ASB2012-2721) published results of two non-guidelines rat developmental toxicity studies, in which a glyphosate based formulation containing POEA was evaluated. However, reporting deficiencies and inconsistencies pose difficulties in data interpretation. These studies are dicussed in detail in the Appendix on tallowamines (please refer to B.6.13).

Romano *et al.* (2010, ASB2012-12012) evaluated a glyphosate based formulation in a male pubertal-like assay in Wistar rats, reporting decreased preputial separation, reduced seminiferous epithelial height, increased luminal diameter of seminiferous tubules, and increased relative testicular and adrenal weights. Given the gravity of the reported findings in this publication, a review was undertaken by Kelce *et al.* (2010, ASB2012-11867). Most recently, Romano *et al.* (2012, ASB2012-12011) reported additional findings in male ratsafter supposed *in utero* and *post natal* exposures which include "behavioral changes and histological and endocrine problems in reproductive parameters and these changes are reflected by a hypersecretion of androgens and increased gonadal activity, sperm production and libido". As in their first publication, Romano *et al.* (2012, ASB2012-12011) base their hypothesis on selectively discussed literature implicating glyphosate as an endocrine disruptor, predominantly with citations to research from the Seralini laboratory.

Kimmel *et al.* (2013, ASB2013-3462) analyzed the information from 7 unpublished developmental studies in rabbits and 6 developmental toxicity studies in rats to determine if glyphosate poses a risk for cardiovascular malformations. In summary, assessment of the reviewed data fails to support a potential risk for increased cardiovascular defects as a result of glyphosate exposure during pregnancy.
Chruscielska *et al.* (2000, ASB2013-9831) submitted a teratogenicity study in Wistar outbred rats. The used test guideline was not indicated. Doses of 0-750-1500-3000 mg/kg bw/dayhave been administered from day 7-14 of pregnancy to 20 females per dose group. No embryotoxic and no teratogenic effects have been administered.
Omran and Salama (2013, ASB2014-7614) report that the exposition of snails to atrazine or glyphosate resultet in signs of endocrine disruption and cellular toxicity. However, in this study only the formulation "Herfosate" was used and no pure active substance glyphosate.

Razi *et al.* (2012, ASB2014-9390) consider that glyphosate (125 mg/kg bw/d oral administered for 10, 20, 30 & 40 days) effects testicular tissue and sperm parameters in male Wistar rats. Clear effects were already seen after 10 days administration and thereafter, however accompanied by significant clinical symptoms (decreased movement, staggering gait, occasional trembling, diarrhea) and reduced body weight gain of 20 %. These findings are in contrast to those in rat studies submitted for EU evaluation. For comparison, the current EU evaluation of glyphosate proposes an overall subchronic (90-d) NOAEL of 414 mg/kg bw/d (rats) and for reproductive toxicity of 351 mg/kg bw/d, albeit generated from feeding studies. Similarily, after oral administration in female rats an NOAEL of 300 mg/kg bw/d for maternal and developmental effects was established, toxic effects were observed at much higher dose levels, only. The high toxicity described in the present publication is hardly to explain, because the publication does not give any information whether technical material or a glyphosated based formulation was tested. To conclude, the results of the publication does not affect the current assessment of glyphosate.
Cassault-Meyer *et al.* (2014, ASB2014-5615) investigated the effects of a glyphosate-based herbicide (Roundup Grand Travaux Plus) after an 8-day exposure of adult rats. Endocrine (aromatase, estrogen and androgen receptors, Gperl in testicular and sperm mRNAs) and testicular functions (organ weight, sperm parameters and expression of the blood-testis barrier markers) were monitored at day 68, 87, and 122 after treatment, spermiogenesis and spermatogenesis. A significant and differential expression of aromatase in testis and a diminution of mRNA expression of nuclear markers in spermatozoa were observed. The authors conclude that results suggest changes in androgen/estrogen balance and in sperm nuclear quality.

POEA DART Studies

Polyethoxylated alkylamine (POEA) surfactants are a class of non-ionic surfactant, containing a tertiary amine, an aliphatic group of variable carbon chain length and two separate sets of ethoxy (EO) chains of variable length. A dietary exposure assessment of POEAs was submitted by Bleeke *et al.* (2010, ASB2010-6123). This exposure assessment report also refers to the US EPA Alky Amine Polyalkoxylates Human Health Risk Assessment, which includes POEAs (http://www regulations.gov/search/Regs/home.html#documentDetail?R=09000064809b983b). Williams *et al.* (2012, ASB2012-12052) recently evaluated and detailed the results of DART studies with two different POEA surfactants.
Furthermore, a detailed comparison of the toxicity of tallowamine and glyphosate was submitted in the appendix "Toxicological evaluation of the POE-tallowamine surfactant (CAS no. 61791-26-2)" which is attached to this report.

Epidemiology Glyphosate DART/ED Publications

Several epidemiology studies in which glyphosate exposure was considered have evaluated the following range of reproductive outcomes: miscarriage, fecundity, pre-term delivery, gestational diabetes mellitus, birth weights, congenital malformations, neural tube defects, attention-deficit disorder / attention-deficit hyperactive disorder (ADD/ADHD). In most instances, glyphosate and reproductive outcomes lack a statistically significant positive association, as described in a recent review of glyphosate non-cancer endpoint publications (Mink *et al.*, 2011, ASB2012-11904). In evaluating ADD/ADHD, a positive association with glyphosate use was reported by Garry *et al.* (2002, ASB2012-11626), but cases were reported by parents with no clinical confirmation and the reported incidence rate of approximately 1% for the study population was well below the general population incidence rate of approximately 7%. Regarding *in utero* exposures, McQueen *et al.* (2012, ASB2012-11898) report very low measured dietary exposures, from 0.005% to 2% of the current glyphosate ADI in Europe. Given the low perfusion rate of glyphosate across the placenta (Mose *et al.*, 2008, ASB2012-11914), human *in utero* exposures would be very limited. Campana *et al.* (2010, ASB2013-10559) estimated the frequency of 27 birth defects in 7 geographical regions of Argentina. A sample of 21,844 newborn with birth defects was selected, ascertained from 855,220 births, between 1994 and 2007, in 59 hospitals. The study results suggested that frequencies of 14 of the 27 examined birth defects were higher in one or more regions. This study was discussed in some publications in relation to the use of glyphosate pesticides. However, Campana *et al.* (2010, ASB2013-10559) commented on secular trends, altitude above sea level, folic acid fortification and ethnic factors and further variables. It was not indicated that any of these variables was associated with an increased occurrence of any type of birth defects.

Two studies of residential proximity to agriculture-related pesticide applications (California) by Carmichael *et al.* (2013, ASB2014-9307) and Yang *et al.* (2013, ASB2014-9644) examined whether early gestational exposure to pesticides were associated with an increased risk of hypospadia, neural tube defects or orofacial clefts in offspring. In both studies formulated glyphosate was mentioned only as one out of five chemicals to which controls were most frequently exposed. The authors of both studies concluded the few positive findings on chemicals, but other than glyphosate, should be interpreted with caution and need to be repeated in other populations.

Manfo *et al.* (2010, ASB2014-9611) examined the effect of pesticides use on male reproductive function in a study on farmers in Cameroon. The farmers of Djutitsa (West Cameroon) used 25 active substances (in 57 preparations) amongst others glyphosate in different formulations and were exposed to agro-pesticides due to inappropriate handling and improper protective tools. Furthermore, the authors concluded, that male farmers, who are exposed to pesticides might have impaired reproductive function through inhibition of testosterone synthesis. Serum biochemical parameter (total testosterone, estradiol/testosterone, androstenedione) were altered compared to the unexposed control group, but these alterations of chemical parameter cannot be related to single pesticides, e.g. glyphosate. Moreover, the fungicides were the most used active ingredients. However, considering the obvious alterations, the authors concluded, that there is urgent need for more training to enable improvement of equipment and efficiency of application to minimize exposure risks.

Further reviews on DART

Antoniou *et al.* (2012, ASB2012-15927) submitted a review article on "Teratogenic Effects of Glyphosate-Based Herbicides: Divergence of Regulatory Decisions from Scientific Evidence". According to the authors published studies "have raised concern regarding the potential for glyphosate and its commercial formulations to cause birth defects and other reproductive problems". The "draft assessment report revealed that industry tests contained clear evidence of glyphosate-mediated teratogenicity and reproductive toxicity". The EU adopted "an acceptable daily intake (ADI) for glyphosate that is unreliable and could potentially result in exposures that cause harm to humans." The authors suggest that a "new risk assessment should be conducted with full public transparency by scientists who are independent of industry."

Lopez *et al.* (2012, ASB2013-10534) submitted a review article on "Pesticides used in South American GMO-Based Agriculture: a review on their effects on humans and animal models". The authors discuss the results of genetic studies in agricultural regions in the province of Cordoba, Argentina, biomarkers in agricultural regions in the province of Santa Fe, Argentina and congenital malformations and genotoxicity in populations exposed to pesticides in Paraguay. According to the authors, human health in these areas was damaged by pesticides. However, a relation to glyphosate or another substance or pesticide was not evidenced. Nevertheless, based on the results of Paganelli *et al.* (2010, ASB2012-11986), it was concluded that glyphosate-based herbicides) would be linked to an increased activity of the retinoic acid signaling pathways and this might explain the higher incidence of embryonic malformations and spontaneous abortions observed in populations exposed to pesticides.

Basrur (2006, ASB2014-7492) submitted a review on disrupted sex differentiation and feminization of men. In this review the studies of Arbuckle and associates are cited which report a relation between pesticide exposure (including glyphosate) and reproductive risk.

Vandenberg *et al.* (2012, ASB2014-9635) submitted a review on low dose effects an nonmonotonic dose responses of hormones and endocrine disrupting chemicals. The authors reviewed two major concepts on EDC studies: low dose and nonmonotonicity. They conclude that nonmonotonic responses and low-dose effects would be remarkably common in studies of natural hormones and EDSs. Whether low doses of EDCs influence certain human disorders would be no longer conjecture, because epidemiologic studies would show that environmental exposures to ECDs would be associated with human diseases and disabilities. The authors demand that fundamental changes in chemical testing and safety determination woud be needed to protect human health.

In a direct response on the article of Vandenberg *et al.* (2012, ASB2014-9635) a discussion paper was

submitted by Rhomberg and Goodman (2012, ASB2014-9391). These authors conclude that Vandenberg *et al.* (2012, ASB2014-9635) presented examples as anecdotes without attempting to review all available pertinent data, selectively citing studies without evaluating most of them or examining whether their putative examples are consistent and coherent with other relevant information. Many of their examples have been questioned by many scientists. Overall, Vandenberg *et al.* (2012, ASB2014-9635) put forth many asserted illustrations of their two conclusions without providing sufficient evidence to make the case for either and while overlooking evidence that suggest the contrary.
Lamb *et al.* (2014, ASB2014-9605) submitted a review with critical comments on the WHO- UNEP state of the science of endocrine disrupting chemicals – 2012. The authors conclude that the 2012 report does not provide a balanced perspective, nor does it accurately reflect the state fo the science on endocrine disruption.

Borgert *et al.* (2013, ASB2014-9292) reviewed literature on thresholds of endocrine activity. The brief review highlights how the fundamental principles governing hormonal effects – affinity, potency, and mass action – dictate the existence of thresholds and why these principles also define the potential that exogenous chemicals might have to interfere with normal endocrine functioning.
The review by Sengupta and Banerjee, (2013, ASB2014-9730) is related to impacts of pesticides on male fertility. With respect to glyphosate the authors only cited in vitro data published by Richard *et al.* (2005, ASB2009-9025), and these have been already reported and evaluated in the present renewal assessment report (please refer to 'In vitro Glyphosate DART/ED Publications').
Kumar (2011, ASB2014-9725) submitted a review on occupational, environmental and lifestyle factors associated with spontaneous abortion. In this review Arbuckle *et al.* (2001, ASB2012-11545) was cited who reported a relation between pesticide exposure (including glyphosate) and reproductive risk. This publication was already reported and discussed under 'Epidemiology DART/ED Publication'.
The extensive review by Wigle *et al.* (2008, ASB2014-9637) summarised the level of epidemiologic evidence of relationships between reproductive and child health outcomes and environmental chemical contaminants. Several references related to glyphosate were cited by the authors [(Curtis *et al.*1999, cited in Arbuckle *et al.* (2001, ASB2012-11545), Arbuckle *et al.* (2001, ASB2012-11545), Savitz *et al.* (1997, ASB2012-12022), Garry (2002, ASB2012-
11626))], which were already reported and discussed under to 'Epidemiology DART/ED Publication'.
The mechanism based short review by Jamkhande *et al.* (2014, ASB2014-9573) summarised common human teratogenic agents. With respect to glyphosate (-based formulations) the authors cited merely data published by Antoniou *et al.* (2012, ASB2012-15927); Paganelli *et al.* (2010, ASB2012-11986). Both publications were already reported and evaluated in the present renewal assessment report (please refer to 'Further reviews on DART').
The English abstract of a Chinese publication by Zhang *et al.* (2013, ASB2014-9643) give notice of a summary on reproductive and developmental toxicity studies on glyphosate and the related mechanisms on humans and animals to provide suggestions for further research.

Comparison of the active substance glyphosate and glyphosate containing formulations concerning DART and ED
For the active substance glyphosate a very comprehensive data package of guideline conform studies on developmental and reproductive toxicity is available. This data package was prepared over the last decades and updated within the last years.
In these submitted studies it was demonstrated that glyphosate is not a teratogenic substance. NOEL values for developmental toxicity and reproductive toxicity can be derived from the results of these studies. There are no relevant indications of an endocrine disrupting activity of the active substance glyphosate. Additionally, also in the further guideline conform toxicological studies (e.g. the subchronic and chronic toxicity studies) no indications of an endocrine disrupting activity of glyphosate (e.g. organ weight and histology of sexual organs, behaviour etc.) have been observed. Therefore, on basis of this comprehensive and high quality data package the active substance glyphosate is not considered to be an endocrine disruptor or a teratogenic substance.
Additionally to the studies which have been performed according to validated EU- and OECD guidelines a large number of studies has been published on DART and ED. Most of these studies use glyphosate containing preparations instead of the pure active substance glyphosate. However, some studies directly

compare the toxicity of the active substance glyphosate and glyphosate conaining preparations. Furthermore, studies have been performed on the toxicity of surfactants which are used in preparations together with glyphosate, especially tallowamine. The results of these surfactant studies can be compared with the results of the above mentioned guideline conform studies on glyphosate.

In result of these comparisons it can clearly be concluded that the toxicity of preparations and the toxicity of surfactants like tallowamine / polyethoxylated alkylamine is significantly higher than the toxicity of the active substance glyphosate.

A detailed comparison of the toxicity of tallowamin and glyphosate was submitted in the appendix "Toxicological evaluation of the POE-tallowamine surfactant (CAS no. 61791-26-2)" which is attached to this report. In this evaluation is clearly demonstrated that there is a significantly higher toxicity of the surfactant tallowamin with regard to all of the following endpoints investigated:

- acute oral toxicity
- acute dermal toxicity
- skin irritation
- eye irritation
- skin sensitization
- short term toxicity, rat
- short term toxicity, dog
- reproduction toxicity study, parental toxicity
- reproduction toxicity study, reproductive toxicity
- reproduction toxicity study, offspring toxicity
- developmental toxicity, rat, fetal effects

Walsh *et al.* (2000, ASB2012-12046) published research claiming that a glyphosate based formulation, but not glyphosate alone, adversely affected the steroidogenesis pathway by inhibiting progesterone production resulting in downstream reduction in mitochondrial levels of StAR protein. Subsequent research by Levine *et al.* (2007, ASB2009-9030) demonstrated no synergism between glyphosate and the surfactant since the cytotoxic effects were completely independent of glyphosate. Identical dose-response curves were noted for formulated product with and without the glyphosate active ingredient.

Further research addressing the steroidogenesis pathway confirmed glyphosate lacked endocrine disruption potential specific to this pathway. Quassinti *et al.* (2009, ASB2012- 12007) evaluated effects on gonadal steroidogenesis in frog testis and ovaries on glyphosate and another active substance, noting that glyphosate unequivocally demonstrated no effect. Forgacs *et al.* (2012, ASB2012-11621) also tested glyphosate alone and demonstrated no effect on testosterone levels in BLTK1 murine leydig cells *in vitro*. Furthermore, the OECD multi-laboratory validation of the Steroidogenesis Assay used for Tier 1 screening of the US EPA EDSP, evaluated glyphosate and concluded no impact on steroidogenesis (Hecker *et al.*, 2011, ASB2012-11840). Consequently, the US EPA considered reference to the OECD validation report sufficient for meeting the glyphosate Steroidogenesis Assay Test Order in the EDSP Tier 1 screening of glyphosate.
Recently, the first publicly data available from the glyphosate Tier 1 assays under the US EPA Endocrine Disruptor Screening Program, were reported at the 2012 Society of Toxicology meeting (Saltmiras & Tobia, 2012, ASB2012-12016) for the Hershberger and Uterotrophic assays. No effects were noted for any potential for the active substance glyphosate to interact with androgenic or estrogenic pathways under these GLP studies following the US EPA 890 Series Test Guidelines.
Richard *et al.* (2005, TOX2005-1743) studied effects of glyphosate and roundup on human placental cells and aromatase. Summarising their results they stated that "roundup is always more toxic than its active ingredient."
In a further study from the same institute Benachour *et al.* (2007, ASB2009-9018) studied time- and dose-dependent effects of roundup on human embryonic and placental cells. They summarized that "in all instances, roundup is more efficient than its active ingredient, glyphosate…". And in a further publication by Benachour and Seralini (2009, ASB2012- 11561) it was stated "this work clearly confirms that the

adjuvants in roundup formulations are not inert." In a response to this publication by the French Agency for Food Safety (AFSSA, 2009, ASB2012-11532) it was answered that surfactant effects are known to increase membrane permeability, causing cytotoxicity and induction of apoptosis. In the most recent publication from the same institute, Mesnage *et al.* (in press, ASB2012-13917) the potential active principle for toxicity on human cells for 9 glyphosate-based formulations was studied. The authors summarized that "ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity."

In a comprehensive analysis of the available literature in developmenat and reproductive outcomes in humans and animals after glyphosate exposure, Williams *et al.* (2012, ASB2012- 12052) summarized: "An evaluation of this database found no consistent effects of glyphosate exposure on reproductive health or the developing offspring. Furthermore, no plausible mechanism of action for such effects were elucidated. Although toxicity was observed in studies that used glyphosate-based formulations, the data strongly suggest that such effects were due to surfactants present in the formulations and not the direct result of glyphosate exposure."

***In vitro* DART/ED publications**

Author(s)	Year	Study title
Walsh, L.P. McCormick, C. Martin, C. Stocco, D.M.	2000	Roundup inhibits steroidogenesis by disrupting steroidogenic acute regulatory (StAR) protein expression. Environmental Health Perspectives Volume: 108 Number: 8 Pages: 769-776 ASB2012-12046

Abstract*
Recent reports demonstrate that many currently used pesticides have the capacity to disrupt reproductive function in animals. Although this reproductive dysfunction is typically characterized by alterations in serum steroid hormone levels, disruptions in spermatogenesis, and loss of fertility, the mechanisms involved in pesticide-induced infertility remain unclear. Because testicular Leydig cells play a crucial role in male reproductive function by producing testosterone, we used the mouse MA-10 Leydig tumor cell line to study the molecular events involved in pesticide-induced alterations in steroid hormone biosynthesis. We previously showed that the organochlorine insecticide lindane and the organophosphate insecticide Dimethoate directly inhibit steroidogenesis in Leydig cells by disrupting expression of the steroidogenic acute regulatory (StAR) protein. StAR protein mediates the rate-limiting and acutely regulated step in steroidogenesis, the transfer of cholesterol from the outer to the inner mitochondrial membrane where the cytochrome P450 side chain cleavage (P450scc) enzyme initiates the synthesis of all steroid hormones. In the present study, we screened eight currently used pesticide formulations for their ability to inhibit steroidogenesis, concentrating on their effects on StAR expression in MA-10 cells. In addition, we determined the effects of these compounds on the levels and activities of the P450scc enzyme (which converts cholesterol to pregnenolone) and the 3 β-hydroxysteroid dehydrogenase (3 β-HSD) enzyme (which converts pregnenolone to progesterone). Of the pesticides screened, only the pesticide Roundup inhibited dibutyryl [(Bu)2]cAMP-stimulated progesterone production in MA-10 cells without causing cellular toxicity. Roundup inhibited steroidogenesis by disrupting StAR protein expression, further demonstrating the susceptibility of StAR to environmental pollutants.

*Quoted from article

Klimisch evaluation

Reliability of study:	Reliable with restrictions
Comment:	Non-standard test systems, but publication meets basic

	scientific principles. However, surfactant blend in Roundup confounds results.
Relevance of study:	Relevant with restrictions: Different effects of glyphosate alone and glyphosate formulations were observed. No conclusion can be drawn that the observed effects are result of glyphosate exposure.
Klimisch code:	2

Additional comments:
Glyphosate did not affect steroidogenesis in the test system.
Roundup formulation data was confounded by mitochondrial membrane damage, attributable to the surfactant in the tested formulation.
Roundup results were comprehensively addressed in Levine *et al.* (2007, ASB2009-9030): Roundup formulation containing glyphosate and Roundup formulation blank without the active ingredient was shown to have "indistinguishable" dose response curves for reductions in progesterone production in hCG stimulated MA-10 Leydig cells. Therefore the effect on progesterone levels shown by Walsh (2000, ASB2012-12046) were independent of glyphosate and attributable to the surfactant component of the formulation.
Comparable rates of progesterone inhibition for several different surfactants suggest a common mode of action for surfactants.
Roundup formulation containing glyphosate and Roundup formulation blank without the active ingredient was shown to have almost identical concentration-dependent decreases in MTT activity in MA-10 cells, suggesting the surfactant alone was responsible for the observed cytotoxicity and effect on mitochondrial function.
The JC-1 assay demonstrated the decreased progesterone production in MA-10 Leydig cells was accompanied by loss of mitochondrial membrane potential. These results confirm StAR protein function and steroidogenesis require intact mitochondrial membrane potential.
StAR protein expression were not affected by treatments, indicating that perturbed mitochondrial membrane, not StAR protein inhibition, was responsible for the effects noted by Walsh *et al.* (2000, ASB2012-12046).

Author(s)	Year	Study title
Paganelli, A. Gnazzo, V. Acosta H. Lopez, S.L. Carrasco, A.E.	2010	Glyphosate-Based Herbicides Produce Teratogenic Effects on Vertebrates by Impairing Retinoic Acid Signalling Chemical Research in Toxicology Volume: 23 Pages: 1586-1595 ASB2010-11410

Abstract*
The broad spectrum herbicide glyphosate is widely used in agriculture worldwide. There has been ongoing controversy regarding the possible adverse effects of glyphosate on the environment and on human health. Reports of neural defects and craniofacial malformations from regions where glyphosatebased herbicides (GBH) are used led us to undertake an embryological approach to explore the effects of low doses of glyphosate in development. *Xenopus laevis* embryos were incubated with 1/5000 dilutions of a commercial GBH. The treated embryos were highly abnormal with marked alterations in cephalic and neural crest development and shortening of the anterior-posterior (A-P) axis. Alterations on neural crest markers were later correlated with deformities in the cranial cartilages at tadpole stages. Embryos injected with pure glyphosate showed very similar phenotypes. Moreover, GBH produced similar effects in chicken embryos, showing a gradual loss of rhombomere domains, reduction of the optic vesicles, and microcephaly. This suggests that glyphosate itself was responsible for the phenotypes observed, rather than a surfactant or other

component of the commercial formulation. A reporter gene assay revealed that GBH treatment increased endogenous retinoic acid (RA) activity in *Xenopus* embryos and cotreatment with a RA antagonist rescued the teratogenic effects of the GBH. Therefore, we conclude that the phenotypes produced by GBH are mainly a consequence of the increase of endogenous retinoid activity. This is consistent with the decrease of Sonic hedgehog (Shh) signaling from the embryonic dorsal midline, with the inhibition of *otx2* expression and with the disruption of cephalic neural crest development. The direct effect of glyphosate on early mechanisms of morphogenesis in vertebrate embryos opens concerns about the clinical findings from human offspring in populations exposed to GBH in agricultural fields.

[k] Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Non-guideline study that is not sufficiently described for assessment. Inadequate positive and negative control experiments.
Relevance of study:	Not relevant: Irrelevant routes of exposure and inappropriately high doses. Test system not adequate for human risk assessment.
Klimisch code:	3

Additional comments:

Response 1 – summarized from Williams *et al.* (2012, ASB2012-12052)

No pH adjustment for doses and thus effects may be in response to the acidic nature of glyphosate technical acid.

Inappropriate and irrelevant routes of exposure.

Data requires further substantiation before consideration in risk assessment.

Response 2 – Saltmiras et al. (2011, ASB2012-12015) letter to the Editor

Multiple high quality toxicological studies and expert review panels consistently agree glyphosate is not a teratogen or reproductive toxicant.

The authors' justification for this research is flawed, providing no valid basis, other than an opinion, of an increase in the rate of birth defects in Argentina.

Direct injection of frog embryos and through chicken shells do not reflect real world exposure scenarios to either environmental species or humans.

Doses were excessively high and irrelevant for risk assessment purposes. Frog embryos were also bathed in glyphosate formulation at doses 9-15 times greater than the acute LC50 same species of frog. Calculating equivalent oral doses based on pharmacokinetics studies, such doses are 150000000 times greater than worst case human exposure monitoring data.

"…. the results from this research cannot be used in isolation to reach the conclusions expressed in the publication. Instead, the type of data in this research paper must be interpreted relative to all other available data on the specific materials under study and with balanced consideration for higher tier apical studies."

Response 3 – Mulet (2011, ASB2012-11916) letter to the Editor

Notes the premise for this research is falsely based on an incorrectly cited local pediatric bulletin from Paraguay.

"…. this article refers to a study in a single hospital in Paraguay showing a correlation between pesticide use (not herbicides as mentioned by Paganelli *et al.*, ASB2010-11410) and birth malformations. In the cited study, (Benitez *et al.*, ASB2012-11563), the authors state that the results are preliminary and must be confirmed. Is important to remark that the Benitez *et al.* study does not include any mention to glyphosate, so does not account for what the authors are stating in the introduction…..This journal is also wrongly cited in the discussion referring to increased malformations due to herbicides, which is not the result of the study."

Response 4 – comments from BVL (2010, ASB2012-11579)
Highly artificial experimental conditions.
Inappropriate models to replace validated mammalian reproductive and developmental toxicity testing methods for use in human health risk assessment.
Inappropriate routes of exposure.
Lack of corroborative evidence in humans.
"In spite of long-lasting use of glyphosate-based herbicides worldwide, no evidence of teratogenicity in humans has been obtained so far."

Response 5 – comments from European Commission Standing Committee on the Food Chain and Animal Health (2011, ASB2012-11615)
The EU commission supports the German Authorities position, "that that there is a comprehensive and reliable toxicological database for glyphosate and the effects observed have not been revealed in mammalian studies, nor evidenced epidemiologically in humans." "…. the Commission does not consider there is currently a solid basis to ban or impose specific restrictions on the use of glyphosate in the EU."
Response 6 – Palma, G. (2010, ASB2012-11989) letter to the Editor
The author of the letter claims that the study by Paganelli *et al.*, 2010 (ASB2010-11410), described effects of glyphosate only at unrealistic high concentrations or via unrealistic routes of exposure. The data are thought to be inconsistent with the literature, and therefore not suitable or relevant for the risk assessment for humans and wildlife. Furthermore the author asserts that findings do not support the extrapolation to human health as stated in the publication.

Author(s)	Year	Study title
Richard, S. Moslemi, S. Sipahutar, H. Benachour, N. Seralini, G.E.	2005	Differential effects of glyphosate and roundup on human placental cells and aromatase. Environmental Health Perspectives Volume: 113 Pages: 716-720 TOX2005-1743

Abstract*
Roundup is a glyphosate-based herbicide used worldwide, including on most genetically modified plants that have been designed to tolerate it. Its residues may thus enter the food chain, and glyphosate is found as a contaminant in rivers. Some agricultural workers using glyphosate have pregnancy problems, but its mechanism of action in mammals is questioned. Here we show that glyphosate is toxic to human placental JEG3 cells within 18 hr with concentrations lower than those found with agricultural use, and this effect increases with concentration and time or in the presence of Roundup adjuvants. Surprisingly, Roundup is always more toxic than its active ingredient. We tested the effects of glyphosate and Roundup at lower nontoxic concentrations on aromatase, the enzyme responsible for estrogen synthesis. The glyphosate-based herbicide disrupts aromatase activity and mRNA levels and interacts with the active site of the purified enzyme, but the effects of glyphosate are facilitated by the Roundup formulation in microsomes or in cell culture. We conclude that endocrine and toxic effects of Roundup, not just glyphosate, can be observed in mammals. We suggest that the presence of Roundup adjuvants enhances glyphosate bioavailability and/or bioaccumulation.

* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Study design is insufficient for risk assessment of real exposure concentrations. Methodological deficiencies (no controls were included). Exceedingly high doses above the limit dose for this study type. Inappropriate test system for

	formulations containing surfactant; cytoxic membrane disruption potential of surfactants are well known for in vitro test systems. EPA Test Guideline OCSPP 890.1200 specifically notes that microsomes are denatured by detergents (i.e. surfactants) and that all glassware should be thoroughly rinsed.
Relevance of study:	Not relevant: Excessive doses exceed typical *in vitro* limit doses. *In vitro* test system is inappropriate with surfactants.
Klimisch code:	3

Additional comments:

Response 1 – summarized from Williams *et al.* (2012, ASB2012-12052)
Glyphosate at non-cytotoxic concentrations in this test system was demonstrated to have no effects on aromatase activity.
Likewise, did not affect mRNA levels after 18 hours treatment at ≤ 0.1% glyphosate.
Roundup aromatase activity measurements are confounded by surfactant effects on microsomes.
The *in vitro* test system is non-validated Physiologically irrelevant concentrations tested.
Testing surfactant-like substances in such systems is now recognized to be not valid.

Response 2 – summarized from the French Ministry of Agriculture and Fish, Committee for Study of Toxicity (2005, ASB2009-9025)
Major methodological gaps.
JEG3 cells, a choriocarcinoma human cell line (average of 70 chromosomes vs 46 in normal human cells).
Concentrations of Roundup used in the various experiments considered to be extremely high. In consideration of limiting factors (oral absorption, 30%; skin absorption, 0.3%; rapid elimination kinetics), such levels would involve considerable human exposure, or several dozen liters of Roundup diluted at 2%. concentrations of Roundup that trigger an effect on aromatase (0.5% - 2%) are at least 1000 times more effective than those of known aromatase inhibitors, such as azole derivatives Study design does not make it possible to show the influence of the adjuvants, nor synergism of adjuvants and glyphosate.
Multiple non-specific effects of surfactant agents on a broad range of cellular targets not discussed.
No comparison with comparable surfactant agents intended for household use. Multiple instances of bias in its arguments and its interpretation of the data.
The authors over-interpret their results in the area of potential health consequences for humans (unsuitable references, non-sustained in vitro-in vivo extrapolation, etc.).

Author(s)	Year	Study title
Benachour, N. Sipahutar, H. Moslerni, S. Gasnier, C. Travert, C. Seralini, G. E.	2007	Time- and dose-dependent effects of roundup on human embryonic and placental cells. Archives of Environmental Contamination and Toxicology Volume: 53 Pages: 126-133 ASB2009-9018

Abstract*
Roundup® is the major herbicide used worldwide, in particular on genetically modified plants that have been designed to tolerate it. We have tested the toxicity and endocrine disruption potential of Roundup (Bioforce®) on human embryonic 293 and placental-derived JEG3 cells, but also on normal human placenta and equine testis. The cell lines have proven to be suitable to estimate hormonal activity and toxicity of pollutants. The median lethal dose (LD_{50}) of Roundup with embryonic cells is 0.3% within 1 h in serum-free medium, and it decreases to reach 0.06% (containing among other compounds 1.27 mM glyphosate) after 72 h in the presence of serum. In these conditions, the embryonic cells appear to be 2-4

times more sensitive than the placental ones. In all instances, Roundup (generally used in agriculture at 1 – 2%, i.e., with 21-42 mM glyphosate) is more efficient than its active ingredient, glyphosate, suggesting a synergistic effect provoked by the adjuvants present in Roundup. We demonstrated that serum-free cultures, even on a short-term basis (1 h), reveal the xenobiotic impacts that are visible 1-2 days later in serum. We also document at lower non-overtly toxic doses, from 0.01% (with 210 µM glyphosate) in 24 h, that Roundup is an aromatase disruptor. The direct inhibition is temperature-dependent and is confirmed in different tissues and species (cell lines from placenta or embryonic kidney, equine testicular, or human fresh placental extracts). Furthermore, glyphosate acts directly as a partial inactivator on microsomal aromatase, independently of its acidity, and in a dose-dependent manner. The cytotoxic, and potentially endocrine-disrupting effects of Roundup are thus amplified with time. Taken together, these data suggest that Roundup exposure may affect human reproduction and fetal development in case of contamination. Chemical mixtures in formulations appear to be underestimated regarding their toxic or hormonal impact.

* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Study report has several reporting deficiencies in the methods section (e.g. test conditions for the pH- and temperature dependent assay not reported). There is no information on the suitability of the used HEK 293 cell line for assessment of hormonal activity. Exceedingly high doses above the limit dose for this study type. Inappropriate test system for formulations containing surfactant; cytoxic membrane disruption potential of surfactants are well known for in vitro test systems.
Relevance of study:	Not relevant: Excessive doses exceed typical *in vitro* limit doses. *In vitro* test system is inappropriate with surfactants.
Klimisch code:	3

Additional comments:
Glyphosate at and above relevant concentrations for this test system was demonstrated to have no effects on aromatase activity.
Roundup aromatase activity measurements are confounded by surfactant effects on microsomes.
Comparable research to Richard et al (2005, TOX2005-1743), but with an additional cell line, HEK 293, derived from aborted human embryo kidneys, transformed by inserting adenovirus DNA.
Excessively high doses tested, not environmentally relevant for human health or environmental risk assessment.
Aromatase production within the steroidogenesis pathway. Therefore, aromatase inhibition would be detected in the steroidogenesis assay. The OECD multi-laboratory validation of the steroidogenesis assay evaluated glyphosate, demonstrating no impact on the steroidogenesis pathway (Hecker *et al.*, 2011, ASB2012-11840).

Response – summarized from Williams *et al.* (2012, ASB2012-12052)
pH of test system not adjusted to physiologically appropriate levels; Negative controls were not pH adjusted to appropriate levels.
Confounding surfactant effects due to cell membrane damage render data generated with formulated products in this test system null.

Author(s)	Year	Study title

Benachour, N. Seralini, G. E.	2009	Glyphosate formulations induce apoptosis and necrosis in human umbilical, embryonic, and placental cells. Chemical Research in toxicology Volume: 22, Pages: 97-105 ASB2012-11561

Abstract*

We have evaluated the toxicity of four glyphosate (G)-based herbicides in Roundup formulations, from 10(5) times dilutions, on three different human cell types. This dilution level is far below agricultural recommendations and corresponds to low levels of residues in food or feed. The formulations have been compared to G alone and with its main metabolite AMPA or with one known adjuvant of R formulations, POEA. HUVEC primary neonate umbilical cord vein cells have been tested with 293 embryonic kidney and JEG3 placental cell lines. All R formulations cause total cell death within 24 h, through an inhibition of the mitochondrial succinate dehydrogenase activity, and necrosis, by release of cytosolic adenylate kinase measuring membrane damage. They also induce apoptosis via activation of enzymatic caspases 3/7 activity. This is confirmed by characteristic DNA fragmentation, nuclear shrinkage (pyknosis), and nuclear fragmentation (karyorrhexis), which is demonstrated by DAPI in apoptotic round cells. G provokes only apoptosis, and HUVEC are 100 times more sensitive overall at this level. The deleterious effects are not proportional to G concentrations but rather depend on the nature of the adjuvants. AMPA and POEA separately and synergistically damage cell membranes like R but at different concentrations. Their mixtures are generally even more harmful with G. In conclusion, the R adjuvants like POEA change human cell permeability and amplify toxicity induced already by G, through apoptosis and necrosis. The real threshold of G toxicity must take into account the presence of adjuvants but also G metabolism and time-amplified effects or bioaccumulation. This should be discussed when analyzing the in vivo toxic actions of R. This work clearly confirms that the adjuvants in Roundup formulations are not inert. Moreover, the proprietary mixtures available on the market could cause cell damage and even death around residual levels to be expected, especially in food and feed derived from formulation-treated crops.

* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Exceedingly high doses above the limit dose for this study type. Inappropriate test system for formulations containing surfactant; cytoxic membrane disruption potential of surfactants are well known for in vitro test systems. No positive controls were included.
Relevance of study:	Not relevant (Excessive doses exceed typical *in vitro* limit doses. *In vitro* test system is inappropriate with surfactants)
Klimisch code:	3

Additional comments:

Response – summarized from the French Agency for Food Safety (AFSSA, 2009, ASB2012-11532)

Cell lines used present characteristics which may be at the source of a significant bias in the interpretation of the results.

Experiments were conducted with 24 hours exposure in a medium without serum, which could lead to disturbance of the physiological state of the cells.

The glyphosate used in the study is glyphosate acid, whereas in the preparations tested it is in the form of an isopropylamine salt. No precise information is given about the pH of test concentrations except the highest dose.

No mention of any positive evidence for the apoptosis test.

Cytoxicity and induction of apoptosis may due to pH and/or variations in osmotic pressure on cell survival at the high doses tested.
Surfactant (tensoactive) effects and increased osmolality are known to increase membrane permeability, causing cytotoxicity and induction of apoptosis.
Conclusions are based on unvalidated, non-representative cell models (in particular tumour or transformed cell lines) directly exposed to extremely high product concentrations in culture conditions which do not observe normal cell physiological conditions.
No new information is presented on mechanism of action of glyphosate and preparations containing glyphosate.
The authors over-interpret their results with regard to potential health consequences for humans, based in particular on an unsupported *in vitro–in vivo* extrapolation.
The cytotoxic effects of glyphosate, its metabolite AMPA, the tensioactive POAE and other glyphosate-based preparations proposed by Benachour and Seralini do not add any pertinent new facts which call into question the conclusions of the European assessment of glyphosate or those of the national assessment of the preparations.

Author(s)	Year	Study title
Gasnier, C., Dumont, C., Benachour, N., Clair, E., Chagnon, M. C., Seralini, G. E	2009	Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines. Toxicology Volume: 262, Number: 3, Pages: 184-191 ASB2012-11629

Abstract*
Glyphosate-based herbicides are the most widely used across the world; they are commercialised in different formulations. Their residues are frequent pollutants in the environment. In addition, these herbicides are spread on most eaten transgenic plants, modified to tolerate high levels of these compounds in their cells. Up to 400 ppm of their residues are accepted in some feed. We exposed human liver HepG2 cells, a well-known model to study xenobiotic toxicity, to four different formulations and to glyphosate, which is usually tested alone in chronic in vivo regulatory studies. We measured cytotoxicity with three assays (Alamar Blue, MTT, ToxiLight), plus genotoxicity (comet assay), anti-estrogenic (on ERα, ERß) and anti-androgenic effects (on AR) using gene reporter tests. We also checked androgen to estrogen conversion by aromatase activity and mRNA. All parameters were disrupted at sub-agricultural doses with all formulations within 24h. These effects were more dependent on the formulation than on the glyphosate concentration. First, we observed a human cell endocrine disruption from 0.5 ppm on the androgen receptor in MDA-MB453-kb2 cells for the most active formulation (R400), then from 2 ppm the transcriptional activities on both estrogen receptors were also inhibited on HepG2. Aromatase transcription and activity were disrupted from 10 ppm. Cytotoxic effects started at 10 ppm with Alamar Blue assay (the most sensitive), and DNA damages at 5 ppm. A real cell impact of glyphosate-based herbicides residues in food, feed or in the environment has thus to be considered, and their classifications as carcinogens/mutagens/reprotoxics is discussed.

* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Due to reporting deficiencies (e.g. correlation between concentration used in toxicity tests and concentrations used in comet assay) assessment of results difficult. Exceedingly high doses above the limit dose for this study type. Inappropriate test system for formulations containing surfactant; cytoxic membrane disruption potential of surfactants are well known for in vitro test systems.

Relevance of study:	Not relevant: Excessive doses exceed typical *in vitro* limit doses. *In vitro* test system is inappropriate with surfactants.
Klimisch code:	3

Additional comments:

Response 1 – summarized from Williams *et al.* (2012, ASB2012-12052)
Glyphosate demonstrated no significant anti-estrogenic potential.
Glyphosate demonstrated some anti-androgenic potential at lower concentrations, but not as doses increased and therefore results are considered unrelated to treatment.
Four glyphosate based formulations demonstrated both estrogenic and androgenic activity. Results are confounded due to surfactants within the formulated products tested, which affect cell membrane integrity and produces false findings.

Response 2 – summarized from BfR Review (2009, ASB2012-11565)
Numerous methodological flaws are noted. Test substance(s) not characterized.
Source of materials for cell culture not provided. Dosing concentrations not well described.
Serum free media only appropriate for short term (3-4 hour) *in vitro* exposures. pH control of dilutions not clear.
Osmolality of test solutions not reported.
Electrophoresis parameters insufficiently or inaccurately reported. Numerous reporting deficiencies are noted.
Influence of serum-free cell culturing on endpoints can not be determined
Incomplete data reporting, in cluding β-galactosirase activity, cototoxicity for select assays. Positive control data not reported.
Confusion between maximum residue levels verses systemic concentrations in humans.

Author(s)	Year	Study title
Clair, E., Mesnage, R., Travert, C., Seralini, G.E.	2012	A glyphosate-based herbicide induces necrosis and apoptosis in mature rat testicular cells *in vitro*, and testosterone decrease at lower levels. Toxicology in Vitro Volume: 26, Number: 2, Pages: 269-279 ASB2012-1628

Abstract*
The major herbicide used worldwide, Roundup, is a glyphosate-based pesticide with adjuvants. Glyphosate, its active ingredient in plants and its main metabolite (AMPA) are among the first contaminants of surface waters. Roundup is being used increasingly in particular on genetically modified plants grown for food and feed that contain its residues. Here we tested glyphosate and its formulation on mature rat fresh testicular cells from 1 to 10000 ppm, thus from the range in some human urine and in environment to agricultural levels. We show that from 1 to 48 h of Roundup exposure Leydig cells are damaged. Within 24–48 h this formulation is also toxic on the other cells, mainly by necrosis, by contrast to glyphosate alone which is essentially toxic on Sertoli cells. Later, it also induces apoptosis at higher doses in germ cells and in Sertoli/germ cells co-cultures. At lower non toxic concentrations of Roundup and glyphosate (1 ppm), the main endocrine disruption is a testosterone decrease by 35%. The pesticide has thus an endocrine impact at very low environmental doses, but only a high contamination appears to provoke an acute rat testicular toxicity. This does not anticipate the chronic toxicity which is insufficiently tested and only with glyphosate in regulatory tests.

* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Non-guideline *in vitro* test with methodological (i.e. no positive controls included) and reporting deficiencies (e.g. dose levels not always specified).
Relevance of study:	Not relevant (Due to reliability. In addition, *in vitro* data do not reflect real *in vivo* exposure situations, and therefore not relevant for human risk assessment purposes.)
Klimisch code:	3

Additional comments:
In vitro test with methodological (i.e. no positive controls included) and reporting deficiencies (e.g. dose levels not always specified). The concentrations used in these experiments are not relevant to human exposures to glyphosate and the experimental system used is not relevant to whole animal outcomes. Importantly, the alleged impacts on endocrine function have not been observed in animal studies of glyphosate or other components of glyphosate formulations at relevant concentrations. Authors state that the lowest concentration of glyphosate tested was 50 ppm, several orders of magnitude higher than an anticipated human intake (based on pharmacokinetics described in Anadon *et al.*, 2009, ASB2012-11542) following worst case dietary exposure at the ADI.

Author(s)	Year	Study title
Hokanson, R. Fudge, R. Chowdhary, R. Busbee, D.	2007	Alteration of estrogen-regulated gene expression in human cells induced by the agricultural and horticultural herbicide glyphosate. Human & Experimental Toxicology Volume: 26, Pages: 747-752, ASB2012-11846

Abstract*
Gene expression is altered in mammalian cells (MCF-7 cells), by exposure to a variety of chemicals that mimic steroid hormones or interact with endocrine receptors or their co-factors. Among those populations chronically exposed to these endocrine disruptive chemicals are persons, and their families, who are employed in agriculture or horticulture, or who use agricultural/horticultural chemicals. Among the chemicals most commonly used, both commercially and in the home, is the herbicide glyphosate. Although glyphosate is commonly considered to be relatively non-toxic, we utilized *in vitro* DNA microarray analysis of this chemical to evaluate its capacity to alter the expression of a variety of genes in human cells. We selected a group of genes, determined by DNA microarray analysis to be dysregulated, and used quantitative real-time PCR to corroborate their altered states of expression. We discussed the reported function of those genes, with emphasis on altered physiological states that are capable of initiating adverse health effects that might be anticipated if gene expression were significantly altered in either adults or embryos exposed *in utero*.

* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Not acceptable *in vitro* methods for test mixtures containing surfactant. Well documented study publication, but surfactants are inappropriate test substance in cell lines.
Relevance of study:	Not relevant Temporal altered gene expression is not a biomarker for toxicity, but rather, may be within the range of normal biological responses of homeostasis. *In vitro* cytotoxicity of surfactants, however, is a significant confounder in data interpretation. Data do not reflect real *in*

	vivo exposure situations, and therefore not relevant for human risk assessment purposes.
Klimisch code:	3

Additional comments:
In vitro cytotoxicity of surfactants is a significant confounder in data interpretation. Relevance of altered gene expression in a cell line derived from a breast cancer should not be extrapolated to reflect human health endpoints. Altered gene expression should not be confused with adverse health outcomes. Rather altered gene expression may equally be considered a biological response within the range of normal homeostasis.

In vivo DART/ED publications

Author(s)	Year	Study title
Yousef, M.I., Salem, M.H., Ibrahim, H.Z., Helmi, S., Seehy, M.A., Bertheussen, K.	1995	Toxic Effects of Carbofuran and Glyphosate on Semen Characteristics in Rabbits, Journal of Environmental Science and Health. Part B. Volume: 30, Number: 4, Pages: 513-534 ASB2012-12058

Abstract*
The present study was undertaken to investigate the effect of chronic treatment with two sublethal doses of Carbofuran (carbamate insecticide) and Glyphosate (organophosphorus herbicide) on body weight and semen characteristics in mature male New Zealand white rabbits. Pesticide treatment resulted in a decline in body weight, libido, ejaculate volume, sperm concentration, semen initial fructose and semen osmolality. This was accompanied with increases in the abnormal and dead sperm and semen methylene blue reduction time. The hazardous effect of these pesticides on semen quality continued during the recovery period, and was dose-dependent. These effects on sperm quality may be due to the direct cytotoxic effects of these pesticides on spermatogenesis and/or indirectly via hypothalami-pituitary- testis axis which control the reproductive efficiency.

* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Non-GLP, non-guideline study with major reporting deficiencies. Dose-levels poorly defined as 1/10 and 1/100 LD50. Purity of the test substances, source of animals, environmental conditions, mortality and clinical signs not reported. No testis and epididymis weights were determined or reported and no histopathological examination conducted. In addition, stability and homogeneity assessment of test substance preparations were not done or not reported. Rabbits have low body weights at study start, suggesting impaired health status.
Relevance of study:	Not relevant (Due to low confidence in study conduct and the inadequacy of reporting).
Klimisch code:	3

Additional comments:

Response – summarized from Williams *et al.* (2000, ASB2012-12053)
Numerous serious deficiencies in the design, conduct, and reporting of this study which make the results uninterpretable.
Only four rabbits per treatment group were used, and therefore statistics are questionable. Rabbits appeared to be small for their age; at study start (32 weeks) tested animals had 16 - 25% lower body weight than historical weights for commercially bred animals of the same age and strain.
Low body weights as study start suggest compromised health status of the animals at initiation.
Dose levels were not quantified.
Purity of glyphosate and composition of the glyphosate formulation were not reported. Inadequate description of test material administration.
Improper semen collection technique reported.
Report is unclear whether control animal sham handling was undertaken, a critical factor in stress related outcomes in this species.
Food consumption of test and control groups not adequately reported.
Variability not adequately reported for endpoint measurements in test and control groups, preventing statistical analysis to support the author's conclusions.
Dose-responses not observed, despite the wide dose spread.
Sperm concentrations of all groups within normal ranges for this strain of rabbit. No meaningful conclusions can be drawn from this publication.

Author(s)	Year	Study title
Daruich, J. Zirulnik, F. Gimenez, M. S.	2001	Effect of the herbicide glyphosate on enzymatic activity in pregnant rats and their fetuses Environmental Research Volume: 85 Pages: 226-231 ASB2012-11601

Abstract*
To prevent health risk from environmental chemicals, particularly for progeny, we have studied the effects of the herbicide glyphosate on several enzymes of pregnant rats. Glyphosate is an organo-phosphorated nonselective agrochemical widely used in many countries including Argentina and acts after the sprout in a systemic way. We have studied three cytosolic enzymes: isocitrate dehydrogenase-NADP dependent, glucose-6-phosphate dehydrogenase, and malic dehydrogenase in liver, heart, and brain of pregnant Wistar rats. The treatment was administered during the 21 days of pregnancy, with 1 week as an acclimation period. The results suggest that maternal exposure to agrochemicals during pregnancy induces a variety of functional abnormalities in the specific activity of the enzymes in the studied organs of the pregnant rats and their fetuses.

* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Basic data given, however, the study is performed with methodological and reporting deficiencies (unknown exposure levels, only cytosolic enzymes measured, inappropriate controls, lack of consistent dose-response data).
Relevance of study:	Not relevant (Due to reliability. In addition, study was performed with a glyphosate formulation (commercialised in Argentina) and not with glyphosate).
Klimisch code:	3

Additional comments:

The study was performed with a glyphosate formulation (commercialised in Argentina) and not with glyphosate. Test substance administration is poorly described, but rough calculations on approximate surfactant intake show excessively high and unrealistic exposures when compared to DART systemic parental and reproductive/developmental NOAEL values for POEA formulation surfactants.

Response summarized from Williams *et al.* (2012, ASB2012-12052)
Test substance and doses not adequately described. Inappropriate control groups.
Results suggest that the effect of treatment on body and organ weights may be due to reduced food and water intakes.
A consistent effect of treatment was not observed and dose-response relationships were generally lacking
The information gathered may be misleading because the enzymes monitored are found in both the cytosol and mitochondria.
Food restriction affects the activity of many enzymes, including those examined in this study.

Author(s)	Year	Study title
Romano, R.M. Romano, M.A. Bernardi, M.M. Furtado, P.V. Oliveira, C.A.	2010	Prepubertal exposure to commercial formulation of the herbicide glyphosate alters testosterone levels and testicular morphology. Archives of Toxicology Volume: 84, Pages: 309-317 ASB2012-12012

Abstract*
Glyphosate is a herbicide widely used to kill weeds both in agricultural and non-agricultural landscapes. Its reproductive toxicity is related to the inhibition of a StAR protein and an aromatase enzyme, which causes an in vitro reduction in testosterone and estradiol synthesis. Studies in vivo about this herbicide effects in prepubertal Wistar rats reproductive development were not performed at this moment. Evaluations included the progression of puberty, body development, the hormonal production of testosterone, estradiol and corticosterone, and the morphology of the testis. Results showed that the herbicide (1) significantly changed the progression of puberty in a dose-dependent manner; (2) reduced the testosterone production, in semineferous tubules' morphology, decreased significantly the epithelium height (P < 0.001; control = 85.8 ± 2.8 µm; 5 mg/kg = 71.9 ± 5.3 µm; 50 mg/kg = 69.1 ± 1.7 µm; 250 mg/kg = 65.2 ± 1.3 µm) and increased the luminal diameter (P < 0.01; control = 94.0 ± 5.7 µm; 5 mg/kg = 116.6 ± 6.6 µm; 50 mg/kg = 114.3 ± 3.1 µm; 250 mg kg = 130.3 ± 4.8 µm); (4) no difference in tubular diameter was observed; and (5) relative to the controls, no differences in serum corticosterone or estradiol levels were detected, but the concentrations of testosterone serum were lower in all treated groups (P < 0.001; control = 154.5 ± 12.9 ng/dL; 5 mg/kg = 108.6 ± 19.6 ng/dL; 50 mg/dL = 84.5 ± 12.2 ng/dL; 250 mg/kg = 76.9 ± 14.2 ng/dL). These results suggest that commercial formulation of glyphosate is a potent endocrine disruptor in vivo, causing disturbances in the reproductive development of rats when the exposure was performed during the puberty period.

* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Study with methodological and reporting deficiencies or conflicting findings (e.g., increased relative testicular weights, but decreased testosterone measurements.
Relevance of study:	Relevant study type for investigating male reproductive endpoints, but questionable relevance of this specific study based on low reliability of data and interpretation. Not relevant for glyphosate (test material was a formulated product, not glyphosate).

Klimisch code: 3

Additional comments:
Test material was a formulated product, not glyphosate. The authors failed to measure many of the key parameters in the validated pubertal male assay protocol and hence generated data that were internally inconsistent or incomplete.

Author(s)	Year	Study title
Romano, M.A. Romano, R.M. Santos, L.D. Wisniewski, P. Campos, D.A. de Souza, P.B. Viau, P. Bernardi, M.M. Nunes, M.T. de Oliviera, C.A.	2012	Glyphosate impairs male offspring reproductive development by disrupting gonadotropin expression Archives of Toxicology Volume: 86, Number: 4, Pages: 663-673 ASB2012-12011

Abstract*
Sexual differentiation in the brain takes place from late gestation to the early postnatal days. This is dependent on the conversion of circulating testosterone into estradiol by the enzyme aromatase. The glyphosate was shown to alter aromatase activity and decrease serum testosterone concentrations. Thus, the aim of this study was to investigate the effect of gestational maternal glyphosate exposure (50 mg/kg, NOAEL for reproductive toxicity) on the reproductive development of male offspring. Sixty-day-old male rat offspring were evaluated for sexual behavior and partner preference; serum testosterone concentrations, estradiol, FSH and LH; the mRNA and protein content of LH and FSH; sperm production and the morphology of the seminiferous epithelium; and the weight of the testes, epididymis and seminal vesicles. The growth, the weight and age at puberty of the animals were also recorded to evaluate the effect of the treatment. The most important findings were increases in sexual partner preference scores and the latency time to the first mount; testosterone and estradiol serum concentrations; the mRNA expression and protein content in the pituitary gland and the serum concentration of LH; sperm production and reserves; and the height of the germinal epithelium of seminiferous tubules. We also observed an early onset of puberty but no effect on the body growth in these animals. These results suggest that maternal exposure to glyphosate disturbed the masculinization process and promoted behavioral changes and histological and endocrine problems in reproductive parameters. These changes associated with the hypersecretion of androgens increased gonadal activity and sperm production.

[k] Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Non-guideline, non-GLP study meeting scientific principles. Unusual and short dosing regiment commencing towards the end of pregnancy (GD18, rather than GD6 as per OECD Test Guidelines 414) through post natal day 5. *In vivo* study with reporting deficiencies (detailed strain description, source of animals, housing conditions, no information if clinical signs were assessed, stability and homogeneity assessment of test substance preparations, no of male offspring evaluated in individual tests evaluated). A number of atypical endpoints evaluated.
Relevance of study:	Not relevant (due to questionable dosing regimen and atypical

array of endpoints measured).

Klimisch code: 3

Additional comments:
Study with some reporting deficiencies (detailed strain description, source of animals, housing conditions, no information if clinical signs were assessed, stability and homogeneity assessment of test substance preparations, no of male offspring evaluated in individual tests evaluated). Dosing was limited to dams, starting on gestation day 18, well after organogenisis, through post natal day 5. No controls for litter effects appear to be reported, confounding interpretation of results. With the very short window of maternal exposure, biological plausibility of any test substance related effects in the mature offspring is questionable. However, the normal variability of some unusual or atypical endpoint measurements, such as "sexual partner preference" along with mRNA and protein expression, is not known. Of particular concern, however, are differences in critical endpoints for control animals reported in Romano *et al.* (2010, ASB2012-12012) compared to Romano *et al.* (2012, ASB2012- 12011); these include increased day of preputional sepratarion (PPS) of control male rate (37 days in 2010; 47 days in 2012), body weight at day of PPS (146 grams in 2010; 245 grams in 2012), serum testosterone concentrations (155 ng/dL in 2010; 63 ng/dL in 2012), estradiol concentrations (32 pg/mL in 2010; 1.4 pg/mL in 2012), subular diameter (266 μm in 2010; 479 μm in 2012), epithelial height (86 μm in 2010; 92 μmin 2012) and luminal height (94 μm in 2010; 257μm in 2012). Therefore, results are difficult to interpret, particularly for relevance to human health risk assessment.

A letter to the editor by DeSesso and Williams, (2012, ASB2014-9369) concluded as follows: "Taken together, the shortcomings in this paper erode any confidence that these experiments are able to demonstrate disruption in the development or function of the male reproductive system in offspring whose dame were treated with glyphosate". Romano and Romano (2012, ASB2014-9396) rebutted these comments and conclusions.

Epidemiology DART/ED Publications

Author(s)	Year	Study title
Arbuckle, T. E. Lin, Z. Mery, L. S.	2001	An exploratory analysis of the effect of pesticide exposure on the risk of spontaneous abortion in an Ontario farm population Environmental Health Perspectives Volume: 109 Pages: 851-857 ASB2012-11545

Abstract*
The toxicity of pesticides on human reproduction is largely unknown—particularly how mixtures of pesticide products might affect fetal toxicity. The Ontario Farm Family Health Study collected data by questionnaire on the identity and timing of pesticide use on the farm, lifestyle factors, and a complete reproductive history from the farm operator and eligible couples living on the farm. A total of 2,110 women provided information on 3,936 pregnancies, including 395 spontaneous abortions. To explore critical windows of exposure and target sites for toxicity, we examined exposures separately for preconception (3 months before and up to month of conception) and postconception (first trimester) windows and for early (< 12 weeks) and late (12–19 weeks) spontaneous abortions. We observed moderate increases in risk of early abortions for preconception exposures to phenoxy acetic acid herbicides [odds ratio (OR) = 1.5; 95% confidence interval (CI), 1.1–2.1], triazines (OR = 1.4; 95% CI, 1.0–2.0), and any herbicide (OR = 1.4; 95% CI, 1.1–1.9). For late abortions, preconception exposure to glyphosate (OR = 1.7; 95% CI, 1.0–2.9), thiocarbamates (OR = 1.8; 95% CI, 1.1–3.0), and the miscellaneous class of pesticides (OR = 1.5; 95% CI, 1.0–2.4) was associated with elevated risks. Postconception exposures were generally associated with late spontaneous abortions. Older maternal age (> 34 years of age) was the strongest risk factor for spontaneous abortions, and we observed several interactions between pesticides in the older age group using Classification and Regression Tree analysis. This study shows that timing of exposure and restricting

analyses to more homogeneous endpoints are important in characterizing the reproductive toxicity of pesticides.

* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	No information about exposure duration, used glyphosate products and application rates. No information if the subjects used more than one pesticide.
Relevance of study:	Not relevant (Study design is not suitable for assessment of glyphosate exposure).
Klimisch code:	3

Additional comments:
Pre-conception glyphosate exposure odds ratio for spontaneous abortion is considered of borderline significance (OR = 1.4). Post-conception glyphosate exposure was not associated with spontantous abortin (OR = 1.1). Authors note multiple limitations of the study relating to exposure, likely misclassification of pesticides and correct assignment of exposure window to pre- of/and post-conception OFFHS information gathering methodology has high potential recall bias. Blair and Zahm (1993, ASB2012-11567) report 60% accuracy when comparing self reported pesticide usage with purchasing records.
OFFHS relied exclusively on maternal self-reports of adverse pregnancy outcomes, not all of which were confirmed via medical or other records.
Three highly relevant confounding factors were not considered in the OFFHS questionnaire: history of previous spontaneous abortion(s), maternal age and smoking.

Response summarized from Williams *et al.* (2012, ASB2012-12052)
395 spontaneous abortions were reported out of 3936 pregnancies; rate of spontaneous aborting in Arbuckle *et al.* (2001, ASB2012-11545) was 10%.
The baseline rate of spontaneous abortions in the general populations is much higher, ranging from 12% to 25%.
Recall bias is reflected in the recall of spontaneous abortion over the previous 5 years (64% of all spontaneous abortions reported) being much higher than the recall of those greater than 10 years prior to the survey (34% of all spontaneous abortions reported).
Substantial exposure misclassification may have occurred (pre- versus post-conception) due to likely author extrapolation of exposure data.
Strong confounding variables are not apparent in previous data analyses published by the authors of the OFFHS, and therefore odds ratios are crude.
Published results fail to demonstrate a significant association of glyphosate exposure spontaneous abortion risk and therefore must be considered cautiously.

Author(s)	Year	Study title
Savitz, D.A. Arbuckle, T. Kaczor, D. Curtis, K.M.	1997	Male pesticide exposure and pregnancy outcome. American Journal of Epidemiology Volume: 146, Number: 12, Pages: 1025-1036 ASB2012-12022

Abstract*
Potential health effects of agricultural pesticide use include reproductive outcomes. For the Ontario Farm Family Health Study, the authors sampled Ontario farms from the 1986 Canadian Census of Agriculture, identified farm couples, and obtained questionnaire data concerning farm activities, reproductive health experience, and chemical applications. Male farm activities in the period from 3 months before conception through the month of conception were evaluated in relation to miscarriage, preterm delivery, and small-

for- gestational-age births. Among the 1,898 couples with complete data (64% response), 3,984 eligible pregnancies were identified. Miscarriage was not associated with chemical activities overall but was increased in combination with reported use of thiocarbamates, carbaryl, and unclassified pesticides on the farm. Preterm delivery was also not strongly associated with farm chemical activities overall, except for mixing or applying yard herbicides (odds ratio = 2.1, 95% confidence interval 1.0-4.4), Combinations of activities with a variety of chemicals (atrazine, glyphosate, organophosphates, 4-[2,4-dichlorophenoxy] butyric acid, and insecticides) generated odds ratios of two or greater. No associations were found between farm chemicals and small-for-gestational-age births or altered sex ratio. Based on these data, despite limitations in exposure assessment, the authors encourage continued evaluation of male exposures, particularly in relation to miscarriage and preterm delivery.

[k] Quoted from article

Klimisch evaluation

Reliability of study:	Not Reliable
Comment:	No information about exposure duration, used glyphosate products and application rates. No information, if the subjects used more than one pesticide. Due to study design and evaluation methods, study results are not reliable.
Relevance of study:	Not Relevant (Study design is not suitable for assessment of glyphosate exposure).
Klimisch code:	3

Additional comments:
Glyphosate is one of many pesticides mentioned in three epidemiological reports that examine possible links between on-farm pesticide use and reproductive outcomes. All three reports - Savitz *et al.* (1997, ASB2012-12022), Curtis *et al.* (1999, cited in ASB2012-11545) and Arbuckle *et al.* (2001, ASB2012-11545) - use data from the Ontario Farm Family Health Study (OFFHS) (Arbuckle 1994, cited in ASB2012-11545). Savitz *et al.* (1997, ASB2012- 12022) investigated associations between reported pesticide use by males and pregnancy outcomes, specifically: miscarriage, pre-term delivery and small-for-gestational-age birth. Curtis *et al.* (1999, cited in ASB2012-11545) studied whether reported pesticide use by males or females was associated with delayed pregnancy, while Arbuckle *et al.* (2001, ASB2012- 11545) looked for associations between reported pesticide use and spontaneous abortion.

In the study by Savitz *et al.* (1997, ASB2012-12022), a number of specific pesticides had weak statistical associations with miscarriages and pre-term deliveries, but pesticides tended not to be associated with small for gestational age births. There were no statistically significant findings for glyphosate. In the study by Curtis *et al.* (1999, cited in ASB2012- 11545), for farms on which glyphosate was used, there was no significant association for women being engaged in pesticide activities. For men, glyphosate use was associated with a slight, but statistically significant, decrease in time to pregnancy. The authors dismissed this finding, which was contrary to their hypothesis that pesticide exposure delayed pregnancy, as probably due to uncontrolled factors or chance. Arbuckle *et al.* (2001, ASB2012-11545) found that reported preconception use of phenoxyacetic acids, triazines, glyphosate, and thiocarbamates were weakly, but statistically significantly, associated with spontaneous abortions. Post conception reported use was not associated with increased risk. The authors characterized the associations between pesticides and spontaneous abortions as "hypothesis generating" pending confirmation from other epidemiologic studies. These studies are not convincing evidence of a relationship between glyphosate exposure and adverse pregnancy outcomes for a number of reasons:

There was no actual exposure data per se in these three epidemiologic studies. Exposures were assumed based on questionnaire responses by study subjects about farm activities and pesticide use. This type of information can be inaccurate. For example, according to a study by the National Cancer Institute, self-reports of pesticide usage were found to be only 60 percent accurate when compared with purchasing

records (Blair & Zahm 1993, ASB2012- 11567). Further increasing the potential for inaccuracy is the fact that study subjects were only asked about pesticide use for the 5 years before the OFFS survey. These responses were assumed to be applicable to the entire farming careers of study subjects, an assumption inconsistent with changes in agricultural practice. Lastly, basing exposure estimation on questionnaire responses has the potential to be influenced by what epidemiologists call "recall bias." This refers to the likelihood that families that experienced an adverse reproductive outcome are more likely to remember use of certain pesticides than families that had only normal births.
The most widely used pesticides, like atrazine, glyphosate, and 2,4-D, are most easily recalled and most likely to be over-reported.
The OFFHS study relied exclusively on maternal self-reports of adverse pregnancy outcomes with no medical or other validation. Generally, scientists place less confidence in reports of health outcomes that are not validated with medical records.
A confounding factor is a cause of a disease that is correlated with another exposure being studied. Failure to control confounding factors, especially those that are strong causes of a disease, can create spurious associations between benign exposures and diseases. In the Arbuckle study, there were at least three important potential confounding factors that were not controlled: history of previous spontaneous abortion, maternal age, and smoking. Even a weak correlation between these factors and use (or recall of use) of pesticides would produce spurious associations. In addition, in all three studies, the authors did not control the putative effect of one pesticide for the putative effects of other pesticides. So, for example, since farmers tend to use 4 or more pesticides each year, a disease that is associated with one pesticide will likely be associated with all, since their use patterns are correlated. In the absence of an analysis that controls for multiple pesticides, the best that can be said is that the findings for any individual pesticide might be due to its correlation with another pesticide.

In summary, three publications based on data collected in the OFFHS found associations between several pesticides and various adverse reproductive outcomes. There was no actual exposure data per se in these three epidemiologic studies. Exposures were assumed based on questionnaire responses by study subjects about farm activities and pesticide use. This type of information can be inaccurate. Glyphosate was not significantly associated with adverse reproductive outcomes in two of these studies (Savitz *et al.* 1997, ASB2012-12022, Curtis *et al.* 1999, cited in ASB2012-11545). Glyphosate and other pesticides were weakly associated with spontaneous abortion in the study by Arbuckle (2001, ASB2012-11545). However, the author did not control for important personal confounding factors or for multiple exposures and no actual exposure data was used, casting doubt on the validity of the findings in this study.
Biomonitoring data for glyphosate collected as part of the Farm Family Exposure Study (FFES), provide assurance that human health effects related to glyphosate exposure are very unlikely. In the FFES, researchers from the University of Minnesota collected 5 days of urine samples from 48 farm families before, during, and after a glyphosate application (Mandel *et al.,* 2005, ASB2012-11893, accepted for publication). Only 60% of farmers showed detectable exposure to glyphosate, with a 1 part per billion limit of detection, and the maximum estimated absorbed dose was 0.004 *mg/kg* (Acquavella *et al.,* 2004, ASB2012-11528). For farmers who apply glyphosate 10 times per year for 40 years, this maximum dose is more than 30,000-fold less than the EPA reference dose1 of 2 mg/kg/day. For spouses, only 4% showed detectable exposures and the maximum systemic dose was 0.00004 mg/kg/day. Since glyphosate is not a reproductive toxic in high dose animal studies and since actual exposures on farms are so low, it is very unlikely that glyphosate would cause adverse reproductive outcomes for farmers or their spouses.

Author(s)	Year	Study title
Garry, V. F. Harkins, M. E. Erickson, L. L. Long-Simpson, L. K. Holland, S. E. Burroughs, B. L.	2002	Birth defects, season of conception, and sex of children born to pesticide applicators living in the Red River Valley of Minnesota, USA. Environmental Health Perspectives Volume: 110 Pages: 441-449 ASB2012-11626

Abstract*
We previously demonstrated that the frequency of birth defects among children of residents of the Red River Valley (RRV), Minnesota, USA, was significantly higher than in other major agricultural regions of the state during the years 1989-1991, with children born to male pesticide applicators having the highest risk. The present, smaller cross-sectional study of 695 families and 1,532 children, conducted during 1997-1998, provides a more detailed examination of reproductive health outcomes in farm families ascertained from parent- reported birth defects. In the present study, in the first year of life, the birth defect rate was 31.3 births per 1,000, with 83% of the total reported birth defects confirmed by medical records. Inclusion of children identified with birth or developmental disorders within the first 3 years of life and later led to a rate of 47.0 per 1,000 (72 children from 1,532 live births). Conceptions in spring resulted in significantly more children with birth defects than found in any other season (7.6 vs. 3.7%). Twelve families had more than one child with a birth defect (n = 28 children). Forty-two percent of the children from families with recurrent birth defects were conceived in spring, a significantly higher rate than that for any other season. Three families in the kinships defined contributed a first-degree relative other than a sibling with the same or similar birth defect, consistent with a Mendelian inheritance pattern. The remaining nine families did not follow a Mendelian inheritance pattern. The sex ratio of children with birth defects born to applicator families shows a male predominance (1.75 to 1) across specific pesticide class use and exposure categories exclusive of fungicides. In the fungicide exposure category, normal female births significantly exceed male births (1.25 to 1). Similarly, the proportion of male to female children with birth defects is significantly lower (0.57 to 1; p = 0.02). Adverse neurologic and neurobehavioral developmental effects clustered among the children born to applicators of the fumigant phosphine (odds ratio [OR] = 2.48; confidence interval [CI], 1.2-5. 1). Use of the herbicide glyphosate yielded an OR of 3.6 (CI, 1.3-9.6) in the neurobehavioral category. Finally, these studies point out that a) herbicides applied in the spring may be a factor in the birth defects observed and b) fungicides can be a significant factor in the determination of sex of the children of the families of the RRV. Thus, two distinct classes of pesticides seem to have adverse effects on different reproductive outcomes. Biologically based confirmatory studies are needed.

[k] Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Epidemiological study with some methodological / reporting deficiencies (selection of study subjects, no information about exposure duration, exposure concentration, pesticide use frequency).
Relevance of study:	Not relevant because of methodological deficiencies.
Klimisch code:	3

Additional comments:

Response 1 – summary from Mink *et al.* (2011) (ASB2012-11904)
Publication reports on different classes of pesticides and several birth defects and developmental outcomes. Paternal use of glyphosate was associated with parent-reported ADD/ADHD in children (OR = 3.6). Six out of 14 children with parent reported ADD/ADHD also reported exposure to glyphosate.
Diagnoses of ADD/AHDH were not all confirmed. However, overall rate for the sample population (14/1532) was well below ADD/ADHD rates for the general population (7%). Variables in statistical model analyses were not reported.

Response 2 – summary from Williams *et al.* (2012, ASB2012-12052)
Health data obtained via parent reporting for 695 families via written questionnaire and confirmed where possible.
Pesticide use information obtained initially via telephone then followed up by written questionnaire.
Reproductive health outcomes for births occurring between 1968 and 1998 were obtained for 1532 live

births. Over half the births occurred prior to 1978, approximately 20 years after study initiation.
All pesticide use classes (herbicide only; herbicide and insecticide; herbicide, insecticide and fungicide; herbicide, insecticide and fumigant) were associated with birth defects.
Authors state neurobehavioral disorder would not be considered based lack consistent diagnoses. However, a detailed analysis was conducted for ADD/ADHD.
43% (6/14) parent reported children with ADD/ADHD were associated with glyphosate formulation use.
14 cases of ADD/ADHD reported out of 1532 live births, which is substantially lower that the diagnosed incidence of 7% for the general population.
No conclusions regarding glyphosate exposure and ADD/ADHD outcome can be drawn. No other glyphosate specific data were reported.

Author(s)	Year	Study title
Garry, V.F., Holland, S.E., Erickson, L.L., Burroughs, B.L.	2003	Male Reproductive Hormones and Thyroid Function in Pesticide Applicators in the Red River Valley of Minnesota Journal of Toxicology and Environmental Health, Part A Volume: 66, Number: 11, Pages: 965-986 ASB2012-11627

Abstract*
In the present effort, 144 pesticide applicators and 49 urban control subjects who reported no chronic disease were studied. Applicators provided records of the season's pesticides used by product, volumes, dates, and methods of application. Blood specimens for examination of hormone levels were obtained in summer and fall. In the herbicide-only applicator group, significant increases in testosterone levels in fall compared to summer and also elevated levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in the fall were noted. With respect to fungicide use, in an earlier cross-sectional epidemiologic study, data demonstrated that historic fungicide use was associated with a significant alteration of the sex ratio of children borne to applicators. As before, among current study subjects it was noted that historic fungicide use was associated with increased numbers of girls being born. Lower mean total testosterone concentrations by quartile were also correlated with increased numbers of live-born female infants. A downward summer to fall seasonal shift in thyroid-stimulating hormone (TSH) concentrations occurred among applicators but not among controls. Farmers who had aerial application of fungicides to their land in the current season showed a significant shift in TSH values (from 1.75 to 1.11 mU/L). Subclinical hypothyroidism was noted in 5/144 applicators (TSH values >4.5 mU/L), but not in urban control subjects. Based on current and past studies, it was concluded that, in addition to pesticide exposure, individual susceptibility and perhaps economic factors may play a supporting role in the reported results.

* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Epidemiological study with some methodological / reporting deficiencies (e.g. selection of control subjects/samples, no details of exposure). Documentation is insufficient for assessment.
Relevance of study:	Not relevant for glyphosate (due to reliability; in addition, no direct assessment of glyphosate exposure was made).
Klimisch code:	3

Additional comments:
The publication brings little information on endpoints attributable to glyphosate.
Given the subjects were pesticide applicators, little can be drawn from the findings other than perhaps

certain endpoints which may be associated with this specific occupation exposed to multiple chemical substances. Of the 136 participants volunteering blood samples, only one individual (subject D) was noted with one abnormally high thyroid hormone levels associated with glyphosate use; thyroid stimulating hormone (FSH) was about double the normal range in the fall and thyroid stimulating hormone (TSH) higher than normal in the summer.
Another individual (subject E) had abnormally high TSH levels associated with multiple pesticide usage of 12 different active ingredients.

Author(s)	Year	Study title
Bell, E.M. Hertz-Picciotto, I. Beaumont, J.J.	2001	A Case-Control Study of Pesticides and Fetal Death Due to Congenital Anomalies Epidemiology Volume: 12, Number: 2, Pages: 148-156 ASB2012-11559

Abstract*
We examined the association between late fetal death due to congenital anomalies (73 cases, 611 controls) and maternal residential proximity to pesticide applications in ten California counties. A statewide database of all applications of restricted pesticides was linked to maternal address to determine daily exposure status. We examined five pesticide chemical classes. The odds ratios from logistic regression models, adjusted for maternal age and county, showed a consistent pattern with respect to timing of exposure; the largest risks for fetal death due to congenital anomalies were from pesticide exposure during the 3rd – 8th weeks of pregnancy. For exposure either in the square mile of the maternal residence or in one of the adjacent 8 square miles, odds ratios ranged from 1.4 (95% confidence interval = 0.8 – 2.4) for phosphates, carbamates, and endocrine disruptors to 2.2 (95% confidence interval = 1.3 – 3.9) for halogenated hydrocarbons. Similar odds ratios were observed when a more restrictive definition of nonexposure (not exposed to any of the five pesticide classes during the 3rd– 8th weeks of pregnancy) was used. The odds ratios for all pesticide classes increased when exposure occurred within the same square mile of maternal residence.

* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Epidemoliogical study with methodological deficiencies (e.g. glyphosate was included in the pesticide class of phosphates, thiophosphates, phosphonates; no differentiation between single and multiple exposures).
Relevance of study:	Not relevant (No glyphosate-specific results.)
Klimisch code:	3

Additional comments:

Response – summary from Williams *et al.* (2012, ASB2012-12052)
Classes of pesticides were evaluated in this study, with glyphosate included as one of 47 active ingredients in the broad category of "phosphates/thiophosphates/phosphonates".
Of the 47 active ingredients, many were organophosphate insecticide with known mammalian modes of action. The glyphosate mode of action is on the EPSPS enzyme in plants, which is not present in the animal kingdom.
Given the very low volatility of glyphosate and the low potential for inhalation exposures to aerosol sprays up to two miles away from the subjects, systemic doses to glyphosate would be considered negligible.
Mose *et al.*, (2008, ASB2012-11914) demonstrated a low perfusion rate of glyphosate across the placenta. Coupled with the known low dermal and gastrointestinal absorption of glyphosate and the rapid elimination of systemic doses of glyphosate in the urine, human *in utero* exposures would be extremely limited.

The reported congenital anomalies associated with fetal death in Bell *et al.* (2001, ASB2012- 11559) can in no way be linked to glyphosate exposure.

Author(s)	Year	Study title
Aris, A. Leblanc, S.	2011	Maternal and fetal exposure to pesticides associated to genetically modified foods in Eastern Townships of Quebec, Canada. Reproductive toxicology Volume: 31, Pages: 528-533 ASB2012-11547

Abstract*
Pesticides associated to genetically modified foods (PAGMF), are engineered to tolerate herbicides such as glyphosate (GLYP) and gluphosinate (GLUF) or insecticides such as the bacterial toxin bacillus thuringiensis (Bt). The aim of this study was to evaluate the correlation between maternal and fetal exposure, and to determine exposure levels of GLYP and its metabolite aminomethyl phosphoric acid (AMPA), GLUF and its metabolite 3- methylphosphinicopropionic acid (3-MPPA) and Cry1Ab protein (a Bt toxin) in Eastern Townships of Quebec, Canada. Blood of thirty pregnant women (PW) and thirty-nine nonpregnant women (NPW) were studied. Serum GLYP and GLUF were detected in NPW and not detected in PW. Serum 3-MPPA and CryAb1 toxin were detected in PW, their fetuses and NPW. This is the first study to reveal the presence of circulating PAGMF in women with and without pregnancy, paving the way for a new field in reproductive toxicology including nutrition and utero-placental toxicities.

* Quoted from article

Klimisch evaluation

Reliability of study: Not reliable

Comment: Exact levels of PAGMF, glyphosate or AMPA in the diets were not determined. It is not clear if the measured concentrations could have been resulted from other exposure routes.

Relevance of study: Relevant with restrictions (Provides real life actual exposure concentrations in humans. Data are limited due to the absence of any information on applied pesticides, application rates, etc.).

Klimisch code: 3

Author(s)	Year	Study title
Benítez-Leite, S. Macchi, ML and Acosta, M.	2009	Malformaciones congénitas asociadas a agrotóxicos. Arch Pediatr Urug Volume: 80, Number: 3, Pages: 237-247 ASB2012-11563

Abstract*
Introduction: exposure to pesticides is a known risk for human health. This paper describes the relationship between parental exposure and congenital malformations in the newborn. Objective: to study the association between exposure to pesticides and congenital malformations in neonates born in the Regional Hospital of Encarnación, in the Department of Itapúa, Paraguay. Materials and methods: a prospective case-controlled study carried out from March 2006 to February 2007. Cases included all newborns with congenital malformations, and controls were all healthy children of the same sex born immediately thereafter. Births outside the hospital were not counted. Exposure was considered to be any contact with agricultural chemicals, in addition to other known risk factors for congenital defects. Results: a total of 52 cases and 87 controls were analyzed. The average number of births each month was 216. The significantly associated risk factors were: living near treated fields (OR 2,46, CI95% 1,09-5,57, p<0,02), dwelling

located less than 1 km (OR 2,66, CI95% 1,19-5,97, p<0,008), storage of pesticides in the home (OR 15,35, CI95% 1,96-701,63), p<0,003), direct or accidental contact with pesticides (OR 3,19, CI95% 0,97-11,4, p<0,04), and family history of malformation (OR 6,81, CI95% 1,94-30,56, p<0,001). Other known risk factors for malformations did not show statistical significance. Conclusion: the results show an association between exposure to pesticides and congenital malformations. Further studies are required to confirm these findings.

[k] Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Study design of epidemiological study for developmental toxicity insufficient for assessment, as well as methological and reporting deficiencies (no assessment to which pesticides / active substances the mothers were exposed, use frequency not specified, selection of control group after study period is questionable, no information on exposure situation of mother for this control group assessed, etc.).
Relevance of study:	Not relevant (The exposure to several pesticides was assessed in general, but no pesticide or active substance, including glyphosate, was specified or assessed).
Klimisch code:	3

B.6.5 Delayed neurotoxicity (Annex IIA 5.7.2)

B.6.5.1 Published data on neurotoxicity

Several publications over the last decade have evaluated glyphosate with respect to neurotoxicity endpoints. Three papers report a total of two human cases of Parkinson's disease. The first case followed acute exposure to a glyphosate formulation while spraying a garden (Barbosa *et al.*, 2001, ASB2012-11557; da Costa et al, 2003, ASB2012-11598). The second case reported chronic exposures to a factory worker in China, where a variety of pesticides including glyphosate were produced (Wang et al, 2011, ASB2012-12047). Several questions arise in attempting to link glyphosate exposures with each case of Parkinson's disease. Firstly, significant systemic exposures to glyphosate in each instance are questionable, given the poor dermal absorption and low volatility of the compound. Secondly, if glyphosate was a causative agent of this fairly common disease, a significant number of cases associated with either acute and/or chronic exposures would be evident. Glyphosate formulations are sometimes readily accessible for suicide attempts, which are usually unsuccessful, as less than 10% of glyphosate self administered ingestions result in death. No reports of Parkinson's disease in survivors following very acute ingestions of glyphosate products have been documented. Glyphosate has been manufactured and widely used in agriculture and consumer markets for approximately 40 years, so a single case of a pesticide factory worker developing Parkinson's disease, while unfortunate, does not constitute cause and effect; there is no evidence of a higher frequency of Parkinson's disease in glyphosate production workers.

Multiple long term animal studies with glyphosate have failed to demonstrate any evidence of neurotoxicity, and certainly have not shown evidence of Parkinson's-like abnormalities. While some studies have suggested statistical associations with general pesticide exposure or general insecticide or herbicide exposure (Engel *et al.*, 2001, ASB2012-11612), there is no evidence suggesting a specific association between glyphosate and Parkinson's disease. In the largest study to date of US Farmers (Agricultural Health Study), no increased risk of Parkinson's disease was found in association with reported glyphosate use (Kamel *et al.*, 2007, ASB2012-11862). Human non-cancer epidemiologic outcomes related to glyphosate have recently been reviewed (Mink *et al.* 2011, ASB2012-11904), and there is no convincing evidence for an increased incidence of Parkinson's disease or other neurological disorders in individuals reporting glyphosate exposure.

Several publications open with the premise that pesticide exposures are linked with Parkinson's disease, and then proceed to report *a priori* research linking glyphosate with a measurable endpoint. This endpoint is then extrapolated to link with Parkinson's disease in humans. Despite the lack of compelling human associations between glyphosate exposure and Parkinson's disease, such research continues to be published. Astiz *et al.*, (2009, ASB2012-11549), Negga *et al.* (2011, ASB2012-11923) and Gui *et al.* (2012, ASB2012-11835) all conducted glyphosate research in the above mentioned manner, all in very different test systems. Negga *et al.* (2011, ASB2012-11923) notes neurodegeneration in *Caenorhabditis elegans* worms following exposure to glyphosate (trimesium form, which has a different toxicology profile than glyphosate) uses concentrations equal to the LD25, LD50 and LD75, or actual concentrations of glyphosate of 3 to 10 percent, i.e.- the high concentration is approximately 10-fold higher than concentrations applied directly in the field. The relevance of such high-dose exposures to the trimesium salt in this experimental model to human Parkinson's disease is highly questionable and irrelevant to the Annex 1 renewal of glyphosate technical acid. Astiz *et al.* (2009, ASB2012-11549) and Gui *et al.* (2012, ASB2012-11835) both affirm their test models (in rats and in PC-12 cells respectively) for evaluating neurodegenerative disorders, then directly link their research results to Parkinson's disease in humans; these two studies are addressed below.

Cole *et al.* (2004, ASB2012-11594) evaluated 15 different pesticides for neurotoxic endpoints in *C. elegans* with analytical grade active ingredients, noting reduced cholinesterase for pesticides with this mode of action, but not glyphosate. Interestingly, the authors report a low pH effect resulting in reduced cholinesterase activity in the high dose of glyphosate and a plant growth promoter. Glyphosate formulations contain salt forms of glyphosate, not the technical acid and thus do not have a low pH. Additionally, human incidents of self induced glyphosate poisonings do not report the common symptoms of acute acetylcholinesterase inhibition; salivation, lacrimation, urination and defecation (SLUD).

After preparation of the original DAR in 2013, the following publications became available:

Cattani *et al.* (2014, ASB2014-3919) studied neurotoxic effects of the formulation Roundup in the hippocampus on immature rats following acute (30 min) and chronic (pregnancy and lactation) exposure. Results showed that acute exposure to Roundup increased CA2+ influx leading to oxidative stress and neural cell death. Taken together, the results demonstrate that Roundup might lead to excessive extracellular glutamate levels and to glutamate excitotoxicity and oxidative stress in rat hippocampus.

Chorfa *et al.* (2013, ASB2014-9328) studied the effects of four pesticides (paraquat, rotenone, maneb and glyphosate) on different molecular events in cell lines which are considered to be related to Parkinson`s disease. Three of the four pesticides triggered molecular events involved in Parkinson`s disease. However, glyphosate was not active in this study.

Seneff *et al.* (2013, ASB2014-9729) reviewed literature on autism. In conclusion they submit a theory on the biochemical mechanisms which could lead to autism. According to this theory, several environmental factors including pesticide exposure (glyphosate and other pesticides

are mentioned, together with, e.g., aluminium, mercury, intake of 'processed food', vaccinations) would promote the 'encephalopathy of autism'. The contribution of glyphosate is ascribed to a disturbance of gut microflora rather than to a direct effect on the neuronal system. Thus, there assumptions are based on certain in vitro results (e.g., Shehata *et al.*, 2013, ASB2012-16301) that are discussed in depth in section B.6.8.3.3. The authors propose dietary and lifestyle changes to prevent autism.

Narayan *et al.* (2013, ASB2014-9620) reviewed literature on Parkinson`s disease. The authors conclude that houshold use of organophosphorus pesticides is associated with increased risk of developing Parkinson`s disease. Glyphosate is considered by these authors to be an organophosphorus pesticide.

McConnell *et al.* (2012, ASB2014-9615) tested multi-well microelectrode arrays for neurotoxicity screening. In result of these tests glyphosate was considered negative concerning neurotoxic effects.

LeFew *et al.* (2013, ASB2014-9608) evaluated microelectrode array data using Bayesian modeling as an approach to screening and prioritization for neurotoxicity testing. Glyphosate was identified to be negative in these neurotoxicity tests.

Kim *et al.* (2013, ASB2014-9592) studied the relation between depressive symptoms and severity of acute occupational pesticide poisoning among male farmers in South Korea. Among the pesticides causing the poisonings, paraquat dichloride was found to be a significant predictor of depressive symptoms. Glyphosate did not cause significant effects.

Kamel *et al.* (2012, ASB2014-9586) summarized the literature on the association of amyotrophic lateral sclerosis (ALS) with pesticides. The meta-analysis suggest that ALS risk is associated with use of pesticides. ALS was associated with aldrin, dieldrin, DDT and toxaphene. However, no relevant association was evidenced for glyphosate.

Freire and Koifmann (2012, ASB2014-9479) conducted a review of the epidemologic literature over the past decade. A significantly increased Parkinson`s disease risk was observed in 13 out of 23 case-control studies. An increased risk has been associated with different pesticides. However, no relevant increase of the risk was evidenced for glyphosate.

Faria *et al.* (2014, ASB2014-9477) analysed the association between occupational exposures to pesticides, nicotine and minor psychiatric disorders (MPD) among tobacco farmers in southern Brazil. The study reinforces the evidence of the association between pesticide poisoning and mental health disorders. In this study organophosphates were the only chemical group positively asscíated with MPD. Glyphosate was not associated with MPD.

The study by Harrill *et al.* (2011, ASB2014-9558) compared the performance of two culture models, a rat primary cortical culture and a human embryonic stem cell-derived neural culture to be used as tools for screening potential developmental neurotoxicants. The authors concluded that based upon the small training set evaluated in their study, neither of the culture models performed better than the other across the determined criteria: the data demonstrated that the culture models performed differently in terms of reproducibility, dynamic range and sensitivity to neurite outgrowth inhibitors. In this study glyphosate was used as one out of six 'negative' chemicals and none of them inhibited neurite outgrowth in either model.

The study by Culbret *et al.* (2012, ASB2014-9355) compared the sensitivity of human (ReN CX) and mouse (mCNS) neuroprogenitor cell lines to chemicals using a multiplex assay for proliferation and apoptosis, endpoints critical for neural development. According to the authors, eleven chemicals reported to affect proliferation and/or apoptosis, and 5 chemicals, amongst others glyphosate, with no reports of effects on either endpoint were examined in concentrations of 0.001 up to 100 µM. High-content screening of markers for proliferation (BrdU incorporation) and apoptosis (activated caspase 3 and p53) was used to assess the effect of chemicals in both cell lines. Under the conditions described, the hypothesis that human cells are significantly more sensitive than rodent cells to chemical insult on proliferation and apoptosis was not supported by these results. Interestingly, the 'negative' chemical, glyphosate (technical material, purity > 99 %), reached the threshold for p53 activation in mCNS at 30 µM, but not in the human cell line.

The review by Grandjean and Landrigan (2014, ASB2014-9494) on neurobehavioural effects of developmental toxicity emphasise that the total number of neurotoxic substances now recognised almost certainly represents an underestimate of the true number of developmental neurotoxicants that have been released into the global environment. In this context, the authors considered glyphosate as a human toxicant based on a case report of a 71-year old male who attempted suicide with a glyphosate formulation published by Malhotra *et al.* (2010, ASB2012-11890, please refer to B.6.9.2. Reports on clinical cases and poisoning

incidents). This interpretation was disagreed by Goldstein and Saltmiras (2014, ASB2014-9493) and Malhotra *et al.* were cited, that this case raises "a suspicion of direct cerebral toxicity", but no conclusion was drawn on glyphosate to be a recognised neurotoxicant, but inquiry into other components of the ingested product was considered to be indicated.

Further studies are reported more detailed:

Author(s)	Year	Study title
Barbosa, E.R. Leiros da Costa M.D. Bacheschi, L.A. Scaff M.	2001	Parkinsonism After Glycine-Derivate Exposure Movement Disorders Volume: 16, Number: 3, Pages: 565-568 ASB2012-11557

Abstract*
This 54-year-old man accidentally sprayed himself with the chemical agent glyphosate, an herbicide derived from the amino acid glycine. He developed disseminated skin lesions 6 hours after the accident. One month later, he developed a symmetrical parkinsonian syndrome. Two years after the initial exposure to glyphosate, magnetic resonance imaging revealed hyperintense signal in the globus pallidus and substantia nigra, bilaterally, on T2-weighted images. Levodopa/benserazide 500/125 mg daily provided satisfactory clinical outcome.

* Quoted from article

Klimisch evaluation

Reliability of study: Not assignable
Comment: Medical case report, single incident
Relevance of study: Relevant with restrictions (Data are limited due to the absence of any information on purity and application concentrations of glyphosate formulation, as well as co- formulations.)
Klimisch code: 4

Author(s)	Year	Study title
Wang, G., Xiao-Ning, F., Yu-Yan, T., Qi, Ch., Shen-Di, CH.	2011	Parkinsonism after chronic occupational exposure to glyphosate. Parkinsonism and related disorders ASB2012-12047

Abstract*
Here we report a patient with Parkinsonism following chronic occupational exposure to glyphosate. A previously healthy 44-year-old woman presented with rigidity, slowness and resting tremor in all four limbs with no impairment of short-term memory, after sustaining long term chemical exposure to glyphosate for 3 years as a worker in a chemical factory. The chemical plant produced a range of herbicides including: glyphosate, gibberilins, and dimethyl hydrogen phosphite; however, the patient worked exclusively in the glyphosate production division. She only wore basic protection such as gloves or a face mask for 50 h each week in the plant where glyphosate vapor was generated. She frequently felt weak.
Physical examination revealed a parkinsonian syndrome.
We cannot exclude the coincidence of idiopathic PD with exposure to glyphosate on our patient.

* Quoted from article

Klimisch evaluation

Reliability of study:	Not assignable
Comment:	Medical case report, single incident
Relevance of study:	Relevant with restrictions
Klimisch code:	4

Author(s)	Year	Study title
Astiz, M. de Alaniz, M.J. Marra, C.A.	2009	Effect of pesticides on cell survival in liver and brain rat tissues Ecotoxicology and Environmental Safety Volume: 72, Pages: 2025-2032, ASB2012-11549

Abstract*
Pesticides are the main environmental factor associated with the etiology of human neurodegenerative disorders such as Parkinson's disease. Our laboratory has previously demonstrated that the treatment of rats with low doses of dimethoate, zineb or glyphosate alone or in combination induces oxidative stress (OS) in liver and brain. The aim of the present work was to investigate if the pesticide-induced OS was able to affect brain and liver cell survival. The treatment of Wistar rats with the pesticides (i.p. 1/250 LD50, three times a week for 5 weeks) caused loss of mitochondrial transmembrane potential and cardiolipin content, especially in substantia nigra (SN), with a concomitant increase of fatty acid peroxidation. The activation of calpain apoptotic cascade (instead of the caspase-dependent pathway) would be responsible for the DNA fragmentation pattern observed.
Thus, these results may contribute to understand the effect(s) of chronic and simultaneous exposure to pesticides on cell survival.

* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Unsuitable test system (i.p exposure route is not relevant for human exposure). No information on purities of test substances used. Small group size (4 males/dose group), reporting deficiencies
Relevance of study:	Not relevant (intraperitoneal injection is a non-relevant route of exposure for humans)
Klimisch code:	3

Additional comments
This non-guideline study utilized very small group numbers (4 rats/group) and therefore is not sufficiently robust to appropriately identify changes attributable to the test material administration.
The test materials are not well described, without indication of whether a glyphosate salt form or acid was used and purity was not reported.
The publication focuses on the post necropsy data analysis and reporting. Data on animal husbandry, clinical observations, feed and water intake, weekly body weight were not reported, but the authors note there were no adverse observations.
No statistically significant effects were noted for liver endpoints, yet the liver is in close proximity to test material administration via intraperitoneal injection.
Statistically significant effects were noted for brain tissue endpoints in the substantia nigra and cerebral cortex. However, there is a lack of biological plausibility for brain exposures to glyphosate, given the necessity to pass the blood-brain barrier and the known rapid elimination kinetics of this polar molecule

via urine.

Author(s)	Year	Study title
Gui, Y.X., Fan, X.N., Wang, H.M., Wang, G., Chen, S.D.	2012	Glyphosate induced cell death through apoptotic and autophagic mechanisms. Neurotoxicology and teratology Volume: not specified (*accepted manuscript*) Pages: not specified ASB2012-11835

Abstract*
Herbicides have been recognized as the main environmental factor associated with human neurodegenerative disorders such as Parkinson's disease (PD). Previous studies indicated that the exposure to glyphosate, a widely used herbicide, is possibly linked to Parkinsonism, however the underlying mechanism remains unclear. We investigated the neurotoxic effects of glyphosate in differentiated PC12 cells and discovered that it inhibited viability of differentiated PC12 cells in dose-and time-dependent manners. Furthermore, the results showed that glyphosate induced cell death via autophagy pathways in addition to activating apoptotic pathways. Interestingly, deactivation of Beclin-1 gene attenuated both apoptosis and autophagy in glyphosate treated differentiated PC12 cells, suggesting that Beclin-1 gene is involved in the crosstalk between the two mechanisms.

* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Documentation insufficient for assessment (not clearly stated dose levels and duration of exposure, as well as treatment conditions for all tests. In addition, tested doses were much higher than real in vivo concentrations).
Relevance of study:	Not relevant (Due to reliability)
Klimisch code:	3

Additional comments:
In this paper, the authors apply glyphosate to adrenal cancer cells in culture at concentrations sufficient to cause cell death. Two major interacting pathways leading to cell death (autolysis and apoptosis) are evaluated, and the results are hardly surprising - the cells do indeed die via known mechanisms leading to cell death. The authors use these observations, and the fact that Parkinson's disease involves the death of certain nerve cells in the brain, to try and create a link between glyphosate and Parkinson's disease. There are, however, many problems with this extrapolation.
The cells used are not the neurons involved in Parkinson's, but rather a cell line derived from an adrenal gland cancer (pheochromocytoma), and the doses used are very high- the high dose killed nearly 50 % of cells in 72 hours, and the low dose was ¼ this level. The high dose equates to approximately 1/10 the concentration applied directly in the field, and is far higher than any internal glyphosate concentration that could ever occur following glyphosate use. A sufficiently high dose of every substance will kill cells - but this does not mean that every substance causes Parkinson's disease.
Unprotected cells in culture are highly susceptible to changes in pH and other non-specific effects, and it is not clear that the researchers assessed or accounted for these possible effects.
This being said, the concentrations of glyphosate used (40 mM) are known to kill other cell types in culture (Heu *et al.*, 2012, ASB2012-11844) via induction of apoptosis. Thus, no particular specificity or neuronally-specific susceptibility exists for the cell line tested. While 40 mM glyphosate is toxic to cells in culture, the LD50 in rodents is over 5000 mg/kg and *C. elegans* will have a 25 % survival following

exposure to a 10 % solution of glyphosate. *In- vitro* results do not appear to reflect *in vivo* events.
Anadon *et al.* (2009, ASB2012-11542) dosed rates with 400 mg/kg of glyphosate, a massive dose relative to any environmental exposure, and achieved glyphosate peak modeled plasma concentrations of approximately 5 µg/mL (5 ppm). Assuming linear kinetics, the current maximum allowable EU daily intake (0.3 mg/kg/day) would give an approximated blood concentration of 0.17 ppm (170 ppb). This is conservative, as McQueen et al (2012, ASB2012-11898) recently evaluated glyphosate exposure to pregnant women and concluded that estimated exposures based on actual measurements in food were only 0.4 % of the current European acceptable daily intake.
The lowest glyphosate concentration used in this experiment is 5mM (830 ppm), or 5000 times higher than the estimated blood concentration following the current EU maximum allowable daily exposure. It is also 166 times higher than the concentrations Anadon *et al.* (2009, ASB2012-11542) achieved using doses of 400 mg/kg glyphosate. In short, the concentrations used in this work are massively higher than any concentration is blood (let alone brain tissue) that can be achieved following normal human exposures.

B.6.5 Further toxicological studies
B.6.5.1 Toxicity of the metabolite AMPA
B.6.5.2 Mechanistic studies on certain aspects of the toxicity of glyphosate
B.6.5.2.1 Studies on mechanism of salivary gladn findings
B.6.5.2.2 Further studies
B.6.5.3 Studies in farm animals
B.6.5.3.1 Acute toxicity in goats
B.6.5.3.2 Subacute toxicity in cows
B.6.5.3.3 Published data

Urinary concentrations of glyphosate in cattle and other species
Krüger *et al.* (2013, ASB2013-11599) reported the abundance of glyphosate in the urine of a total of 240 cows from Danmark. From each of eight dairy farms, the same number of 30 cows (15 fresh calving, 15 high yielding cows, i.e., at the top level of milk production) was selected. All these 240 cows excreted glyphosate in the urine, however, at very different concentrations. Urine samples were diluted 1:20 with distilled water and tested for glyphosate by means of an ELISA kit (Abraxis, USA). The limit of detection (LOD) or a limit of quantification (LOQ) were, unfortunately, not mentioned. However, it is stated in the paper that validation of test results had been done by a comparison with GC-MS which is considered a more suitable method. It was mentioned that the correlation coefficient between the two methods was 0.96 and, thus, sufficiently high but this validation data was not shown in this paper. However, for further interpretation of the results, it is assumed that the method was in fact valid and that the measured values were reliable.
It is worth mentioning that the cows (breed not given) were 4 to 7 years old and had an average body weight between 550 – 600 kg and that the total number of cows in the farms ranged from 140 to 400 animals. The average daily milk yield in the different farms ranged from 8.6 to 11.2 kg

Mean urinary glyphosate concentrations differed very much among the eight farms, ranging from 10 µg/L up to 103.3 µg/L. It is a reasonable assumption that urinary excretion of glyphoate was due to dietary exposure and, thus, detection of glyphosate in the urine of cattle is not surprising. Residues of glyphosate may occur in feedstuffs for ruminants and, so far the maximum residue limits (MRLs) are not exceeded, are allowed by law and of no concern. (Therefore, the word “contamination” as used by the authors is not correct and somehow misleading.) The reason for the remarkable differences between the farms is unknown but most probably the diet was different, including a high variability in glyphosate residues. It is one of the main deficiencies of the publication that no details on feeding regime have been given and that the diets were not analysed for glyphosate content.
It is interesting to note that the mean urinary concentrations in cows exceeded the mean value in human urines of 0.2 µg/L as found by Hoppe (2013, ASB2013-8037, see B.6.9.3) by more than 500 times suggesting higher residues of glyphosate in cattle rations than in human diet. This big difference is also confirmed by comparison to further data on glyphosate findings in human urine samples (see B.6.9.3)

The maximum mean value of *ca* 103 µg/L can be used to calculate a systemic dose to which the cows in that farm had been exposed to since glyphosate does not accumulate but is rapidly excreted. The study authors have estimated a maximal glyphosate excretion via urine of 3.1 mg/day. If an oral absorption rate of only 20% is assumed instead of 30% as used by the study authors (for justification, see B.6.1), the maximum daily oral intake should have been in the magnitude of 15 – 16 mg. This dose might be compared either to the ADI (even though but it is not clear if a reference dose established for humans is per se also applicable to farm animals) and in particular to ruminants.

Thus, the maximum expected systemic dose was better compared to the NOAELs in subacute studies with either the isopropylamine salt of glyphosate (540 mg/kg bw/day according to Rowe *et al.*, 1987, TOX9552424) or a Roundup formulation (400 mg/kg bw/day according to Rowe *et al.*, 1987, ASB2010-8131). For a cow of 550 kg, the latter NOAEL would correspond to a daily intake of 220 g. In the study description (see above), a glyphosate content of 30.5% is mentioned. Thus, a daily glyphosate intake of 67 g can be calculated. If this amount of 67000 mg is compared to the expected maximum systemic exposure of 15 or 16 mg per day in the study in Danish cows, a margin of safety of about 1:4200 would result. Based on these considerations, an impairment of animal health in the Danish cows is very unlikely.

In contrast, the authors reported increased activities of the enzymes creatine kinase (CK), glutamate oxaloacetate transaminase (GOT, synonymous to ASAT), and glutamate dehydrogenase (GLDH) in blood serum but also changes in cholesterol levels and an increase in blood urea concentrations. values of unknown origin. For this comparison, they used reference values of unknown origin with the most of them not precisely mentioned. A relationship with the detection of glyphosate in urine was postulated interpreting the alterations in laboratory parameters as indicative for liver damage and neprhrotoxicity.

Leaving aside the serious methodical deficiencies of the study (*e.g.*, the absence of a control group with no glyphosate residues in the urine), there is no evidence that the altered clinical chemistry parameters in cows were indicative of any health deterioration. There could be many different reasons of such alterations and, taking into account the very low glyphosate concentrations (see above), exposure to glyphosate is not a likely one. Moreover, the statistical correlation between glyphosate excretion in urine and changed clinical chemistry parameters as claimed by the autors was in fact rather poor. (Thus, for the comparison of glyphosate with creatine kinase, an R value of 0.135 was considered in the articleas indicative of "positive correlation" which in fact is not the case. In contrast, higher R values of up to 0.809 were obtained when two different clinical chemistry parameters such as zinc and cobalt concentrations were measured and may indicate some correlation between them but apparently not with glyphosate excretion. Unfortunately, due to the way of reporting, the reader may be misleaded here to assume correlation with glyphosate.)

Since, in parallel, the authors claimed very low serum levels of several trace elements such as cobalt or manganese, a possible chelating mode of action of glyphosate was suspected. However, these considerations appear purely speculative, in particular against the background of the very low exposure. Even if glyphosate would have chelating properties, the ingested and absorbed amount is not expected to bind trace elements to such an extent that clinical signs might be expected to occur even though such an effect was suspected by scientists from the Aarhus university uin Danmark (Sørensen *et al.*, 2014, ASB2014-5761).

Again, statistical correlation was rather weak. Indeed, the almost complete absence of the two elements in cattle from all farms rather points to either an analytical problem or to a deficiency in the diet.

Thus, to conclude, the urinary levels in Danish cows might well reflect the abundance of glyphosate residues in their feed. The systemic exposure that may be calculated is very low and no health concern is anticipated. Final conclusion on the clinical chemistry findings is not possible but, on one hand, it seems not proven that they were adverse and, on the other, it is very unlikely that they might be related to glyphosate.

In a second paper dealing with determinations of glyphosate in the urine of cattle, Krüger *et al.* (2014, ASB2014-5024) reported, again, data from Danmark. The sample number was 242. It is not clearly stated but can be assumed that the 240 cows mentioned in the first paper were completely or at least partly included. These urine measurements were compared to samples from Germany. The German urine samples were collected from 343 cows from "conventional husbandry" and from 32 cows of which husbandry was

not described but which were kept in so-called "GM free" regions. (There are regions in Germany where farmers try to avoid feed that is or might be produced abroad on the basis of genetically modified crops.) The samples were analysed by means of a not further specified ELISA (Abraxis, USA) but, again, an LOD or LOQ were not mentioned. Instead, a comparison between values obtained by this ELISA and GC-MS was provided and, this time, explained in greater detail. The comparison revealed a very good correlation (R2 of 0.96 for cattle urine, based on 21 samples that were measured in parallel by both methods). Neither individual numeric values for urine concentrations nor statistical parameters such as the mean, median or standard deviation are given in this very brief paper but only figures. It seems from these printed figures that the mean urinary concentration in Danish cows was slighly above 40 µg/L as compared to only ca 20 µg/L in the German cows from conventional husbandry. This difference was statistically significant. The German cows from "GM free" regions had hardly any glyphosate in their urines. In contrast to what is said in the article, this finding is not surprising since imported feedstuffs will most certainly contain higher residues of glyphosate.
As discussed above with regard to Krüger *et al.* (2013, ASB2013-11599), urinary concentrations in this magnitude are of no health concern for cattle.

Furthermore, glyphosate was found in the urine of fattening rabbits (n = 77) in the magnitude of about 60 µg/L (mean value, standard deviation showing values ouf up to ca 120 µg/L). Less glyphosate (mean of 20-30 µg/L) was determined in the urine of hares (n = 193). Nothing is known on the origin of these samples and no conclusions can be drawn.
(In human samples that were analysed by the same group, the mean concentration was nearly 2 µg/L with a maximum in the magnitude of 5 µg/L. For details, see section B.6.9.3.) Information on glyphosate residues in the organs of slaughtered cows was also given in this paper and is reported in chapter B.7.

Possible impact on the microflora in ruminant's GIT
A number of papers has been published recently in which a possible causal link of glyphosate exposure and subsequent Clostridium botulinum (C. botulinum) overgrowth with a new disease in cattle is suggested. The scientific background of this assumption is the herbicidal mode of action of glyphosate. In plants, the enzyme 5-enolpyruvylshikimic acid-3-phosphate synthase (EPSP synthase) is inhibited resulting in a lack of formation of aromatic amino acids by the shikimate pathway that is common in the plant kingdom but not does not occur in animals. However, this pathway is operative in most bacteria and yeast and many protozoan species. Thus, an impact of glyphosate on microflora, e.g., in the intestines, is at least conceivable. In line with that, concern on this issue was expressed by scientists from the Aarhus University in Denmark (Sørensen *et al.*, 2014, ASB2014-5761).

Rodloff and Krüger (2012, ASB2013-13311) hypothesised that an emerging new disease in cattle but also symptoms in a small number of farmers might be caused by Clostridium botulinum. This animal disease of so far unknown etiology and pathogenesis was reported to have occurred from the late 1990ies onwards in cattle mainly from some parts of Germany but, according to the authors, cases had been observed also in France, the Netherlands, and the U.K. even though references were not given. Clinical signs in cattle are predominately seen in the perinatal period and comprise indigestion with alternating constipation and diarrhea, apathy, ataxia, paralysis, retracted abdomen, breathing difficulties, a decrease in milk yield, and death. In some farmers taking care of affected herds, symptoms such as dizziness, weakness, fatigue, blurred vision, nausea, and difficulties to speak, to swallow and to breathe have been occasionally reported. The authors suggested a causal link to Clostridium botulinum because these signs and symptoms appear similar to what is known from the rarely occurring cases of botulism in animals and man that are not food-borne (i.e., not caused by acute intoxication with the bacterial toxin). By means of an ELISA (no details given), they have detected Clostridium botulinum neurotoxin (BoNT) in fecal samples (in total in 16 out of 33) obtained from cattle on six German farms where the suspicious clinical signs or even fatalities had been noted but not in a total of 10 samples from two farms without any evidence of this disease. Toxin types were different (A, B, C, D, and E, in various combinations). Health state of the donor animals was not reported. In addition, neurotoxin of different types was found in various organs such as the rumen and the liver in 15 sick cows after slaughter. However, it is not known howmany cows were examined in total

post mortem and by which method and if they belonged to the group of which fecal samples had been taken before.
In man, 16 out of 77 fecal samples were positive for BoNT that were taken from humans who were reported to have been in close contact with diseased cows. It is not clear if one or more samples were obtained from the same person and, thus, the total number of humans under investigation is not known. Again, health status of the involved people was not reported. With regard to neurotoxin types, mainly type E had been found.
Unfortunately, these numbers do not completely match with a table in the paper in which 12 BoNT-positive human fecal samples in a total number of 33 and 29 BoNT-positive bovine fecal samples out of 118 under examination were mentioned to be obtained from 7 "selected" German cattle farms.

Conclusion by RMS:
This paper does not contribute anything to risk assessment of glyphosate and, in fact, this herbicidal active ingredient was not mentioned therein. However, it is important for understanding of subsequent publications and, therefore, is referred to in this RAR.
In itself, this paper is adequate to suggest a scientific hypothesis, based on some data that might require further research. However, no causal relationship between a new disease in cattle and C. botulinum has been established. C. botulinum neurotoxin could not be quantified. Qualitative detection of the neurotoxin might be also a random co-incidence. The publication is flawed by many reporting deficiencies. In particular, the method by which the neurotoxin was detected and its different types were distinguished, is not described.

In a similar paper, Krüger *et al.* (2012, ASB2013-13312) reported the abundance of (different types) of the micro-organism *C. botulinum* itself in 44 out of 196 bovine fecal samples (22.5%) and in 17 out of 77 human fecal samples (22%) but also in silages (9 / 21 = 47%), concentrate feed specimens (4 / 14 = 28.6%), and in all 7 tested house dust specimens from a total of 41 dairy farms in the German Federal State of Schleswig-Holstein. This finding was based on the immunological detection of antigens of *C. botulinum* by an ELISA technique using polyclonal antibodies. All the cattle had been reported to have shown clinical signs as described above. In addition, in four of the involved humans, symptoms were claimed to have occurred but without further specification or medical confirmation.

Conclusion by RMS:
Again, this paper, in principle, is not relevant for risk assessment of glyphosate but it is mentioned therein that ingested spores might germinate in the intestinal tract if protective indigenous bacterial flora is lacking (as observed in cases of infant botulism) or altered and this might be linked to the glyphosate hypothesis explained below. Therefore, it is mentioned here despite its many reporting deficiencies. The findings rather point to ubiquitious occurrence of C. botulinum but are not suitable to prove a causal relationship of its abundance to clinical signs or symptoms.

By the same group, it was published that different bacteria such as *Enterococcus faecalis*, *Enterococcus faecium* or *Bacillus badius* were able to inhibit growth of *C. botulinum* and/or the production of its neurotoxins *in vitro* whereas other bacterial species did not exhibit such an effect (Shehata *et al.*, 2012, ASB2013-8529). Subsequently, Krüger *et al.* (2013, ASB2013-8527) reported that glyphosate (analytical grade) and the herbicide Roundup UltraMax® containing 450 glyphosate/mL was able to suppress this antagonizing effect of *Enterococcus* species on *C. botulinum in vitro*. What they actually observed was a growth inhibition of both *C. botulinum* (and a reduction in neurotoxin type B production) and *Enterococcus faecalis* by glyphosate and Roundup herbicide but at different concentrations. While growth of *E. faecalis* was completely inhibited by 0.1 mg glyphosate or Roundup/mL, the same effect on *C. botulinum* was seen only at a concentration of 1 mg Roundup/mL or of 10 mg glyphosate/mL.

Conclusion by RMS:
This data suggests a different susceptibility of E. faecalis and C. botulinum to cytotoxic effects of glyphosate and a glyphosate-based herbicide in vitro. With regard to C. botulinum, the Roundup UltraMax formulation was more toxic than the active ingredient confirming similar evidence from various fields of toxicological

testing. No conclusions can be drawn if similar effects might occur in vivo because the situation in, e.g., the GITs of ruminants or monogastric animals is different with hundreds or thousands of mirobial species co-existing. In any case, even the lowest tested concentration of 0.1 mg/mL appears extremely high if a (maximum) systemic dose of 15 or 16 mg per cow is assumed to result from feeding the animals with a ration containing glyphosate residues (based on Krüger et al., 2013, ASB2013-11599, see above). Thus, the possible impact of glyphosate (herbicides) on bacteria due to inhibition of the enzyme EPSP was somehow confirmed in vitro but there is no health concern and no impact on realistic risk assessment.

A different toxicity of Roundup UltraMax® to various microbial species was also observed by Shehata *et al.* (2013, ASB2012-16301) who measured the effect of different concentrations on 23 bacterial species and strains mostly of chicken origin and also on sporulated *Eimeria tenella* (*i.e.*, a protozoon in poulty) oocytes *in vitro*. It is not clear whether the mentioned concentratios ranging from 0.075 mg to up to 5 (bacteria) or 1.2 (*Eimeria tenella*) mg per mL are related to the herbicide formulation or had been adjusted to the active ingredient. In general, the authours found lower minimum inhibitory concentrations (0.075 – 0.6 mg/mL) for beneficial bacteria whereas, in contrast, some pathogenic germs such as *Clostridium perfringens* or several *Salmonella* species appeared much less sensitive with growth inhition seen only at the highest tested concentration of 5 mg/mL. With regard to *Eimeria tenella*, the threshold for an effects was around 0.3 mg/mL with a clear effect to be seen at 0.6 mg/mL.

Conclusion by RMS:
Different cytototoxicity of a glyphosate-based herbicide to micro-organisms was confirmed once more and might be due to either the active ingredient or, e.g., a surfactant. (It is not known whether a tallowamine surfactant was contained.) However, antibiotic activity of the herbicide (expressed in the minimum inhibitory concentrations) was lower than that of known antibiotics that are used in veterinary medicine. Even the lowest effect concentrations in this study were by far higher than the expected glyphosate concentrations in poultry feed (GTF, 2013; ASB2013-11007) and, thus, must be considered unrealistically high. Furthermore, in vitro exposure of selected individual species and strains to a herbicide might be not a good model for complex interactions in GIT of poultry when residues are ingested.

To conclude, a link of glyphosate residues in ruminant's diet to a new disease in cattle has not been established and is not likely. Furthermore, there is no convincing proof that clinical signs in cattle (of which the occurrence cannot be doubted) were indeed caused by *C. botulinum* or its toxins. Meanwhile, a comprehensive case-control study on a possible causal relationship between C. botulinum and that chronic disease in cattle has been conducted in Germany. Preliminary results suggest that this was not the case even though further investigations have been considered necessary. In addition, use of glyphosate on the included farms had no impact on the occurrence of clincial signs (Seyboldt and Hoedemaker, 2014, ASB2014-10736).

However, because of the growing public concern about this cattle disease especially in Germany and because an effect on micro-organisms due to EPSP inhibition cannot be excluded, the German Federal Institute for Risk Assessment (BfR) has commisioned a study with a glyphosate-based herbicide (containing a tallowamine surfactant) in an artifical rumen system (RUSITEC) to investigate wheter (1) quantitative composition of ruminal microflora might be compromised and (2) there is evidence of *C. botulinum* overgrowth. Unfortunately, results of this project have not been published so far. However, an internal research report of the Veterinary High School in Hannover (Riede *et al.*, 2013; ASB2013-14684) has been submitted to the Federal Institute for Risk Assessment and is reported here in brief. Two different experiments were performed. In the first one, the effects of a glyphosate-based herbicide (Plantaclean® XL; 360 g/l glyphosate, containing a tallowamine surfactant) on rumen fermentative parameters were studied. Total glyphosate doses per day were 0.26 or 2.31 mg per fermentation vessel. No major changes in rumen parameters were detected except slight decreases in NH3-N concentrations and increases in isovalerate production in response to the high dosage. There was an increase in (beneficial) Bifidobacterium spp. but, in general, the microbial communities were not affected. In the second trial, no effects of the herbicide on the growth of C. sporogenes was found that had been artificially added to serve as a surrogate for C. botulinum.

Malformations in piglets with suspected correlation with glyphosate

Krüger *et al.* (2014, ASB2014-8935) reported glyphosate residues in different organs/tissues (brain, gut wall, heart, kidneys, liver, lungs, and muscle tissue) from a total of 38 malformed one-day old piglets (breed not specified) which had been brought in by a Danish farmer. The same farmer had complained about reproduction and developmental problems already in the past, e.g., in an Internet publication by the NGO "GM-Free Cymru (Wales)" in which health effects in pigs including malformations were mainly ascribed to the feeding of genetically modified soy (Anon., 2012, ASB2014-3921) and, apparently, his observations have been also referred to by Sørensen *et al.* (2014, ASB2014-5761). The malformations were very much different including craniofacial but also visceral and leg anomalies. For determination of glyphosate, apparently the same ELISA as for urine measurements (Abraxis, USA) was used after mincing and diluting tissue samples from the various organs. Mean glyphosate concentrations between 2.1 ppm (liver) and 12.9 ppm (heart) were found. In most organs, standard deviation was extremely large and individual values in single animals ranged from 0 (liver) and 0.1 ppm (kidney) to occasional findings as high as 80 ppm (in lung and heart). The authors speculated if there was a correlation between the malformations and intake of glyphosate residues to which the piglets might have become exposed via the placenta. The farmer claimed that the rate of malformed piglets had increased from 1:1432 when the sows had been fed a diet containing 0.25 ppm glyphosate to 1:260 when the sows received a diet with a glyhosate content of 0.87-1.13 ppm during the first 40 days of pregnancy.

Conclusion by RMS:

This publication cannot be considered to describe a reliable scientific study. First, the analytical data obtained from the piglets appear questionable since no information was given whether the ELISA had been modified for these investigations or validated for analysing tissue samples. No LOD or LOQ was mentioned. For the changing glyphosate content in the diet as claimed by the farmer, there is no confirmation in the publication.

The second main weakness of the study is that only malformed piglets had been investigated for glyphosate concentrations in their organs. Thus, there was no control group to (possibly) prove the hypothesis of a potential correlation.

For the following considerations, such a correlation is unlikely:

-In a multitude of developmental studies and multi-generation studies in rats, no evidence of teratogenicity was obtained. Even in rabbits which proved more vulnerable, developmental effects were confined to exaggerated dose levels causing also clear maternal toxicity (see sction B.6.6). It is very unlikely that pigs, receiving much lower amounts of glyphosate by ingestion of residues in the diet, should be that much more sensitive and, if so, it is hardly conceivable that such effects would not have become apparent before and also in other countries and on other farms.

-Many different malformations were reported. However, most chemical teratogens produce a specific teratogenic effect or a certain pattern of findings. Moreover, teratogenic effects usually follow a dose response. In this case, the glyphosate concentrations in the organs and tissues were so variable that such a dose response may be excluded.

-Malformations in piglets are quiet frequent and have often a genetic background. Infectious diseases may also play a role. There is no indication in the paper that a differential diagnosis has been considered.

B.6.5.1 Further published data (released since 2000)

Introduction by RMS:

A large number of studies on toxicity of glyphosate and its formulations was published since 2000. Most of these studies are presented in the chapters on genotoxicity, carcinogenicity, reproductive toxicity and neurotoxicity of this report because they are discussed there in context with these endpoints. However, some additional studies are presented below that could not be allocated to these endpoints.

Additionally to the reviews reported above in the chapters on different end points of this RAR some further toxicological reviews on glyphosate have been published since 2000. The content of these reviews is a

broad range of possible effects which are attributed by the authors to glyphosate, glyphosate formulations or generally to pesticides. However, some reviewers summarised effects described in the original studies without to differentiate between clearly evidenced effects and questionable or highly doubtful effects. The quality of the reviewed literature is in many cases not sufficiently discussed. On this basis some autors come to conclusions which deviate from conclusions of the rapporteur of this RAR.

Samsel and Seneff, 2013 (ASB2013-8535) reviewed toxicological literature on glyphosate and concluded that glyphosate inhibits cytochrome P450 enzymes. The authors believe that this activity would result in nearly all diseases as inflammatory diseases, obesity, depression, ADHD, autism, Alzheimer`s disease, Parkinson`s disease, ALS, multiple sclerosis, cancer, cachxia, infertility, developmental diseases, gastrointestinal disorders, heart disease, diabetes. Antoniou *et al.* (2011, ASB2011-7202) reviewed toxicological literature on Roundup and glyphosate. The authors conclude that Roundup and glyphosate would cause endocrine disruption, damage to DNA, reproductive developmental toxicity, neurotoxicity and cancer as well as birth defects. Many of these effects would be found at very low doses, comparable to levels of pesticide residues found in food and the environment. Mostafalou and Abdollahi (2013, ASB2014-9618) published a review on the relation between pesticides and elevated ranges of a broad range of different diseases. According to the authors pesticides cause diseases as different types of cancers, diabetes, neurodegenerative disorders like Parkinson, Alzheimer and amyotrophic lateral sclerosis, birth defects, and reproductive disorders, resipiratory problems, cardiovascular diseases, chronic nephropaties, autoimmune diseases, chronicfatique syndrome and aging.

Mesnage and Seralini (2014, ASB2014-9616) submitted a review on pesticide toxicity and genetically modified organisms (GMOs) which are used in agriculture. The authors propose to pay more attention on the mixtures of pesticides with further substances and to test relevant combinations of pesticides at levels which occur in genetically modified plants. NABU (2011, ASB2012-8016) reviewed some ecological and toxicological literature on glyphosate and formulations. The active substance, metabolites and further substances in the formulations are considered toxic especially for aquatic organisms. They would disturb human cells and the development of vertebrates. In result of resistance of wild plants the amount of glyphosate products is expected to grow in the future. PANAP (2009, ASB2012-8017) reviewed literature on toxicity, environmental effects and environmental fate of glyphosate. The authors conclude that independent scientific studies and poisonings in Latin America are beginning to reveal that use of glyphosate would not be safe.

Antoniou *et al.* (2010, ASB2012-803) reviewed toxicological and ecological literature on glyphosate and genetically modified Soya. The authors conclude that the toxic activity of glyphosate is increased by the combination with further substances in the formulations. Toxicity was already observed at concentrations which occur in agriculture and environment. The authors conclude that there would be a relation between glyphosate and increased malformations. Furthermore, epidemiologicas studies would demonstrate a relation between glyphosate use and carcinogenicity and genotoxicity.

Brändli and Reinacher (2012, ASB2012-804) submitted a short survey on use and health effects of glyphosate. The authors conclude that the use of glyphosate for siccation would be a scandal and would be considered to be bodily injury by negligence.

Greenpeace (2013, ASB2012-810) reviewed literature on ecological and health effects of glyphosate. The authors conclude that the submitted evidence in this report demonstrates that glyphosate-based products can have advers impacts on human and animal health, and that a review of their safety for human and animal health is urgently needed. The authors demand that no genetically modified glyphosate-tolerant crops should be authorised. They would be linked to unsustainable farming practices that damage the basic natural resources food production is based upon, and their cultivation should be banned.

Altenburger *et al.* (2012, ASB2014-9176) submitted a review that provides an overview on experimental studies from the past decade that address diagnostic and/or mechanistic questions regarding the combined effects of chemical mixtures using toxicogenomic techniques. By joining established mixture effect models with toxicokinetic and –dynamic thinking the authors suggest a conceptual framework that may help to overcome the current limitation of providing mainly anecdotal evidence on mixture effects.

Furthermore, some studies have been published in which the authors investigate the activity of glyphosate

and/or glyphosate formulations on selected biochemical or morphological structures. However, based on the provided information the impact of these results for the in vivo situation of the whole organism of animals or humans with autoregulation and feedback mechanisms is questionable. The dose dependency of the described effects and their importance for real life situations is often not sufficiently discussed by the authors. Many authors conclude that further studies would be necessary.
In some cases the authors compare the toxicity of glyphosate with the toxicity of glyphosate formulations or of surfactants. A frequent result in these cases is a higher toxicity of the formulations or the surfactants. In some further cases only glyphosate formulations have been used in the studies. Some authors use these results for a conclusion concerning the toxicity of the active substance glyphosate but do not consider the activity of surfactants or further substances in the formulation.

Chaufan *et al.*, 2014 (ASB2014-7616) examined the effects of glyphosate, AMPA and the formulation Roundup Ultra Max on oxidative balance and cellular endpoints in HepG2 cells. Only the formulation Roundup Ultra Max had toxic effects while no effects were found with glyphosate and AMPA. The formulation produced an increase in reactive oxygen species, nitrotyrosine formulation, superoxide dismutase activity and glutathione levels.
In an in in vivo study (Larsen *et al.*, 2012, ASB2014-6905) Wistar rats were exposed during 30 or 90 days to low levels of 0.7 and 7 mg/L glyphosate in drinking water. Only 4 animals per dose and sex were used. Levels of glutathione and glutathione peroxidase have been increased which was considered as a protective mechanism by the authors. George *et al.*, 2013 (ASB2014-8034) studied the effects of glyphosate on HaCaT cell proliferation. The authors concluded that glyphosate promotes proliferation in HaCaT cells probably by disrupting the balance between Ca^{2+}-levels and oxidative stress. However, in the study the commercial formulation Roundup was used. Therefore, the results can not be attributed to the substance glyphosate only. Hedberg et Wallin, 2010 (ASB2014-7494) studied the effects of glyphosate, Roundup and further substances on intracellular transport in Xenopus laevis. The chemicals inhibited retrograde transport of melanosomes in the range of 0.5 – 5 mM. Cellular morphology and localization of microtubules and actin filamentes were affected. The effects are pH-dependent. El-Shenawy, 2009 (ASB2012-11611) compared the cytotoxicity of Roundup and the active substance glyphosate. Male rats were i. p. treated with Roundup or glyphosate. The results characterize Roundup as a stronger antioxidant than the active substance glyphosate itself. Caglar and Kolankaya, 2008 (ASB2012-11580) treated rats with formulation Roundup orally during 5 and 13 weeks and studied hepatotoxicity. The authors concluded that high doses of Roundup can be a potential riks for human health. Modesto and Martinez (2010, ASB2012-811) studied effects of Roundup Transorb on fish. They observed haematologic alterations and effects on antioxidant defenses and on acetylcholinesterase activity.
Zhao *et al.* (2013, ASB2014-9645) investigated the effect of different doses of glyphosate on apoptosis and expression of androgen-binding protein and vimentin mRNA in mouse Sertoli cells. The authors conclude that glyphosate can cause cellular damages, inhibit cell proliferation, induce cell apoptosis, and decrease expression of ABP and vimentin mRNAs in mouse Sertoli cells in vitro. Xia *et al.* (2013, ASB2014-9642) studied the induction of vitellogenin gene expression in the fish medaka exposed to glyphosate and potential molecular mechanism. While glyphosate markedly up-regulated VTG transcription levels in both female and male fish, the upward trend was inhibited at the high glyphosate concentrations. Wunnapak *et al.* (2014, ASB2014-9638) used Roundup to induce nephrotoxicity in rats. A panel of kidney injury biomarkers was evaluated in terms of suitability to detect acute kidney injury and dysfunction. Martini *et al.* (2012, ASB2014-9613) used 3Z3-L1 fibroblasts to investigate the effect of a commercial formulation of glyphosate on proliferation, survival and differentiation. According to the results, a glyphosate-based herbicide inhibits proliferation and differentiation in this mammalian cell line and induces apoptosis suggesting GF-mediated cellular damage.

Larsen *et al.* (2014, ASB2014-9606) evaluated the activities of different xenobiotic- metabolizing enzymes in liver subcellular fractions from Wistar rats exposed to a glyphosate based herbicide. The results demonstrated certain biochemical modifications after exposure to a GLP-based herbicide. The authors conclude that the pharmacotoxicological significance of these findings remains to be clarified. Belle *et al.* (2012, ASB2014-9251) write in a letter to the editor that Williams *et al.* (2012, ASB2012-12052) analyzed five of their articles. Williams *et al.* (2012, ASB2012-12052) would minimize

the experimental evidence that glyphosate plays a role in toxicity and would discredit their findings.The article of Williams *et al.* (2012, ASB2012-12052) would contain several errors. Williams *et al.* (2012, ASB2012-12052) have not evidenced that the experiments of Belle *et al.* have been incorrect or biased.

Kim *et al.* (2013, ASB2014-9591) investigated the mechanism of the additive effect of glyphosate and TN-20, a common surfactant in glyphosate herbicides. The results support the possibility that mixtures of glyphosate and TN-20 aggrevate mitochondrial damage and induce apoptosis and necrosis. Throughout this process, TN-20 seems to disrupt the integrity of the cellular barrier to glyphosate uptake, promoting glyphosate-mediated toxicity.

Kilinc *et al.* (2013, ASB2014-9588) studied the influence of pesticide exposure on carbonic anhydrase II from sheep stomach. The authors conclude that both glyphosate isopropylamine and dichlorvos inhibited CA-II isoenzym in a noncompetetive manner.

Jasper *et al.* (2012, ASB2014-9583) evaluated the toxicity of hepatic, haematological, and oxidative effects of glyphosate Roundup on male and female albino Swiss mice. The results of this study indicate that glyphosate-Roundup can promote haematological and hepatic alterations, even at subacute exposure, which could be related to the induction of reactive oxygen species.

Chaufan *et al.* (2014, ASB2014-9314) studied the effects on oxidative formulation in HepG2 cells. The authors conclude that the results confirm that G formulations have adjuvants working together with the active ingredient and causing tolxic effects that are not seen with acid glyphosate.

Gencer *et al.* (2012, ASB2014-9481) studied in vitro effects of Imazethapyr, 2,4-D, glyphosate and propanocarb on human erythrocyte carbonic anhydrase activity. Imazethapyr was the most effective inhibitor for CA-H isoencyme. The lowest inhibition was caused by glyphosate.

Campo *et al.* (2009, ASB2014-9281) evaluated the toxicity of ten pesticides used in the municipality of Popayan., Colombia, using bioassay with Bacillus subtilis. Glyphosate was slightly toxic in this test.

Kwiatkowska *et al.* (2014, ASB2014-9603) published a study that was undertaken to evaluate toxic potential of glyphosate, ist metabolites AMPA, methylphosphonic acid and its impurities N-(phosphonomethyl)iminodiacetic acid (PMIDA), N-methylglyphosate, hydroxymethylphosphonic acid and bis-(phosphonomethyl)amine. The authors evaluated the effect of those compounds on haemolysis, haemoglobin oxidation, reactive oxygen species (ROS) formation and changes in morphology of human eythrocytes. Glyphosate, its metabolites and impurities induced a little haemolysis and haemoglobin oxidation. All changes were very low, even after 24 h incubation. Most of the investigated compounds induced reactive oxygen species formation from 0.25 mM, except the N-methylglyphosate wich caused an increase in ROS formation from 0.5 mM. Moreover, the investigated xenobiotics did no change the size and shape (except bis-(phosphonomethyl)amine) of the human erythrocytes. Changes in human erythrocytes were observed only when high concentrations of the compounds were applied. Some investigated metabolites and impurities caused a slight stronger damage to human erythrocytes than glyphosate.

Mesnage *et al.* (2013, ASB2014-1755) studied the toxicity on human cells in vitro for glyphosate-based formulations and also for polyethoxylated tallowamine POE-15, glyphosate alone and a formulation without glyphosate. Hepatic (HEPG2), embryonic (HEK293) and placental (JEG3) cells were exposed 24 h. POE-15 was the most toxic of the tested substances and combinations. The authors conclude that pesticide formulations should be studied as mixtures for toxic effect with mammals over a 2-year period. They question the use of ethoxylated substances in herbicide formulations, since they appear as active principle for human sell toxicity. The results would challenge guidance values as the ADI because they are only derived from results of studies with the active substance.

Mesnage *et al.* (2012, ASB2012-13917) tested the toxicity of 9 active pesticide substances including glyphosate, comparing with the toxicity of their formulations, including Roundup. The tests have been performed in vitro with 3 human cell lines. The authors conclude that Roundup was found in this experiments to be 125 times more toxic than glyphosate. These results would challenge the relevance of the ADI because this is only based on the toxicity ofthe active substance. Therefore, an additional adjuvant factor of at least 100 could be applied to the present calculation of the ADI. However, this calculation

would never replace the direct study of the commercial formulation with its adjuvants in regulatory tests. Coalova *et al.* (2014, ASB2014-7615) studied the influence of spray adjuvant on the toxicity effects of a glyphosate formulation in Hep-2-cell line. They determined the median lethal concentration of Atanor (glyphosate formulation), Impacto (spray adjuvant) and a mixture of both agrochemicals. The substances and mixtures induced dose- and time-dependent cytotoxicity. The toxicity of a mixture of Atanor and Impacto was additive in Hep2-cells. The authors conclude that the addition of adjuvant to glyphosate formulation would increase the toxicity of the mixture in cell culture. Kwiatkowska *et al.* (2014, ASB2014-8085) investigated the effect of glyphosate, its metabolites and impurities on acetylcholinesterase (AchE) activity (in vitro) in human erythrocytes. The authors conclude that the compounds studied (used in concentrations that are usually determined in the environment) do not disturb function of human erythrocyte acetylcholinesterase.

Hoare (2014, ASB2014-9157) submitted a QSAR assessment on the toxicological properties of glyphosate and its impurities. To assess the toxicological properties of glyphosate and five impurities (AMPA, IBMPA, MAMPA, NMG and IDA) present in the technical grade of material, the QSAR models ACD labs, DEREK NEXUS, TOXTREE, EPA T.E.S.T. VEGA and OECD Toolbox were employed. None of the structures analysed triggered any alerts on DEREK NEXUS for carcinogenicity, chromosome damage, genotoxicity or mutagenicity. Eye and skin irritation were not anticipated in any of the QSAR models evaluated. No alert for skin sensitisation was triggered in DEREK NEXUS for any compound. Equivocal alerts for nephrotoxicity and plausible alerts for hepatotoxicity were triggered by DEREK NEXUS for glyphosate, NMG, MAMPA and AMPA.

A further study was published which describes cases of intoxication in dogs and cats:

Bates and Edwards (2013, ASB2014-9249) inform in a letter about cases of intoxication of dogs and cats by glyphosate in UK, registered by the Veterinary Poisons Information Service. Vomiting, diarrheoea and lethargy were the most common signs in dogs. Vomiting, anorexia and lethargy were the most common signs in cats.

Two further studies have been submitted which investigated ecotoxicological effects of glyphosate:

Relyea (2005, ASB2012-204) examined the impact of four globally common pesticides including glyphosate on the biodiversity of aquatic communities containing algae and animals. In a further study Relya (2012, ASB2012-2791) created wetland communities including water plants and animals and exposed thes communities to Roundup. The author reports different effects on nontarget species.

Further studies are presented in detail below:

Author(s)	Year	Study title
Robert Bellé, Ronan Le Bouffant, Julia Morales, Bertrand Cosson, Patrick Cormier et Odile Mulner-Lorillon	2007	L'embryon d'oursin, le point de surveillance de l'ADN endommagé de la division cellulaire et les mécanismes à l'origine de la cancérisation. Journal de la Société de Biologie, Volume : 201, Number: 3, Pages: 317-327 ASB2012-11560

Abstract*

Sea urchin embryo, DNA-damaged cell cycle checkpoint and the mechanisms initiating cancer development (translation from original article)

Cell division is an essential process for heredity, maintenance and evolution of the whole living kingdom. Sea urchin early development represents an excellent experimental model for the analysis of cell cycle

checkpoint mechanisms since embryonic cells contain a functional DNA-damage checkpoint and since the whole sea urchin genome is sequenced. The DNA- damaged checkpoint is responsible for an arrest in the cell cycle when DNA is damaged or incorrectly replicated, for activation of the DNA repair mechanism, and for commitment to cell death by apoptosis in the case of failure to repair. New insights in cancer biology lead to two fundamental concepts about the very first origin of cancerogenesis. Cancers result from dysfunction of DNA-damaged checkpoints and cancers appear as a result of normal stem cell (NCS) transformation into a cancer stem cell (CSC). The second aspect suggests a new definition of "cancer", since CSC can be detected well before any clinical evidence. Since early development starts from the zygote, which is a primary stem cell, sea urchin early development allows analysis of the early steps of the cancerization process. Although sea urchins do not develop cancers, the model is alternative and complementary to stem cells which are not easy to isolate, do not divide in a short time and do not divide synchronously. In the field of toxicology and incidence on human health, the sea urchin experimental model allows assessment of cancer risk from single or combined molecules long before any epidemiologic evidence is available. Sea urchin embryos were used to test the worldwide used pesticide Roundup that contains glyphosate as the active herbicide agent; it was shown to activate the DNA-damage checkpoint of the first cell cycle of development. The model therefore allows considerable increase in risk evaluation of new products in the field of cancer and offers a tool for the discovery of molecular markers for early diagnostic in cancer biology. Prevention and early diagnosis

* Quoted from article

Klimisch evaluation

Reliability of study: Not assignable

Comment: Documentation insufficient for evaluation.
The publication overview provides information on the general application of the sea urchin embryo model for the prediction of "cancerogenicity". Only a short reference to another study with a glyphosate-containing herbicide is given. Details of the glyphosate product are not provided. Common surfactants have previously shown the same effects in this model. This model is not appropriate for testing materials containing surfactants because surfactant induced cytotoxicity via membrane disruption is well documented using *in vitro* systems.

Relevance of study: Not relevant (Prevention of cell cycle transition was determined for the glyphosate formulation. This model is not appropriate for testing materials containing surfactants.)

Klimisch code: 4

Author(s)	Year	Study title
Marc J., Mulner-Lorillon, O., Boulben, S., Hureau, D., Durand, G., Belle, R.	2002	Pesticide Roundup Provokes Cell Division Dysfunction at the level of CDK1/Cyclin B Activation.. Chem. Res. Toxicol. 2002, 15, 326-331 ASB2013-9838

Abstract*

To assess human health risk from environmental chemicals, we have studied the effect on cell cycle regulation of the widely used glyphosate-conaining pesicide Roundup. As a model system we have used sea urchin embryonic first divisions following fertilization, which are appropriate for the study of universal cell cycle regulation without interference with transcription. We show that 0.8% Roundup (containing 8 mM glyphosate) induces a delay in the kinetic of the first cell cleavage of sea urchin embryos. The delay

is dependent on the concentration of Roundup. The delay in the cell cycle could be induced using increasing glyphosate concentrations (1-10 mM) in the presence of a subthreshold concentration of Roundup 0.2%, while glyphosate alone was ineffective, thus indicating synergy between glyphosate and Roundup formulation products. The effect of Roundup was not lethal and involved a delay in entry into M-phase of the cell cycle, we analysed CDK1/cyclin B activation during the first division of early development. Roundup delayed the activation of CDK1/cyclin B *in vivo*. Roundup inhibited also the global protein synthetic rate without preventing the accumulation of cyclin B. In summary, Roundup affects cell cycle regulation by delaying activation of the CDK1/cyclin B complex, by synergic effect of glyphosate and formulation products. Considering the universality among species of the CDK1/cyclin B regulator, our results question the safety of glyphosate and Roundup on human health.

* Quoted from article

Author(s)	Year	Study title
Marc J., Mulner-Lorillon, O., Durand, G., Belle, R.	2003	Embryonic cell cycle for risk assessment of pesticides at the molecular level. Environmental Chemistry Letters Volume: 1, Number: 1, Pages: 8-12 ASB2009-9013

Abstract*

Cell cycle mechanisms are highly conserved from unicellular eukaryotes to complex metazoans including humans. Abnormalities in the regulation of the cell cycle result in death or diseases such as cancer. Early development of sea urchin has proved to be a powerful model for cell division studies and offers the opportunity to study synchronous cell divisions in the absence of transcriptional control. We have analyzed pesticide induced dysfunctions in the first cell division following fertilization in sea urchin embryos, using Roundup, a widely used pesticide formulation containing isopropylamine glyphosate as the active substance. The pesticide induced cell cycle dysfunction by preventing the *in vivo* activation of the universal cell cycle regulator CDK1/cyclin B. We further show that synthesis of the regulator protein, cyclin B, as well as its association to the catalytic protein, CDK1, were not affected by the pesticide. Therefore, our results suggest that the pollutant impedes the processing of the CDK1/cyclin B complex, which is required in its physiological activation. Our studies demonstrate the relevance of sea urchin embryonic cells as a sensitive model to assess pesticide toxicity at the level of the universal cell cycle checkpoints.

* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Mechanistic study. Outcome with little additional information compared to the authors' previously published work. Non-standard, non-guideline. Commonly used surfactants have previously shown the same effects in this model.
Relevance of study:	Not relevant (Prevention of cell cycle transition was determined for the glyphosate formulation. This model is not appropriate for testing materials containing surfactants because surfactant induced cytotoxicity via membrane disruption is well documented using *in vitro* systems.)
Klimisch code:	3

Author(s)	Year	Study title

Marc, J. Mulner-Lorillon, O. Belle, R.	2004	Glyphosate-based pesticides affect cell cycle regulation Biology of the Cell Volume: 96, Pages: 245-249, ASB2009-9014

Abstract*
Cell-cycle dys-regulation is a hallmark of tumor cells and human cancers. Failure in the cell- cycle checkpoints leads to genomic instability and subsequent development of cancers from the initial affected cell. A worldwide used product Roundup 3plus, based on glyphosate as the active herbicide was suggested to be of human health concern since it induced cell cycle dysfunction as judged from analysis of the first cell division of sea urchin embryos, a recognized model for cell cycle studies. Several glyphosate-based pesticides from different manufacturers were assayed in comparison with Roundup 3plus for their ability to interfere with the cell cycle regulation. All the tested products, Amega, Cargly, Cosmic, and Roundup Biovert induced cell cycle dysfunction. The threshold concentration for induction of cell cycle dysfunction was evaluated for each product and suggests high risk by inhalation for people in the vicinity of the pesticide handling sprayed at 500 to 4000 times higher dose than the cell- cycle adverse concentration.

* Quoted from article

Klimisch evaluation

Reliability of study:	Not Reliable
Comment:	Non-standard, non-guideline study. Commonly used surfactants have previously shown the same effects in this model.
Relevance of study:	Not relevant (Prevention of cell cycle transition was determined for the glyphosate formulation. This model is not appropriate for testing materials containing surfactants because surfactant induced cytotoxicity via membrane disruption is well documented using *in vitro* systems.
Klimisch code:	3

Author(s)	Year	Study title
Marc, J. Belle, R. Morales, J. Cormier, P. Mulner-Lorillon, O.	2004	Formulated glyphosate activates the DNA-response checkpoint of the cell cycle leading to the prevention of G2/M transition. Toxicological Sciences, Volume: 82, Pages: 436-442 ASB2012-11894

Abstract*
A glyphosate containing pesticide impedes at 10 mM glyphosate the G2/M transition as judged from analysis of the first cell cycle of sea urchin development. We show that formulated glyphosate prevented dephosphorylation of Tyr 15 of the cell cycle regulator CDK1/cyclin B in vivo, the end point target of the G2/M cell cycle checkpoint. Formulated glyphosate had no direct effect on the dual specific cdc25 phosphatase activity responsible for Tyr 15 dephosphorylation. At a concentration that efficiently impeded the cell cycle, formulated glyphosate inhibited the synthesis of DNA occurring in S phase of the cell cycle. The extent of the inhibition of DNA synthesis by formulated glyphosate was correlated with the effect on the cell cycle. We conclude that formulated glyphosate's effect on the cell cycle is exerted at the level of the DNA-response checkpoint of S phase. The resulting inhibition of CDK1 cyclin B Tyr 15 dephosphorylation leads to prevention of the G2/M transition and cell cycle progression.

* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Non-standard, non-guideline study. Commonly used surfactants have previously shown the same effects in this model.
Relevance of study:	Not relevant (Prevention of cell cycle transition was determined for the glyphosate formulation. This model is not appropriate for testing materials containing surfactants because surfactant induced cytotoxicity via membrane disruption is well documented using *in vitro* systems.
Klimisch code:	3

Additional comments:
Comments of the notifier are submitted on the web site of Monsanto (2006, ASB2013-5455): http://www monsanto.com/products/Documents/glyphosate-background materials/Response_ISIS_apr_06.pdf
The following two recent publications, by Heu *et al.* (2012, ASB2012-11843 and ASB2012- 11844) are commented on collectively after the second summary/Klimisch rating, below.

Author(s)	Year	Study title
Heu, C., Berquand, A., Elie-Caille, C., Nicod, L.	2012	Glyphosate-induced stiffening of HaCat keratinocytes, a Peak Force Tapping study on living cells. Journal of Structural Biology Volume: 178, Number: 1, Pages: 1-7 ASB2012-11843

Abstract*
The skin is the first physiological barrier, with a complex constitution, that provides defensive functions against multiple physical and chemical aggressions. Glyphosate is an extensively used herbicide that has been shown to increase the risk of cancer. Moreover there is increasing evidence suggesting that the mechanical phenotype plays an important role in malignant transformation. Atomic force microscopy (AFM) has emerged within the last decade as a powerful tool for providing a nanometer-scale resolution imaging of biological samples. Peak Force Tapping (PFT) is a newly released AFM-based investigation technique allowing extraction of chemical and mechanical properties from a wide range of samples at a relatively high speed and a high resolution. The present work uses the PFT technology to investigate HaCaT keratinocytes, a human epidermal cell line, and offers an original approach to study chemically-induced changes in the cellular mechanical properties under near-physiological conditions. These experiments indicate glyphosate induces cell membrane stiffening, and the appearance of cytoskeleton structures at a subcellular level, for low cytotoxic concentrations whereas cells exposed to IC_{50} (inhibitory concentration 50%) treatment exhibit control-like mechanical behavior despite obvious membrane damages. Quercetin, a well-known antioxidant, reverses the glyphosate-induced mechanical phenotype.

* Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable

Comment: Non-guideline *in vitro* tests with no control for low pH effects. Minor reporting deficiencies (source and purity of glyphosate, replicates per dose level)

Relevance of study: Not relevant (*in vitro* data on the effects on an immortalised epidetmal cell-line does consider low exposure potential due to *stratum cornea* protection. Inappropriate test substance if not adjusted for pH; low pH glyphosate acid is not in formulated glyphosate based products)

Klimisch code: 3

Author(s)	Year	Study title
Heu, C., Elie-Caille, C., Mougey, V., Launay, S., Nicod, L.	2012	A step further towards glyphosate-induced epidermal cell death: Involvement of mitochondrial and oxidative mechanisms. Environmental Toxicology and Pharmacology Volume: 34, Number: 2, Pages: 144-153 ASB2012-11844

Abstract*
A deregulation of programmed cell death mechanisms in human epidermis leads to skin pathologies. We previously showed that glyphosate, an extensively used herbicide, provoked cytotoxic effects on cultured human keratinocytes, affecting their antioxidant capacities and impairing morphological and functional cell characteristics. The aim of the present study, carried out on the human epidermal cell line HaCaT, was to examine the part of apoptosis plays in the cytotoxic effects of glyphosate and the intracellular mechanisms involved in the apoptotic events. We have conducted different incubation periods to reveal the specific events in glyphosate-induced cell death. We observed an increase in the number of early apoptotic cells at a low cytotoxicity level (15%), and then, a decrease, in favour of late apoptotic and necrotic cell rates for more severe cytotoxicity conditions. At the same time, we showed that the glyphosate-induced mitochondrial membrane potential disruption could be a cause of apoptosis in keratinocyte cultures.

* Quoted from article

Klimisch evaluation

Reliability of study: Not reliable

Comment: Non-guideline *in vitro* tests with no control for low pH effects. Minor reporting deficiencies (source and purity of glyphosate, replicates per dose level)

Relevance of study: Not relevant (*in vitro* data on the effects on an immortalized epidermal cell-line does consider low exposure potential due to *stratum cornea* protection. Inappropriate test substance if not adjusted for pH; low pH glyphosate acid is not contained in formulated glyphosate based products)

Klimisch code: 3

Additional comments:
Glyphosate technical acid evaluated was not reported to be pH adjusted and therefore does not reflect real world exposures to the more neutral pH formulations, which contain glyphosate salts, not glyphosate acid. The pH range of test concentrations (850 - 1150 mg/L) is very acidic, approximately 1.7-2.2 pH units. Keeping in mind the pH scale is logarithmic, these values are substantially lower than those of viable skin and *in vitro* cell cultures.

Exposure potential to live human epidermal skin cells in the field is likely to be considerably lower than the authors have considered. The epidermis is protected by the *stratum cornea*. Human *in vitro* dermal absorption studies for a range glyphosate formulated products are presented in the chapter on dermal absorption, showing a very low dermal absorption of glyphosate; nearly all of the glyphosate is washed off the skin surface after 24 hour exposures (88% to >99% before *stratum cornea* removal). Therefore, the studies of Heu *et al.*, while representative of glyphosate spray concentrations, are approximately two or more orders of magnitude higher of those which may result for 8-24 hour dermal exposures.

Author(s)	Year	Study title
Axelrad, J.C. Howard, C.V. McLean, W.G.	2003	The effects of acute pesticide exposure on neuroblastoma cells chronically exposed to diazinon Toxicology, Volume: 185, Pages: 67-78 ASB2012-11553

Abstract*
Speculation about potential neurotoxicity due to chronic exposure to low doses of organophosphate (OP) pesticides is not yet supported by experimental evidence. The objective of this work was to use a cell culture model of chronic OP exposure to determine if such exposure can alter the sensitivity of nerve cells to subsequent acute exposure to OPs or other compounds. NB2a neuroblastoma cells were grown in the presence of 25 µM diazinon for 8 weeks. The OP was then withdrawn and the cells were induced to differentiate in the presence of various other pesticides or herbicides, including OPs and OP-containing formulations. The resulting outgrowth of neurite-like structures was measured by light microscopy and quantitative image analysis and the IC_{50} for each OP or formulation was calculated. The IC_{50} values in diazinon-pre-exposed cells were compared with the equivalent values in cells not pre-exposed to diazinon. The IC50 for inhibition of neurite outgrowth by acute application of diazinon, pyrethrum, glyphosate or a commercial formulation of glyphosate was decreased by between 20 and 90% after pre-treatment with diazinon. In contrast, the IC_{50} for pirimiphos methyl was unaffected and those for phosmet or chlorpyrifos were increased by between 1.5- and 3-fold. Treatment of cells with chlorpyrifos or with a second glyphosate-containing formulation led to the formation of abnormal neurite-like structures in diazinon-pre-exposed cells. The data support the view that chronic exposure to an OP may reduce the threshold for toxicity of some, but by no means all, environmental agents.

* Quoted from article

Klimisch Evaluation

Reliability of study: Not reliable
Comment: Incorrect characterisation of glyphosate as an organophosphate pesticide. Inappropriate test system for formulations containing surfactant; cytoxic membrane disruption potential of surfactant are well known for in vitro test systems. Exposure route not relevant for human risk assessment. Rationale for chosen test substance concentration not given.
Relevance of study: Not relevant (*in vitro* data, do not reflect real *in vivo* exposure situations. Pre-exposure to diazinon is not relevant for this submission).
Klimisch code: 3

Author(s)	Year	Study title

Benedetti, A. L. Vituri, C.D. Trentin, A.G. Domingues, M.A.C. Alvarez-Silva, M.	2004	The effects of sub-chronic exposure of Wistar rats to the herbicide Glyphosate-Biocarb® Toxicology Letters, Volume: 153, Pages: 227-232 ASB2012-11562

Abstract*
The object of this study was to analyze the hepatic effects of the herbicide Glyphosate-Biocarbo (as commercialized in Brazil) in Wistar rats. Animals were treated orally with water or 4.87, 48.7, or 487 mg/kg of glyphosate each 2 days, during 75 days. Sub-chronic treatment of animals starting from the lowest dose of glyphosate induced the leakage of hepatic intracellular enzymes, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), suggesting irreversible damage in hepatocytes. We observed the increase of Kupffer cells in hepatic sinusoid of glyphosate-treated animals. This was followed by large deposition of reticulin fibers, composed mainly of collagen type III. We may conclude that Glyphosate-Biocarbo may induce hepatic histological changes as well as AST and ALT leaking from liver to serum in experimental models.

* Quoted from article

Klimisch evaluation

Reliability of study: Not reliable

Comments: Study report meets basic scientific principles. Study design and documentation is insufficient for assessment.

Relevance of study: Not relevant because study design not sufficient for assessment of toxicity of the active substance Glyphosate. Toxicity is attributable to high oral dosing of surfactant component. There are several reporting deficiencies.

Klimisch code: 3

Author(s)	Year	Study title
Mesnage, R. Clair, E. Gress, S. Then, C. Szekacs, A. Seralini, G.E.	2012	Cytotoxicity on human cells of Cry1Ab and Cry 1Ac *Bt* insecticidal toxins alone or with a glyphosate-based herbicide. Journal of Applied Toxicology doi: 10.1002/jat.2712. [Epub ahead of print] ASB2012-11900

Abstract*
The study of combined effects of pesticides represents a challenge for toxicology. In the case of the new growing generation of genetically modified (GM) plants with stacked traits, glyphosate-based herbicides (like Roundup) residues are present in the Roundup-tolerant edible plants (especially corns) and mixed with modified *Bt* insecticidal toxins that are produced by the GM plants themselves. The potential side effects of these combined pesticides on human cells are investigated in this work. Here we have tested for the very first time Cry1Ab and Cry1Ac *Bt* toxins (10 ppb to 100 ppm) on the human embryonic kidney cell line 293, as well as their combined actions with Roundup, within 24 h, on three biomarkers of cell death: measurements of mitochondrial succinate dehydrogenase, adenylate kinase release by membrane alterations and caspase 3/7 inductions. Cry1Ab caused cell death from 100 ppm. For Cry1Ac, under such conditions, no effects were detected. The Roundup tested alone from 1 to 20 000ppm is necrotic and apoptotic from 50 ppm, far below agricultural dilutions (50% lethal concentration 57.5 ppm). The only measured significant combined effect was that Cry1Ab and Cry1Ac reduced caspases 3/7 activations induced by Roundup; this could delay the activation of apoptosis. There was the same tendency for the other markers. In these results, we argue that modified *Bt* toxins are not inert on non-target human cells,

and that they can present combined side-effects with other residues of pesticides specific to GM plants.

[k] Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Non-guideline, non-GLP *in vitro* tests meeting scientific principles. Deficiencies: No positive controls were specified, test conditions not described (referenced to a description elsewhere). Exceedingly high doses and an inappropriate test system for formulations containing surfactant; cytoxic membrane disruption potential of surfactants are well known for in vitro test systems.
Relevance of study:	Relevant with restrictions (Due to reliability. The assessed combinatory effects are of limited relevance)
Klimisch code:	3

Additional comments:

Direct exposure to cells in culture bypasses normal processes limiting absorption and cellular exposure and avoids normal metabolism, excretion, serum protein binding, and other factors that would protect cells in the intact organism. Anadon *et al.* (2009, ASB2012-11542) dosed rates with 400 mg/kg of glyphosate, a massive dose relative to any environmental exposure, and achieved peak modeled plasma concentrations of glyphosate of approximately 5 µg/mL (5 mg/L or 5 ppm). Assuming linear kinetics, the maximum allowable US daily intake (2 mg/kg/day) would give an approximated blood concentration of 0.025 ppm (25 ppb). McQueen *et al.* (2012, ASB2012-11898) recently evaluated glyphosate exposure to pregnant women and concluded that estimated exposures based on actual measurements in food were only 0.4% of the acceptable daily intake.

The "Roundup" LC_{50} concentration used (57.5 ppm) is more than 2000-fold higher than the anticipated concentration (based on Anadon *et al.*, 2009, ASB2012-11542) following maximum allowable intake.

The co-application of Cry protein with the glyphosate-surfactant reduces the apparent degree of cellular injury (as measured by induction of caspase levels). This occurs even at concentrations of Cry1Ab which the authors report to cause cellular injury and membrane disruption. This is worth noting for several reasons:

First, it brings into question the toxicity observations with Cry1Ab, as the argument that membrane disruption and impaired mitochondrial function should be protective seems to be highly untenable, especially in view of the studies (Levine et al, 2007, ASB2009-9030) demonstrating the mitochondrial membrane activity of surfactants.

Second, it should take off the table any implications of a "synergistic effect" of Cry proteins and glyphosate-surfactant herbicides. (The direction is, if anything, antagonistic, but the entire system is fundamentally irrelevant.)

Third, this probably is demonstrating the artificiality of the system itself. As noted above, this is a protein-free medium. Protein protects cells in culture by multiple mechanisms- binding to toxic materials, binding to potential receptor sites, or other non-specific surface-stabilisation effects. It appears from Mesnage's own data that simple addition of protein to their system, even at low concentrations (and even if that protein is a Cry protein) protects from toxicity.

Author(s)	Year	Study title

Clair E., Linn, L., Travert, C., Amiel, C., Seralini, G.E	2012	Effects of Roundup® and Glyphosate on Three Food Microorganisms: *Geotrichum candidum*, *Lactococcus lactis* subsp. *cremoris* and *Lactobacillus delbrueckii* subsp. *bulgaricus* Current Microbiology, Volume: 64, Number: 5, Pages: 486-491 ASB2012-11592

Abstract*
Use of many pesticide products poses the problem of their effects on environment and health. Amongst them, the effects of glyphosate with its adjuvants and its by-products are regularly discussed. The aim of the present study was to shed light on the real impact on biodiversity and ecosystems of Roundup®, a major herbicide used worldwide, and the glyphosate it contains, by the study of their effects on growth and viability of microbial models, namely, on three food microorganisms (*Geotrichum candidum*, *Lactococcus lactis* subsp. *Cremoris delbrueckii* subsp. *bulgaricus*) widely used as starters in traditional and industrial dairy technologies. The presented results evidence that Roundup® has an inhibitory effect on microbial growth and a microbicide effect at lower concentrations than those recommended in agriculture. Interestingly, glyphosate at these levels has no significant effect on the three studied microorganisms. Our work is consistent with previous studies which demonstrated that the toxic effect of glyphosate was amplified by its formulation adjuvants on different human cells and other eukaryotic models. Moreover, these results should be considered in the understanding of the loss of microbiodiversity and microbial concentration observed in raw milk for many years.

[k] Quoted from article

Klimisch evaluation

Reliability of study:	Not reliable
Comment:	Non-validated, non-guideline test with methodological and reporting deficiencies (e.g. dose concentrations in media not specified, no positive controls or controls that show the validity of the test system /and concentration range tested). Inappropriate test system for formulations containing surfactant; cytoxic membrane disruption potential of surfactants are well known for in vitro test systems.
Relevance of study:	Not relevant (Due to reliability)
Klimisch code:	3

Additional Comments
Glyphosate at 1% had no effect on lactobacilli but did impair *Geotrichum*, which is unsurprising as glyphosate at herbicidal concentrations will impact metabolism of many fungi, which (like plants) use the shikimate pathway for aromatic amino acid production.
Surfactants are known to be bacteriostatic, with (for example) quaternary ammonium compounds typically being active in the 30-150 ppm range.

Clair *et al.* demonstrate that surfactants are bacteriostatic for 3 microorganisms at concentration ranges well within the range of concentrations generally found to be useful for sanitation purposes. However, surfactant solutions are routinely used to sanitize food processing equipment at concentrations at or above those tested by Clair *et al.* (2012, ASB2012-11592).

B.6.5 Medical data and information (Annex IIA 5.9)
B.6.5.1 Toxicity of the metabolite AMPA
B.6.5.2 Reports on clinical cases and poisoning incidents

Glyphosate is worldwide used extensively as herbicide. According to this extensive use a large number of poisoning incidents happened and was published. An extensive review of clinical cases was submitted by Bradberry *et al.*, 2004, ASB2012-11576. Another review of cases was published by Lee *et al.*, 2008, ASB2012-11879.

Burger *et al.* (2009, ASB2013-11831) briefly mentioned a total of 60 reports by physicians from Germany on cases of poisoning with glyphosate herbicides since 1990. Without further specification, in the vast majority of 52 cases only slight health impairment was reported. In four cases, health disturbances were considered "moderate" whereas the only one actually life- threatening case was the result of ingestion of 200 mL of a herbicide containing glyphosate and a tallowamine surfactant with suicidal intent. In the three remaining cases, no symptoms were reported or their severity could not be evaluated.

More than 650 cases of intoxication/irritation ascribed to ingestion of contact to glyphosate- based herbicides are mentioned in an overview on poisoning incidents from Brazil that was just recently kindly provided to the RMS by the Brazilian National Health Surveillance Agency (Paumgartten/ANVISA, 2012, ASB2013-13413). This data was collected by up to 10 Brazilian poison centers between 2010 and 2012. It is not clear if it is representative for the whole huge country in which the agricultural conditions in general and also those of pesticide use are extremely different. In addition, there is even a higher number of poison information centers in Brazil that have not provided data and, thus, the number of incidents might underestimate the real incidence. On the first glance, the exposure routes, ingested amounts, circumstances (accident, suicidal attempt?), clinical signs and medical treatment are similar to what is known from Germany and from the literature. The much higher total number of cases (as compared to Germany) seems to reflect the applied amount of glyphosate and its formulations that is by orders of magnitude higher in Brazil. Unfortunately, severity of symptoms is not graded and vey often the final outcome was not reported. Another problem is that the product to which exposure was claimed is not specified. Frequently, "Roundup" is mentioned but different formulations are marketed under this name. In many other cases, the incident is ascribed to"glyphosate". However, this is not credible because there is no simple access to the active ingredient and a certain product must have been applied. Furthermore, it is not clear whether a causal relationship had been actually confirmed. Thus, this potentially most interesting data require further thorough analysis before it may be used for evaluation of health risks.

Jayasumana *et al.* (2014, ASB2014-3085) published a hypothesis on an association between glyphosate exposition, hard water and nephrotoxic metals in an epidemic of chronic kidney disease in Sri Lanka. The authors conclude that although glyphostate alone does not cause an epidemic of chronic kidney disease, it seems to have acquired the ability to destroy the renal tissues when forms complexes with localized geo environmental factor (hardness) and nephrotoxic metals. This conclusion is mainly based on a metal chelating property of glyphosate and the extensive use of glyphosate in Sri Lanka, and the occurance of hard water in the concerned areas. However, the hypothesis was not experimentally evidenced up to now. Sirinathsinghji (2014, ASB2014-10742) report that Sri Lanka is set to partially ban glyphosate-based herbicide use following a study linking it to a fatal chronic kidney disease epidemic affecting the country. This decision is also based on the above described publication of Jayasumana *et al.* (2014, ASB2014-3085).

Zouaoui *et al.* (2012, ASB2014-9734) reported 13 cases of acute intoxication with glyphosate (mostly suicidal). The most common symptoms were oropharyngeal ulceration, nausea and vomiting. The main altered biological parameters were high lactate and acidosis. We also noted respiratory distress, cardiac arrhythmia, hypercaleamia, impaired renal function, hepatic toxicity and altered consciousness. In fatalities, the common symptoms were cardiovascular shock, cardiorespiratory arrest, haemodynamic disturbance, intravascular disseminated coagulation and multiple organ failure. Concentrations of glyphosate and AMPA have been determined in blood and urine.

Sribanditmongkol *et al.* (2012, ASB2014-9731) report a case of a woman who died after ingestion of approximately 500 ml Roundup formulation. Toxic effects of the pesticide were caused by the abilitiy to erode tissues including mucous membranes and linings of the gastrointestinal and respiratory tracts.

A mild degree of pulmonary congestion and edema was observed in both lungs.
Mariager *et al.* (2013, ASB2014-9612) report the case of a 43-year-old man with a history of alcohol abuse. He had used a concentrated glyphosate herbicide and accidentally sprayed the liquid on himself. The patient developed local swelling, bullae and exuding wounds. Neurological impairment followed affecting finger flexion and sensation with reduced nerve conduction. Imaging revealed oedema of the soft tissue and juxta-articular osteopenia

Lee *et al.* (2012, ASB2014-9607) report the case of a 60-year orld patient who intentionally ingested 450 ml Roundup. He experienced cardiac arrest but was successfully resuscitated and treated with continuous venovenous haemodiafiltration.

Knezevic *et al.* (2012, ASB2014-9593) report the case of a 36 year old male patient who attemted suicide by drinking approximately 300 mL of glyphosate formulation. The patient became hypotensive, hypoxic with oliguric acute renal failure. After a single 27.5 hour treatment, clinical condition and renal function parameters did not require further dialysis.

Hour at al. (2012, ASB2014-9571) describe the case of a 66 year old man with a history of alcohol abuse who ingested 500 ml of rum and 350 ml of Roundup. He was hypotensive, diaphoretic and hypoxic. After veno-venous hemodiafiltration his condition improved within 24 hours.

Hinojosa *et al.* (2011, ASB2014-9566) submitted a retrospective study to identify substances involved in poisonings at Lariboisiere hospital. 315 patients were included with 891 announced substances. Only 1 case of glyphosate was identified.

Garlich *et al.* (2014, ASB2014-9480) report the case of a 62-year old man who drank a bottle of 41% glyphosae formulation. He was bradycardic and obtunded with respiratory depression. The patient underwent haemodialysis 16 h post ingestion after which he demonstrated improvement in clinical status.

A case of an inhalative intoxication by glyphosate is reported by BfR (2007, ASB2014-9290). A 59 years old farmer sprayed a glyphosate formulation without protective equipment over approximately 3 hours. He suffered from laboured breathing, cough and fever. A biopsy showed alveolitis and bronchiolitis.

Beswick and Millo (2011, ASB2014-9283) describe a fatal poisoning with a glyphosate surfactant herbicide. A 29-year old man was admitted following deliberate ingestion of approximatly 300 ml of Roundup Ace. He developed severe and persistent lactic acidosis, hyperkalaemia, hypotension, torrential watery diarrhoea and abdominal distension in the first 24 hours. The clinical course was complicated by cardiac arrhythmia and an episode of cardiac arrest. On day three following poisoning, the patient died.
Malhotra *et al.* (2010, ASB2012-11890) report a case of a 71-year old male who attempted suicide with a glyphosate formulation and developed a prolonged but reversible encephalopathy suggestive of acute CNS toxicity. He was in cardiogenic shock with severe metabolic acidosis. Neurologic investigations were performed to exclude structural pathology. CT brain scan was normal. An EEG reading on day 8 demonstrated generalised slow wave acitivity with triphasic sharp and slow wave complex consistent with an encephalopathy although non convulsive seizures could not be excluded.

B.6.5.1 Publications on dermal absorption of glyphosate

Wester *et al.* (2005, ASB2012-12050) compared dermal absorption through human skin *in vitro* after exposure either to a 1% solution (not further specified) or to cotton sheets that were treated with the same solution on the same day or up to 2 days before to reflect the abundance of glyphosate residues infabric that may occur due to applications of this active ingredient in cotton. The authors could show a lower absorption of glyphosate across the skin from the treated cotton as compared to the liquid solution itself but found also remarkable residues on and in the skin when the cotton sheets had been treated on

the same day. Adding of water to the cotton sheets resulted in an increase in absorption rate.